ESTABLISHMENT OF THE URUGUAYAN BIOSAFETY FRAMEWORK AND A REGULATORY PERSPECTIVE OF ENVIRONMENTAL RISK ASSESSMENT FOR TRANSGENIC CROPS ENGINEERED WITH COMPLEX TRAITS

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ABSTRACT

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With increasing world population and anticipated impacts of climate change, there is a need to develop crop varieties that provide increased productivity while preserving biodiversity and the environment. Genetic engineering is one of the techniques available to be used in plant breeding for crop improvement. This technology has the potential to generate a broad range of new products, including organisms with significantly altered morphology and physiology. Use of these new technologies and the potential impact of the resultant products have led to a cautious attitude determining that GE crops follow a different regulatory pathway than new varieties derived from conventional breeding programs. In this thesis I discuss the establishment of a biotechnology biosafety regulatory system in Uruguay, its comparison with other systems in the region and the analysis of environmental safety questions from a regulatory point of view. In recent years the Uruguayan regulatory system for GE organisms has undergone consolidation in response to political support of biotechnology. The legal support is based on Decree 353/008 and is slowly progressing to law. This thesis describes development of an operational system to handle applications including establishment of an administrative system which supports the risk analysis phases of risk assessment; risk management; and risk communication; and an operational system for follow-up actions of monitoring, surveillance and inspection. The Uruguayan approach is next compared with those of Argentina, Brazil and Paraguay, which together form the Southern Common Market (MERCOSUR for its acronym in Spanish). The objective was to

identify from divergences new strategies for improvement of the Uruguayan system, and from commonalities potential approaches for harmonization. Harmonization could potentially achieve a more efficient and effective framework for the use of economic, infrastructure and human resources, and avoid problems of asynchronous authorizations. While difficult to coordinate the approval of GE organisms for commercial use throughout the region, specific suggestions are proposed with actions toward harmonization. Finally, the thesis addresses risk analysis for crops engineered with complex traits such as dehydration stress tolerance. These traits may utilize genes that encode transcription factors, signalling factors, metabolic pathway enzymes, among others, with the potential to initiate a cascade of cellular changes that may produce unanticipated effects on plant metabolism, physiology, and/or development with biosafety implications. As a result, the application review for environmental biosafety may be more complex as we move from the first wave of genetically engineered crops, such as insect- or herbicide tolerant crops for which the gene product directly confers the trait of interest, to crops with more complex traits. It should be noted, however, that while important to evaluate, pleiotropic effects are not a hazard per se. Rather, the same ultimate harms must be evaluated, whether those harms arise due to primary effects of the gene, or pleiotropic or unintended effects. A conceptual framework is developed for regulators when analyzing environmental risk assessments of transgenic crops with complex traits. A case study is performed for release of cucumber expressing the *Arabidopsis thaliana* dehydration stress tolerance transcription factor gene, *CBF*, into the Uruguayan environment.

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LIST OF ABBREVIATIONS

ANII:	National Agency of Research and Innovation
CAAR:	Advisory Committee for Risk Analysis
CAI:	Institutional Coordination Committee
CECTE:	Committee of Ethics in Science and Technology (Argentina)
CERV:	Commission on Risk Assessment of Genetically Engineered Plants
CGR:	Risk Management Commission
CIBIO:	Internal Biosafety Commission (Brazil)
CNBS:	National Biosafety Council (Brazil)
COMBIO:	Agricultural and Forestry Biosafety Commission, (Paraguay)
CONABIA:	National Advisory Commission on Agricultural Biotechnology (Argentina)
CTAUOGM:	Technical Advisory Committee on the Use of Genetically Modified Organisms
CTNBIO:	National Biosafety Technical Commission (Brazil)
DGSA:	General Direction of Agricultural Services
DGSPA:	General Direction of Agricultural Protection Service
DMA:	Directorate of Agrifood Markets (Argentina)
DPA:	Division of Agricultural Protection
ERB:	Biosafety Risk Assessment
GIM:	Interministries Working Group created by Decree No. 037/007
GNBio:	National Biosafety Cabinet
GTA:	Working Group on legal aspects
GTE:	Working Group on labeling
GTI:	Working Group on institutions
GTP:	Working Group on public participation

IIBCE:	Biological Research Institute Clemente Estable
INASE:	National Institute of Seeds
INIA:	National Institute of Agriculture Research
IP:	Institute Pasteur of Montevideo
LATU:	Technological Laboratory of Uruguay
MAG:	Ministry of Agriculture and Livestock (Paraguay)
MAGYP:	Ministry of Agriculture, Livestock and Fisheries (Argentina)
MEC:	Ministry of Education and Culture
MEF:	Ministry of Economy and Finances
MERCOSUR:	Southern Common Market among Argentina, Brazil, Paraguay and Uruguay.
MGAP:	Ministry of Livestock, Agriculture and Fisheries
MINCYT:	Ministry of Science, Technology and Innovation (Argentina)
MSP:	Ministry of Public Health
MVOTMA:	Ministry of Housing, Land and Environment
NBF:	National Biosafety Framework
OERF:	Registration and Monitoring Bodies and Entities (Brazil)
RR:	Round-up Ready
SAGYP :	Secretary of Agriculture, Livestock and Fisheries (Argentina).
SEAM:	Secretary of Environment (Paraguay)
SENACSA:	National Service of Quality and Plant Health and Seeds (Paraguay)
SENASA:	National Health Service and Food Quality (Argentina)
SENASA:	National Service of Quality and Animal Health before named National Service of Animal Health (SENASA), (Paraguay)
SENAVE:	National Service of Quality and Plant Health and Seeds (Paraguay)
SIB:	Biosafety Information System (Brazil)

UDELAR: University of the Republic - public academia

CHAPTER I: LITERATURE REVIEW

INTRODUCTION

We are approaching a new era of genetically engineered (GE) crops which will involve an increased variety and complexity of types of genes and phenotypes incorporated into new crops and planted in new locations. Environmental safety considerations for GE crops vary with crop, gene, trait and location and result from a complex interplay of each of the factors (Figure 1.1).

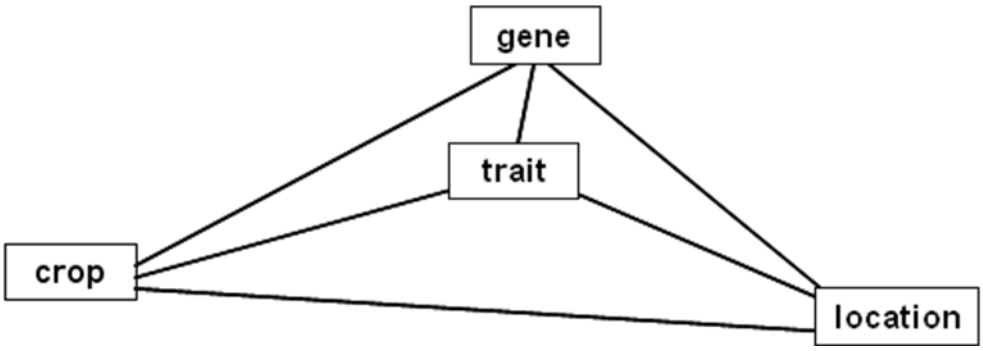


Figure 1.1. Environmental safety considerations for GE crops result from a complex interplay of factors that vary with crop, gene, trait, and location. (Figure from Grumet *et al*., 2011).

Changes are observed when comparing the first decade of commercially approved biotech crops "1996-2005" with the second decade "2006-2015" (Figure 1.2). New types of genes whose mode of action differs from those of the first wave of transgenic crops include genes that encode transcription factors, signal transduction pathway and metabolic enzymes. New traits under development include resistance to biotic and abiotic stresses, increase in valuable compounds, improved availability of nutrients, or a decreased concentration of undesirable substances. New crops include some non-commodity crops such as horticultural species, and 'orphan crops' that have received little breeding or biotechnology attention. As different regions of the world begin to grow these crops, and new kinds of crops are grown, new locations and environmental conditions also will be encountered.

To date, commercially produced GE crops were almost exclusively developed by U.S. or European private corporations and first cultivated in the US and other countries which are producers of those crops, such is the case of soybean in South America or canola in Canada (James, 2012). Additionally the private developer also applied for regulatory approval in strategic countries that import the product as food and/or feed, such as countries of the European Union and Japan (Stein and Rodriguez-Cerezo, 2009). However many developing and transitioning countries whose economies rely on agriculture are incorporating biotechnology and molecular plant breeding in their national plans, to find solutions for increasing national and regional demand for food security and to satisfy global needs for a more productive agriculture (James, 2012).

GE crops are being developed by public national providers in Asia and Latin America designed specifically to solve local agricultural problems and to be commercialized in domestic markets. For example, in Brazil a public/private association between the private companies BASF together with the National Agricultural Research Institute EMBRAPA led to the development of transgenic soybean with tolerance to the herbicide imidazolinona that was approved for commercial release by the Brazilian government in 2009 (Brazil, CTNBio, 2009). More recently the public sector institution EMBRAPA has developed and obtained commercial regulatory approval of GE *Phaseolus* beans with virus resistance (Brazil, CTNBio, 2012). Other examples include Bt brinjal (eggplant) in India, Bt rice and phytase maize in China which has been approved for field trials, Bt maize in Cuba that was planted 3,000 hectares as "regulated commercialization", as well as other examples under development in Cuba, Argentina, and Chile (Cohen, 2005; Grumet *et al*., 2011; James, 2011, 2012). Bt eggplant in India is being developed through a private-public partnership (between Monsanto (Mahyco) and the public sector), while a water efficient maize project in Africa is being performed in partnership with the Bill and Melinda Gates Foundation (James, 2011).

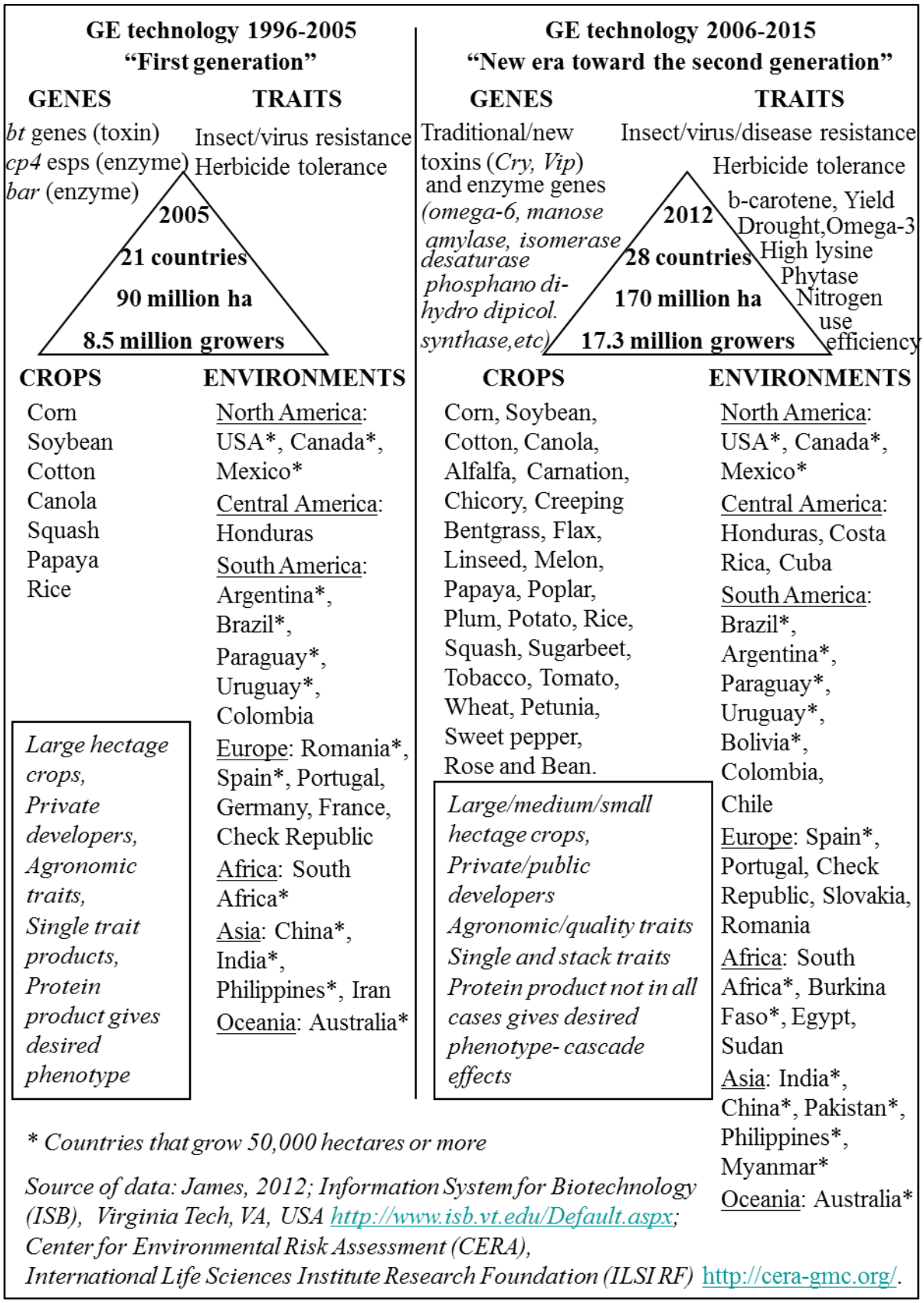


Figure 1.2. Comparison of deregulated or in an advanced stage of risk analysis events between the first two decades of the genetically engineered technology (1996-2005 and 2006-2015).

Bt rice and phytase maize in China have been developed entirely by the public sector, the Chinese Agricultural Academy of Sciences (CAAS). Other examples of China include Beijing University Bt poplar developed by the Research Institute of Forestry and Beijing virus resistance sweet pepper (PK-SP01) developed by the Beijing University (ISAAA homepage, 2013). Bt maize in Cuba with resistance to a major local pest (*Spodoptera frugiperda*) has been developed by the Havana-based Institute for Genetic Engineering and Biotechnology (CICB) (James, 2012). The technology used by Monsanto and BASF to develop the drought tolerant transgenic maize, that will be commercially released in the US in 2013, was donated to a Private/Public sector partnership (WEMA) of sub-Saharan Africa to develop a biotech drought tolerant maize expected for 2017 (James, 2012). Other countries including the Philippines, Bangladesh, Vietnam, Malaysia, Thailand, Egypt, Kenya, South Africa, Mexico, Costa Rica, have records of official field trials of GE plants conducted with a variety of species (Nap *et al*., 2003; Toenniessen *et al*., 2003; James, 2011).

As these sorts of projects involving new kinds of technologies are undertaken by an increasing number of countries throughout the world, the necessary regulatory systems must also be developed and adapted. In this thesis I discuss the establishment of a biotechnology biosafety regulatory system in Uruguay and the analysis of environmental safety questions from a regulatory point of view. Specific portions of the literature review are from *"Future possible genetically engineered crops and traits and their potential environmental impacts"* Grumet R, Wolfenbarger L and Ferenczi A. pp. 47-57 and *"Systems to regulate genetically engineered plants. Similarities and differences among countries"* Hokanson K and Ferenczi A. pp. 147-155 of *"Environmental safety of genetically engineered crops"* 2011. 234p.

CURRENT STATUS OF GLOBAL PRODUCTION OF GENETICALLY ENGINEERED CROPS

Since the first commercially released transgenic crops were planted in the United States in 1996 the total area planted has been constantly increasing, reaching 170.3 million hectares in 2012 (James, 2012). This represents approximately 11.3% of the 1.5 billion hectares of total arable land area worldwide (James, 2011). In 2012 twenty eight (28) countries cultivated GE crops; an additional 31 countries have regulatory approvals for import for food and feed use (James, 2012). The area planted by the 28 countries varies substantially, with 17 countries that plant 50,000 or more hectares. Rapid adoption occurred primarily in countries such as US and Canada in North America; Brazil, Argentina, Paraguay and Uruguay in South America; South Africa in Africa; and China and India in Asia. These are all countries that are major producers of one or more of the handful of primary GE crops, cotton, soybean, maize and canola.

Several Latin American countries are currently producing GE crops. In the countries of the Southern Common Market (MERCOSUR for its acronym in Spanish), there is currently political support for biotechnology. Brazil is the country with the highest number of commercial approvals (36), followed by Argentina (28), Uruguay (14) and Paraguay (6). Uruguay is in the tenth position worldwide with an estimated 1.3 million hectares of total GE crops. Four events in soybean and ten events in corn have been approved for planting and commercialization since 1996 (URUGUAY, GNBio homepage 2013). In the season 2011-12, approximately 900,000 ha of soybean were planted, with 100% GE varieties, and about 120,000 ha of corn, with an estimated 60- 90% GE varieties (Souto, 2010; Uruguay, MGPA-DIEA, 2012). In Brazil, not only the area, but also the number of events approved recently has increased, reaching five in soybean, eighteen in corn, twelve in cotton and one in bean (Brazil, CTNBio, 2013). Argentina also has changed its regulatory system to improve its

efficiency and has already increased the number of events with regulatory approval having approved five in soybean, twenty in corn and three in cotton (Argentina, CONABIA, 2013). Paraguay for its part also has recently adjusted its regulatory system having approved four commercial events in corn and one in soybean (Paraguay, CONBIO, 2013). Table 1.1 compares the commercial approvals in soybean and corn, which are the crops planted in Uruguay, within the countries of MERCOSUR.

Other Latin American countries also support transgenic technology. The case of Mexico approving transgenic maize for field trials is particularly interesting. As Mexico is a center of origin for maize, there are additional challenges for the regulatory system regarding monitoring and control to avoid possible environmental harms due to gene flow through pollen (Ortiz, 2011). Costa Rica recently approved transgenic crops, not for internal market, but for seed export. Chile currently produces seed for export and is now adjusting its biosafety framework to include commercial production of GE crops (Teresa Agüero, 2011). The government of Chile has the will to approve transgenic crops for domestic production, and also foresees potential to release their own transgenic crops solving specific local problems. Research institutions in Chile have developed several projects that are being tested under contained conditions. Commercial release of GE crops can be a disadvantage for the seed industry by creating potential problems with adventitious and low level presence. Currently there is only transgenic seed export production under strict biosafety controlled conditions that is concentrated in a specific area of the country for this purpose.

Table 1.1.Commercial approvals in soybean and corn in the countries of the Southern Common Market (MERCOSUR): Brazil (BRA), Argentina (ARG), Uruguay (URU) and Paraguay (PAR).

Event/Crop	BRA	ARG	URU	PAR
Events in soybean				
40-3-2 (Ht1)	1998	1996	1996	2004
BPS-CV127-9 (Ht3)	2009	2013	---	---
A2704-12 (Ht2)	2010	2011	2012	---
A5547-127 (Ht2)	2010	2011	2012	---
MON89788X87701 (Ht1xIr1)	2010	2012	2012	2013
Events in corn				
176	---	1998	---	---
T25 (Ht2)	2007	1998	---	---
MON810 (Ir1)	2007	1998	2003	2012
BT11 (Ir1,Ht2)	2007	2001	2004	2012
NK603 (Ht1)	2008	2004	2011	---
GA21 (Ht1)	2008	2005	2011	---
TC1507 (Ir1,Ht2)	2008	2005	2011	2012
MIR162 (Ir1)	2009	2011	---	---
MIR604 (Ir2)	---	2012	---	---
MON89034 (Ir1)	2009	2010	---	----
MON88017 (Ir2,Ht1)	2010	2010	---	---
DP-098140-6 (Ht1, Ht4)	---	2011	---	---
MON810XNK603 (Ir1xHt1)	2009	2007	2011	---
BT11XGA21 (Ir1,Ht2xHt1)	2009	2009	2011	---
TC1507XNK603 (Ir1,Ht2xHt1)	2009	2008	2012	---
TC1507XMON810 (Ir1,Ht2xIr1)	2011	---	---	---
MON89034XNK603 (Ir1xHt1)	2010	2012	---	---
MON89034XMON88017 (Ir1xIr2,Ht1)	2011	2010	---	2012
BT11XMIR162XGA21 (Ir1,Ht2xIr1xHt1)	2010	2011	2012	---
MON89034XTC1507XNK603 (Ir1xIr1,Ht2xHt1)	2010	2012	2012	---
TC1507XMON810XNK603 (Ir1,Ht2xIr1xHt1)	2011	---	---	---
BT11XMIR162XMIR604XGA21 (Ir1,Ht2xIr1xIr2xHt1)	---	2012(1)	---	---
(1) Intermediate combinations are also approved.				
Ht = herbicide tolerance (1: glifosate; 2: glufosinate; 3: imidazolinones; 4: acetolactato sintasa) Ir = insect resistance (1: lepidopteran; 2: coleopteran)				
Source of data: National Biosafety Technical Commission (CTNBio), Brazil, http://www.ctnbio.gov.br/index.php/content/view/12482.html; National Advisory Commission on Agricultural Biotechnology (CONABIA), Argentina, http://64.76.123.202/site/agregado_de_valor/biotecnologia/55-OGM_COMERCIALES/index.php; National Biosafety Cabinet (GNBio), Uruguay http://www.mgap.gub.uy/portal/hgxpp001.aspx?7,1,144,O,S,0,MNU;E;121;6;MNU;,; Agricultural and Forestry National Biosafety Commission (CONBIO), Paraguay, http://www.mag.gov.py:1082/conbio/.				

Other Latin American countries have chosen not to produce GE crops. Peru adjusted its biosafety framework in 04/15/2011, when the Ministry of Agriculture established a new Decree, No. 003/2011 (Perú, 2011a). Soon after the promulgation of Decree No. 003/2011 agricultural associations requested that it be revoked. Law No. 29811 was then promulgated establishing a moratorium on GE crops (Perú, 2011b). Since November 2012 Peru has entered a moratorium for ten years prohibiting the use of GE organisms (Peru, 2012). However, GE organisms for research in contained trials for pharmaceutical and veterinary GE products and GE products imported for food and feed are excluded from the moratorium (Peru, 2012). Bolivia has approved herbicide tolerant soybean, but there is ambiguity between a political will to achieve food security by profiting from protecting biodiversity or by supporting production of transgenic crops (Personal Communication, Ing. Agr. Enzo Benech, Subsecretary of the Ministry of Agriculture, Uruguay 2012).

New genes and trends

Many of the new types of traits that are being developed arise from different kinds of genes than were used for the first wave of GE crops. The first wave of transgenic crops primary utilized genes whose protein product was directly responsible for the desired trait (e.g., Bt proteins confer insect resistance; herbicide resistance genes encode proteins that prevent binding of the herbicide or otherwise inactivate the herbicide) (Carpenter *et al*., 2002). These protein products are largely inert with respect to other cellular functions. Possible pleiotropic or epistatic effects are more likely to be due to a position effects rather than gene function.

In contrast, the function of many of the newer genes being tested is to initiate other changes within the cell. They may cause the cell to produce needed compounds to survive, grow and respond to the environment. Such genes may encode transcription factors that

regulate expression of other genes; they may code for signaling factors that initiate response to perceived changes in the cellular environment; or they may produce metabolic pathway enzymes that result in the production of new cellular compounds. Examples include transcription factor genes that have been used to modulate floral development or abiotic stress resistance; signaling or hormonal factor genes that have been used to initiate responses to invading pathogens or modify fruit ripening; and metabolic pathway genes that have been used to modify oil, carbohydrate, or amino acid composition (Figure 1.2).

As a result of their downstream actions, these types of genes would be expected to have broader effects on plant metabolism, physiology, and development than genes for which the protein itself is the final end product (Wolfenbarger and Grumet, 2002; Little *et al*., 2009; Chan *et al*. 2011; Grumet *et al*., 2011). Indeed, it is the ability of these kinds of genes to initiate a cascade of effects that makes them highly valuable for genetic engineering. A single gene can achieve what might otherwise take dozens of genes (Thomashow, 2001). At the same time, however, altered expression of a broad range of genes via introduction of regulatory, signaling or metabolic genes, also has the potential to modify non-target phenotypes within the plant through pleitrophic or epistatic interactions (Wolfenbarger and Grumet, 2002; Little *et al*., 2009). These changes could, in turn, influence fitness or the probability of gene flow of the introduced trait.

New traits and trends

The first generation traits, herbicide tolerance and insect resistance, were rapidly adopted and continue to be the traits with the most regulatory approvals and area planted (James 2011, 2012). More recently, stacked events with two or three traditional traits combined have been increasingly adopted, representing 26% of the global area of GE crops planted in 2012 (Table 1.2).

Table 1.2. Percentage of main traits out of the total GE crops planted in 2012.

Trait	Area planted (million ha)	Percentage of the global area with GE crops (160 million ha)
Herbicide tolerance	93.9	59
Stacked events	43.7	26
Insect resistance	23.9	15
Source: James, 2011, 2012.		

While these first generation GE traits dominate current commercial production, information from field trials can be used as an indicator of which new genes, traits and crops will likely reach the market in the future. Several factors will determine if a GE crop will reach production stage including the success of the trait in conferring the desired phenotype, concerns of consumer opposition, and numerous market issues regarding anticipated adoption and profitability of the product. However, as a rough estimation, field trial-data provide a "biotech observatory" that regulatory systems can use to monitor what may come in the near future.

An OECD (2009) report indicates that agronomic and product quality traits have received increasing attention in recent years, while trials for the first generation trait, herbicide tolerance, stayed constant and insect resistance trials declined. Stein and Rodriguez-Cerezo (2009) predict that insect resistance and herbicide tolerance will still be dominant traits by 2015 but that new agronomic and quality traits will also be available. Trials for product quality traits have remained stable over the past 15 years at about 13% (Table 1.3).

The agronomic traits category refers to plant growth, development and performance traits (growth rates, earliness by altered flowering, yield enhancement, fruit ripening) and environmental or abiotic stress tolerances (frost, cold, heat, drought, salinity) (Wolfenbarger and Grumet, 2002). Agronomic traits that are currently being incorporated include higher nitrogen use efficiency as well as drought and cold tolerance (OECD, 2009; Grumet *et al.*,

2011; James, 2011, 2012). In 2009 a new event, MON87460-4, conferring drought tolerance in corn, was submitted for review. The gene introduced, *cspB* from *Bacillus subtilis,* is an RNA chaperone that preserves RNA secondary structure during conditions of environmental stresses (Castiglioni *et al*., 2008). The resulting phenotype in corn is a reduction in yield loss when the GE plant is under water-limited conditions compared to the conventional crop (Monsanto, 2009). Drought tolerant sugarcane could also be released in Indonesia in a near future (James, 2012).

Table 1.3. Percentage of traits of the total field trials by year.

Trait/Year	**1997**	**2007**	**2011**
Herbicide Tolerance	30.0%	22.3%	19.2%
Insect Resistance	20.3%	19.7%	8.65%
Virus Resistance	13.6%	1.8%	1.33%
Agronomic Properties	7.13%	21.5%	32.0%
Product Quality	14.35%	13.9%	13.5%
Total number of field trials	1066	2018	2565
Source: Information Systems for Biotechnology, Virginia Tech http://www.isb.vt.edu/, 2012.			

More recently the event MON87712-4 incorporating the *BBX32* gene from *Arabidopsis thaliana* to confer enhanced yield in soybean was submitted for deregulation in 2011 (Monsanto, 2011). The BBX32 protein is a member of the B-box zinc finger family acting as a transcriptional accessory protein assisting the function of transcription factors through protein-protein interactions (Monsanto, 2011). The BBX32 protein represses plant responses to the transition from light to dark causing a change in the diurnal metabolism during the reproductive phase of the soybean plant. These changes result in an extended period of photosynthetic activity leading to an increased availability of assimilates and higher yield (Monsanto, 2011). If this event is deregulated would be the first trait for yield improvement *per se*, approved for commercialization. Increased yield to date has been achieved indirectly from events such as pest resistance and herbicide tolerance through reduced crop losses.

Quality traits refer to industrial processing traits and consumer-oriented traits. Industrial processing traits include improved oils and fatty acid composition, modified carbohydrates (sugar and starches), proteins and amino acid content and characteristics for biofuel production. Consumer appeal traits include nutritional quality (vitamins A, E, protein), reduction in targeted components (nicotine, caffeine, allergens), medical products (oral vaccines, antibodies and pharmaceuticals) and altered storage and appearance as well as improvement for animal feed (Wolfenbarger and Grumet, 2002). Some quality categories could overlap, for example "oil and fatty acids" could include changes in fatty acids for industrial processing, or in the "animal feed" category could be changes in proteins or carbohydrates (OECD, 2009). Quality traits that are in the pipe line for commercial use according to James (2011), include β-carotene in rice (golden rice), omega-3 in soybean, high lysine maize and phytase in maize.

From a regulatory standpoint, it is also important to mention the tendency of stacked events to replace the single event crops. Stacking refers to combination of multiple events in one plant by conventional breeding of the original single events. For example, 4-way stacked maize, which combines eight genes (SmartStax) for above and below grown insect pest control plus herbicide tolerance for weed control is currently on the market.

New crops and trends

The majority of GE crops that reach the commercial phase are still primarily varieties of soybean (47% of global biotech area), corn (32%), cotton (15%) and canola (5%) (James, 2011). There are a few examples of horticultural GE crops among the first generation of transgenic crops which include tomato (FlavrSavr) with delayed ripening (which was the first approved commercial release transgenic crop in the US in 1994 but it is no longer in the market); papaya and squash with virus resistance; and carnation in Australia, Japan and the

Netherland (Brookes and Barfoot, 2006; ISAAA website, 2013). However, as of 2012, GE varieties of 25 crops reached commercial regulatory approval (James, 2011, ISAAA website, 2013). These include additional large hectarage crops, like rice and wheat, as well as small and medium hectare high value-added crops such as sugar beet, melon, plum, tomato, beans (Table 1 in Appendix).

The number of engineered crops species that have been registered for field trials is considerably larger than those that have been commercialized. These include safflower, barley, sugarcane, sunflower, Kentucky bluegrass, lettuce, spinach, cassava, onion, sweetpotato, pumpkin, eggplant, peas, grapes, banana, apples, olives, nuts, peanuts, eucalyptus and pine (OECD, 2009; ISB Virginia Tec website, 2012; BCH website, 2013). Many are important crops for nutrition and income in developing countries, or are local or regional species outside the international market dynamic and for which there is less attention from international or regional crop research organizations. However crops such as cassava, sweetpotato and eggplant are valued culturally, adapted to harsh environments, nutritious and diverse in terms of genetic background, agroclimatic adaptation and economic niches for what the potential to extend applications of transgenic technology for these crops improvement may be significant (Naylor *et. al*., 2004).

Small-acreages horticultural crops including fruits and vegetables, together with tree nuts and nursery crops are grouped in what is called specialty crops. Specialty crops have become increasingly important in the U.S. agricultural economy, exceeding the combined value of the five major program crops ($49 and $45.8 billion in sales respectively) (USDA 'Specialty Crop Research Initiative', Farm Bill 2007). However, from a biotechnology point of view, these crops face unique challenges. Small-acreages specialty crops do not have the financial support to face the costs of generating new GE cultivars and the associated regulatory system costs (Kalaitzandonakes *et al*., 2007; Moose and Mumm, 2008). There are

cases in which engineered crops from this category have already been generated or other crops may have potential for biotech crop development, but the regulatory system is a clear limiting factor to reach the commercial phase (US Pew Initiative on Food and Biotechnology, 2007).

New environments and trends

The broader range of traits and crop possibilities brings additional countries into the biotechnology era increasing the interest across the developing world (Nap *et al*., 2003; Grumet *et al*., 2011; James, 2012). Looking globally the countries as "new environments", 28 countries were growing GE crops in 2012 (James, 2012). Since 2005 10 new countries have adopted the technology (Pakistan, Bolivia, Burkina Faso, Myanmar, Chile, Sudan, Cuba, Egypt, Costa Rica and Slovakia).

Each new country that achieves regulatory approval for GE crops implies a specific environment to consider in risk assessment. Factors to be taken into account include the nature of the ecosystem of the receiving environment, whether it is an introduction of a new cultivar of a currently grown crop or if it is a new crop with a new cropping system, and the presence of compatible relatives that may allow for gene exchange (Grumet *et al*., 2011).

ENVIRONMENTAL RISKS FOR CONSIDERATION WHEN ANALYZING GENETICALLY ENGINEERED CROPS

The potential environmental impacts that have been raised for consideration when releasing transgenic crops include both ecological and agronomic concerns (Snow and Palma, 1997; Grumet and Gifford, 1998; Barton and Dracup, 2000; Conner *et al*., 2003). Even though the environmental concerns that are discussed in this thesis are also applicable to crops developed by traditional plant breeding, they had not received much attention until the development of genetically engineered crops (Sanvido *et al*., 2006).

The agronomic concerns relate to increased weediness (invasiveness) either by the GE plant itself or weedy relatives; possible negative effects on non-target organisms; and negative effects on soil functions due to change in agricultural practices. The ecological concerns relate to possible negative effects on natural resources resulting from gene flow to wild relatives influencing invasiveness or biodiversity, or destruction of refuge areas due to agricultural expansion. There are other impacts that can be grouped as "commercial" which include impacts related to gene flow from pollen or due to seed mixing with conventional or organic crop causing coexistence conflicts. These concerns are discussed in chapter 3 with special emphasis on GE plants with genes for dehydration stress tolerance. Each type of these harms has been extensively described in numerous papers and reviews (Keeler, 1989; Snow and Palma, 1997; Grumet and Gifford, 1998; Barton and Dracup, 2000; Wolfenbarger and Phifer, 2000; Ellstrand, 2001, 2003; Dale *et al*., 2002; Conner *et al*., 2003; Hancock, 2003; Jenczewski *et al*., 2003; Pilson and Prendeville, 2004; Hails and Morley, 2005; Snow *et al*., 2005; Nickson, 2008). A brief description of these potential environmental harms and questions asked in risk assessment are included below.

Potential ultimate harms

Weediness and invasiveness

Weediness and invasiveness are the ultimate concerns of transgenic crops which can arise as a result of the direct effect of the transgene, as a result of gene flow, or due to secondary unexpected effects of the transgene. The harms feared are that the crop itself and/or compatible wild relatives through gene flow, can become more persistent in agricultural environments (a weed), or can become more invasive in natural habitats (invasiveness). Gene flow from pollen to wild relatives could also cause decrease abundance leading to extinction of rare wild relatives. Weediness could result if the GE crop becomes more aggressive and harder to eliminate (Ellstrand and Hoffman, 1990; Dale, 1992; Hancock *et al*., 1996; Barton and Dracup, 2000; Wolfenbarger and Phifer, 2000; Stewart *et al*., 2003).

The transgene would pose a hazard if it has the capacity to enhance, directly or indirectly, due to unexpected secondary effects, the ability of the crop or wild relative to become weedy/invasive. From a regulatory point of view, baseline information about the biology of the crop, whether compatible wild relatives are present in the receiving environment, and the phenotypic effect of the transgene on the general plant fitness, are analyzed in order to determine whether the GE crop and/or wild relative has the potential to become weedy/invasive (Hancock, 2003).

Hancock (2003) classifies species and transgenes into different categories according to their invasive potential (species categories) and the fitness impact (transgenes categories). The environmental risk is the result of the combination of these categories. For example, a transgene that confers herbicide tolerance will represent a concern for agriculture if it is engineered in a crop that itself has weedy characteristics or if there is present in that area a compatible wild relative that already has weedy characteristics. In crops that already have weedy characteristics, volunteer plants could become weeds in subsequent crops. In the case

of crops that have compatible weedy relatives, such as sorghum, canola, wheat, sunflower or rice (Hancock, 2003; Snow *et al*., 2003; Stewart *et al*., 2003), a key question is whether the transgene persists in the environment after being introgressed into the wild relative genome (see following section about gene flow). In this example, the transgene for herbicide tolerance will have a high selective pressure in an environment where that herbicide is applied.

In the case of invasiveness, the net effect of the trangene on reproductive fitness can be neutral, detrimental, advantageous or variable depending on the environment and stress encountered (Ellstrand *et al*., 1999; Hancock, 2003). Traits that may confer fitness and competitive advantages to wild relatives include transgenes for biotic or abiotic stressesif those factors represent a limitation in the wild habitat. The selective pressure for insect and disease resistance transgenes will depend on the specific levels of natural control in the wild habitat (Hancock, 2003; Stewart *et al*., 2003).

Characteristics that have been associated with ability of weeds to spread and persist outside their natural geographic range include: broad germination requirements, discontinuous germination (internally controlled) with high seed longevity, a rapid growth through vegetative phase to reproductive phase, continuous seed production, self-pollination or cross-pollinated with unspecialized pollinators or wind pollination, very high seed output under favorable environmental conditions, plasticity in seed production under a wide range of environmental circumstances, short- and long-distance seed dispersal, vigorous vegetative reproduction, propagules with brittleness, vigorous competitors, polyploidy (Baker, 1974). While these characteristics cannot be used to predict weediness (Conner *et al*., 2003), several are evaluated at field experiments when comparing the transgenic plant with the non-transgenic plant counterpart. The characteristics generally included are: seed dormancy, germination, vegetative growth rate, period of phenological phases to determine life cycle, time to flower, period of

seed production and amount of seed produced; persistence of seeds in the soil and presence of volunteer plants in following season (URUGUAY, GNBio website, 2013). Volunteer plants could be a way for the transgene to persist in the agroecosystem and eventually allow hybridization and introgression into wild relatives if they are present in the vicinity and are sexually compatible.

Negative effect on non-target organisms

Non-target organisms include all other organisms the GE crop may impact, with the exception of those the crop was engineered to control, if it is intended to confer pest resistance. The concerns of a negative impact on non-target organisms regarding potential ecological consequences include a change in food web relationships or a change in ecological processes that occur in the soil. The impact on non-target organisms can be studied following a step-wise (tier) approach in which early tier tests are performed in laboratory (García-Alonso *et al.,* 2006; Romeis *et al*., 2006, 2008).

Non-target organisms are fed with a dose in high excess (e.g., 10-fold) of the level normally ingested when eating prey that fed on the GE plant or when directly consuming parts of the GE plant. This approach uses the criterion that if no harm is observed when ingested at high excess, the risk of a negative impact on non-target organisms at levels normally encountered in the field would be low. If a risk is identified during the laboratory tests, the assessment increases in complexity and realism reaching semi-field and field studies in order to determine its ecological relevance. Risk assessment based only on laboratory studies has been considered as being ecologically unrealistic and not able to predict large-scale and long-term effects (Andow and Hilbeck, 2004; Lovei and Arpaia, 2005). It is possible that there could be more subtle potential direct and indirect effects of the insecticidal protein in the plant affecting the non-target organisms, when they interact in the environment

(Romeis *et al*., 2006). Thus, a regulatory authority may require additional field studies including post release monitoring studies.

Processes that may result in environmental harms

Gene flow

The environmental concern defined as "gene flow" refers to transfer of the transgene from the GE crop to a wild relative. The dispersal of the transgene itself is not the ultimate harm to be evaluated. The concern is the phenotypic effect of the transgene if it persists in the environment (agroecosystem or ecosystem). Possible phenotypic effects of the transgene that would be of concern for the environment are the ones already discussed: weediness, invasiveness/ extinction and/or negative impact on non-target organisms, whichever is applicable (Ellstrand *et al*., 1999; Conner *et al*., 2003; Hancock, 2003; Wolfenbarger, 2003; Weebadde and Maredia, 2011).

Gene flow from pollen happens through the normal sexual reproductive process which is referred as vertical gene transfer. For gene flow to have an environmental impact, the transgene must become fixed in the wild relative genome. This requires several steps that may take years or a few generations. The steps leading to transgene fixation include the presence of a compatible wild relative in the receiving environment, a crop biology with the ability to form viable hybrids by cross-pollination, and the ability of the transgene to stably persist in populations of the wild relative (process of introgression) which will be influenced by the selective pressure that determines the phenotypic effect of the transgene. If these steps are met, the critical issue will be to determine whether the introgressed transgene causes ecological consequences specific to the GE trait.

For hybridization to occur, the primary question is whether there are sexually compatible relatives in the vicinity of the GE crop. If relatives are present, additional factors

such as flowering times, rate of out-crossing and self-pollination, rate of pollen dispersal, pollen viability and longevity, wind speed and direction, pollinating agents and their characteristics, distance between GE crop and receiving species, plot size and wild population density, frequency and distribution, will influence the rate of occurrence of hybridization. Subsequent to cross pollination, the resultant hybrid seeds must be viable and able to germinate in the recipient environment. The F1 progeny must, in turn, produce flowers and further hybridize with the wild relative to form backcross hybrids, allowing for introgression of the transgene (Ellstrand *et al*., 1999; Stewart *et al*., 2003). Finally, the backcross hybrids must survive and reproduce through several generations until the transgene persists in populations of the wild relative (Conner *et al*., 2003; Stewart *et al*., 2003).

According to the factors that influence hybridization and introgression, from a regulatory point of view, analysis includes baseline information about proximity to compatible wild relatives, the biology of the crop, and their invasive characteristics, and the phenotypic effect of the transgene on their fitness (Cook, 1999; Hancock, 2003). From the breeding literature we can get information such as which wild species are compatible with specific crops as well as the center of origin and geographical distribution of wild species (Hancock, 2003). Crop biology considerations include: reproductive barriers (breeding system: inbreeding or outcrossing crop), modes of pollination, methods of seed dispersal (natural and through agricultural activity), coincidence in flowering times, and hybrid viability (fertility of hybrids) (Ellstrand *et al*., 1999; Raybould and Gray, 1993; Hancock *et al*., 1996). If hybridization is confirmed, information regarding proximity (frequency and distribution) to wild relatives and size of populations within the area where the GE crop will be cultivated is used to determine appropriate isolation distances in order to manage gene escape to minimize hybrid frequency.

In most cases, if the gene does not confer a selective advantage, the hybrids usually survive for only one season. Presence of the transgene in wild populations would result from a continuing flux of new volunteers but not due to an introgression event (Conner *et al*., 2003).

Gene flow from pollen could be also a concern regarding the presence of non-GE conventional crops in the receiving environment when coexistence regulation exists. In this case, the dispersal of the transgene in itself is the ultimate harm to be evaluated although the phenotypic effect of the transgene does not cause harm to the environment. In this regard, gene flow can also happen through seed mixing referred as adventitious presence (AP) or low level presence (LLP) (Codex Alimentarius Commission, 2003; Demeke *et al*., 2006; Brookes, 2008; McCammon, 2010). The former refers to the case of non-transgenic seed mixed with transgenic seed of an event with regulatory approval. The later refer to the case of imported transgenic or non-transgenic seed mixed with transgenic seed of an event that has not received regulatory approval in the importing country.

Finally, gene flow could also result from horizontal gene transfer between unrelated organisms. Although this manner of gene flow has been raised as a concern (Snow *et al*., 2005), it will not be discussed in this thesis due to its extremely low probability of occurrence (Nielsen *et al*., 1998; Syvanen, 1999, 2012; Kees, 2008).

Secondary unexpected effects

Intended effects are those expected to occur according to the gene introduced, for example insect or virus resistance, herbicide tolerance or intended alterations in the grain composition. Intended effects are quantified in efficacy tests to verify that the introduced gene is expressing the desired phenotype which gives an advantage compared with the conventional counterpart (Raybould *et al*., 2010). Intended effects are also measured by

comparative analysis of phenotypic parameters and plant organ composition with the conventional counterpart (EFSA, 2006, 2011). Phenotypic measures include agronomic performance parameters, yield, disease resistance, or others defined according to the trait introduced and mechanism of gene-action. In the case of alteration in composition, target compounds such as newly expressed proteins, macro- and/or micro-nutrients are quantified (EFSA, 2006).

Unintended effects refer to consistent differences in phenotype and/or plant composition between the GE plant and the corresponding control plants other than the intended effects of the introduced trait (EFSA, 2006). Possible sources of unintended or unanticipated effects include:

a) An effect of the insertion site of the transformation event.

b) An indirect effect of the introduced gene or its protein.

The first case includes transformation-induced-mutations that could occur due to the transgene insertion into the plant genome interrupting other gene expression. According to Wilson *et al* (2006) transformation-induced-mutations can be classified as: changes associated with disruption of the plant genomic DNA caused by the site of insertion; the presence of non-genomic DNA such as DNA sequences from the vector used; and any alteration to the plant genomic DNA, including: base pair changes, duplications, deletions or rearrangements as well as somaclonal variation. These alterations can be caused by the mechanism used to insert the transgene into the plant (mainly *Agrobacterium*-mediated transformation or particle bombardment), or during the plant regeneration process that includes tissue culture.

The other source of unintended effects refers to the unintended phenotypes that the trait and gene itself potentially could cause (Wilson *et al*., 2006). This source of unintended effects is linked to perturbations due to interactions within plant metabolic, signalling or

regulatory pathways and may be more likely with some of the new genes and traits under development (Wolfenbarger and Grumet, 2002; Little *et al*., 2009).

The unexpected effects are not harm 'per se'. Rather, specific harms, as discussed in previous sections, are considered regardless of whether they occur due to a direct effect of the gene product or to unexpected secondary effects (Personal Communication, Sally L. McCammon, Ph.D., Science Advisor, Office of Science Biotechnology Regulatory Services, Animal and Plant Health Inspection Service APHIS-USDA, 2010).

The detection of unexpected effects begins at early stages of the GE crop development during the regular evaluation of risks. The criterion used is the concept that traditionally cultivated crops have a history of safe use regarding food/feed safety and environmental security. This is the criteria of *'familiarity'* generally used in biosafety frameworks. The evaluation of risks is based on a comparative assessment in which the GE plant is compared with its non-GE conventional counterpart. If only intended differences are identified, it is considered that the GE plant is *substantially equivalent* to its counterpart and no further data in necessary. If unintended differences are identified, it is determined whether the difference is biologically relevant and the probability that this difference will determine an environmentally harmful effect (Garcia-Alonso, 2010). If the difference has biological relevance, further analyses are performed focusing on that factor. The analyses performed are the same as if intended or unintended effects were causing the difference. If pre-commercial release field trials show that the transgenic line being tested exhibits unexpected negative agronomic or potential environmental negative effects, that construct, or that event, if it is an insertion effect, would be dropped from further development.

On the other hand, there may be cases where unintended effects are not identified during the comparative assessment stage. It is of concern that such changes may be manifest when the crop is commercially released in large areas. Thus, regulatory systems may request

the applicant to assess the probability of occurrence of unintended effects. This is a challenge for the regulatory authority, since it is unclear what would be the appropriate requirement to the applicant in order to assess the probability of unintended, unidentified effects. The Brazilian and European Union's biosafety frameworks include the consideration of unexpected, unidentified effects by requesting a monitoring plan after the GE crop is authorized for commercial release (BRAZIL, 2011; EFSA, 2006).

Changes in agricultural practices

The gene introduced may confer a trait that determines changes in agricultural practices, usually referred as "associated technology". This is the case for traits such as herbicide tolerance. A commercial release of an herbicide tolerant crop may determine the increase usage of the herbicide for which the trait gives tolerance. An increase in the usage of a particular herbicide may require an adjustment in the analysis of the negative impact on non-target organisms and may require measures to prevent the generation of herbicide resistance because of its increased use (Ríos *et al*., 2005; Cerdeira and Duke, 2006; Young, 2006; Cerdeira *et al*., 2011).

The use of herbicide tolerance also has influenced tillage practices by allowing reduced or no tillage production. It is also possible that the new crop/trait could favor agricultural expansion into natural environments not currently under cultivation, such as areas currently limited by drought or salinity. The implications of the changes in agricultural practices can be considered in the context of the risk assessment process done by the biosafety framework or can be considered by other environmental agencies.

GENETICALLY ENGINEERED CROPS AND A BIOSAFETY FRAMEWORK

Why regulate genetically engineered crops?

Biosafety in its broad sense refers to *"a) the goal of ensuring that the development and use of genetically engineered organisms and products made from them do not negatively affect plant, animal, or human health; genetic resources; or the environment; b) policies and procedures adopted to avoid risk to human health and safety, and to the conservation of the environment, as a result of the use of genetically engineered organisms for research and commerce"*(Traynor *et al*., 2002). *"Biosafety"* is a global concept advisable for a sustainable agricultural development due to its integrated approach. It encompasses not only the introduction and release into the environment of genetically engineered organisms and their products, but also food security, zoonosis, introduction of pests and diseases of animals and plants, and introduction and management of invasive alien species (FAO, 2007). Thus, GE organisms are *one* aspect of the concept of biosafety. This section describes the regulation of the release of GE organisms.

Specifically regarding GE organisms, the Convention on Biological Diversity defines biosafety as the "*means to regulate, manage or control the risks associated with the use and release of living modified organisms (LMOs) resulting from biotechnology which are likely to have adverse environmental impacts that could affect the conservation and sustainable use of biological diversity, taking also into account the risks to human health"* (UNEP/CBD 1992, Article 8(g)). As part of the CBD, it is The Cartagena Protocol (CP) on Biosafety (2000) that is an international treaty that regulates *living modified organisms* (LMOs) resulting from *"modern biotechnology".* The CP refers to LMOs as *"any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology"* and defines *"modern biotechnology" as the application of:*

a. *In vitro nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or*
b. *Fusion of cells beyond the taxonomic family that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection"*

For the Cartagena Protocol the concept of biosafety refers to "*the need to protect human health and the environment from the possible adverse effects of the products of modern biotechnology*" (Secretariat of the Convention on Biological Diversity, 2000).

Genetic engineering technology, also called recombinant DNA technology, has the potential to generate a broad range of new products, including organisms with significantly altered morphology and physiology. Both the process of genetic engineering and the potential impact of the resultant products have led to a cautious attitude. As a result, unlike conventionally bred cultivars, products obtained through the process of genetically engineered are subject to regulation.

The concern regarding GE organisms rose at the beginning of their development from U.S. scientists who were working with recombinant DNA. The Asilomar Conference in February 1975 reviewed the progress in this technique and discussed potential biohazards of working with this methodology (Berg *et al*., 1975). Scientists recognized the potential of this technology for molecular biology and for future (at that time) useful practical applications. The organizing Committee tried to reach a consensus in the appropriate way to deal with the potential risks in laboratory work with recombinant DNA. Recommendations arose with different levels of containment according to the type of experiment (Berg *et al*., 1975).

The following year the US National Institute of Health (NIH) published the guidelines for research that uses recombinant DNA (NIH, 1976, 1978). Since then until 1984, the NIH Recombinant DNA Advisory Committee was the federal entity charged with reviewing and

monitoring DNA research in the US. Progress of this technology in parallel with growth of biotechnology companies and legal issues associated with intellectual property rights led the US government in 1984 to consider and establish policies to guide federal agencies in the regulation of biotechnology research and its products. In 1984 the White House Office of Science and Technology Policy (OSTP) published the "Coordinated Framework for Regulation of Biotechnology" which was finalized in 1986.

How to regulate genetically engineered crops?

Various challenges are presented when implementing a regulatory system for genetically engineered organisms. Human, financial and infrastructure resources are required for an operational biosafety framework. The objective is to develop a regulatory system that is: responsible, appropriate, clear, transparent, consistent, predictable and cost/time-effective (UNEP, 2005). In order to obtain confidence and credibility in the regulatory system it is necessary to have a clear state policy supporting the technology while recognizing the need for regulation. The final regulatory system will depend on each country's own legal system, form of government, obligations, objectives and priorities (UNEP, 2005). Several papers have described and compared regulatory systems of different countries (Flint *et al*., 2000; MacKenzie, 2000; Solleiro and Galvez, 2002; McLean *et al*., 2003; Nap *et al*., 2003; Jaffe, 2004; McHughen and Smyth, 2008; Ramessar *et al*., 2009; Hokanson and Ferenczi, 2011).

The necessary components of a national biosafety framework include: 1) a national biosafety policy; 2) a regulatory regime comprised of laws, acts, decrees and other legal instruments; 3) an administrative system to deal with applications in which the competent authority/ies will carry forward the decision-making procedure (risk analysis); 4) follow-up actions (inspection, enforcement, emergency provisions, etc); and 5) public awareness and participation (UNEP, 2005; García-Huidobro, 2011). These components, and decisions and

considerations required in designing them, have been extensively described in numerous reviews (Traynor *et al*., 2002; UNEP, 2005; FAO, 2007). Some of the key decisions and considerations are discussed below. Table 1.4 summarizes the elements necessary for the implementation of the decision-making procedure.

Table 1.4. Elements necessary for the implementation of the decision-making procedure (modified from UNEP, 2005).

Element	Notes
Objective	To identify what is to be protected and the level of protection to be sought. This refers to the protection of the health and environment being possible to include other objectives such as socioeconomic considerations, coexistence, etc.
Scope	To define the products/organisms and activities to be regulated.
Principles	To define basic principles that will underlie the decision-making procedure.
Institutions	To define responsible institutions for the implementation of the regulatory system.
Administrative system	To define an administrative system that will deal with applications.
Decision-making procedure	To define the methodology for the decision-making procedure.
Monitoring	To define if the framework would provide for monitoring of the effects of an authorized GE crop/product post-releases.
Control	To define what inspections and enforcement actions would be required and what institution would undertake them.
Labeling	To define if there would be specific label requirements.
Liability and redress	To define whether the framework will have a specific provisions on liability and redress
Source: Modified from UNEP, 2005.	

When defining the regulatory regime (laws, decrees, etc), the biosafety framework can be developed using existing laws as it is the case for the US and Canadian systems, where already existing laws were considered sufficient to regulate GE organisms. The decision to regulate under existing laws also implies that the regulatory needs for biotechnology are not different from other types of regulated articles (Hokanson and Ferenczi, 2011). On the other hand, specific regulation for GE organisms can be written, as it is the case in the European

Union (EU), or a mix of already existing laws and decrees may be combined with new regulation written specifically for GE organisms as has been done in several countries from South America and Africa. Each approach has its advantages and disadvantages.

If using existing laws, agencies involved and general configuration of the system are already established. However the specific commitments of an institution must also be linked with the regulation of GE organisms. In the US for example the USDA/APHIS regulates insect and herbicide tolerant GE crops as 'plant pests' because that is within its purview. The US EPA regulates only 'plant incorporated protectants', such as gene products of Bt crop plants, because it regulates traditional insecticides in agriculture (Hokanson and Ferenczi, 2011).

Another approach is the one taken by New Zealand that includes the regulation of GE organisms under the wide concept of *biosafety* (FAO, 2007). New Zealand includes GE organisms as *"new organisms"* under the Hazardous Substances and New Organisms Act (HSNO Act) (NEW ZELAND, 1996) in coordination with their Biosecurity Act (NEW ZELAND, 1993). Thus, New Zealand does not have a specific Act or Law for GE organisms.

Regarding the decision-making procedure, the criteria that will trigger the regulatory process could be *process-based* or *product-based*. The majority of countries that have in place a biosafety framework use the *process-based* criteria. Any organism derived from genetic engineering is considered a regulated product and needs to go through the regulatory process. This is the criterion established by the Cartagena Protocol. Any living organism generated through the process of genetic engineering needs to comply with the regulatory requirements even if the same product, for example a drought tolerant corn variety, obtained by conventional breeding techniques, already has been released into the environment. Canada on the other hand has a clear *product-based* regulatory system in which it is not the process that triggers the regulatory system, but the final product, regardless of the process to obtain it.

In Canada release into the environment of all new genetic combinations that determine a novel trait are regulated. Novel traits in Canada are defined as a trait *which is both new to the Canadian environment and has the potential to affect the specific use and safety of the plant with respect to the environment and human health* (Canada - CFIA, 2013). Thus, not only new products derived from genetic engineering but also through mutagenesis or other *"conventional"* methods are regulated (Nap *et al*., 2003; CERA website, 2013). This approach considers that there are methodologies in conventional breeding with the same potential risks as those posed by recombinant DNA (NAS, 2002).

Specialty crops regulatory challenges

Incorporating biotechnology and molecular plant breeding into national plans requires intellectual and infrastructure capacity, as well as financial resources for the regulatory system. The limited budget and low institutional support for the specialty crops sector has limited ability of this sector to proceed through the expensive regulatory system needed to reach the commercial phase of developed biotech crops (US Pew Initiative on Food and Biotechnology, 2007). Even though these crops may be attractive for commercial companies, depending on the country's market, the cost of discovery, development and authorization for commercial release of a transgenic event is estimated in US$ 135 million (James, 2012), of which between US$ 7 and US$ 10 million correspond to the regulatory approval process (Kalaitzandonakes *et al*., 2007). Regulatory costs vary by country, crop and trait and depend whether the GE crop is developed by a public institution, such is the case of China, or private company such as in US and India (Bayer *et al*., 2010). Bayer *et al.*, (2010) found the cost of regulatory compliance to be between US$ 3 and 12,5 million in the US, US$ 195,000 in India and between US$ 53,000 and 90,000 in China.

The same regulatory system is applied for all situations despite the great diversity among specialty crops and the variety of target traits. The fact that the regulation is required event-by-event may not be as much of a limitation for commodity crops such as corn in which after an event has been approved, other varieties of the same crop with the same event can be generated by cross-breeding and these new events do not need to go through the regulatory system. However, in the case of some specialty crops, such as those that are clonally reproduced, each variety needs to be genetic engineered for the trait to be incorporated and then each event needs to go through the regulatory system. In addition, new varieties of specialty crops are generally in the market for a short time before being replaced (Fernandez and McCammon, 2007), further necessitating an expedited system of regulation.

This situation has inspired the Pew Initiative on Food and Biotechnology and the US Department of Agriculture Animal and Plant Health Inspection Service (USDA-APHIS) who co-sponsored a workshop entitled "Emerging Challenges for Biotech Specialty Crops" (US Pew Initiative on Food and Biotechnology, 2007) to examine the problems that engineered specialty crops encounter with the regulatory system). This workshop was the last of several workshops organized by the Pew Initiative and sponsored by different US governmental agencies to understand particular issues of specialty crops regulation (Pew Initiative on Food and Biotechnology, 2007; Personal Communication Sally L. McCammon, Ph.D., Science Advisor, Office of Science Biotechnology Regulatory Services, Animal and Plant Health Inspection Service APHIS-USDA, 2010, 2013). Government regulators, scientific experts, industry representatives and policy makers from the biotech specialty crops sector had roundtable discussions to identify the challenges and to find possible solutions to improve the regulatory system to making it better adapted to the biotech specialty crops sector.

Table 1.5 lists six proposals reached at a workshop held in 2007 (Fernandez and McCammon, 2007). One suggestion to improve the regulatory system for specialty crops was

development of a tiered risk-based regulatory system (proposal 1 in Table 1.5). Three critical pieces of information were suggested as the basis for regulatory decisions: the geographical range of compatible relatives; the invasiveness/weediness of the crop and its relatives; and the phenotype of the transgene. It was stressed that the critical parameter should be the plant's new phenotype conferred by the transgene. In this way, the lower-tier biotech crops (i.e., no compatible relatives, non-weedy or invasive, new phenotype not likely to cause environmental consequences) would require less data and information while the higher-tier products would require more information to collect.

Another suggestion to adjust the regulatory system for these crops is to switch from the case-by case approach to consider categories of crop-trait-environment (proposal 2 in Table 1.5). The effect of a particular trait on a set of orphan crops with common genetic structures, at a similar limited production environment can be analyzed together.

The necessity to develop information modules (and white papers) that can be used in the application process to accelerate the writing and evaluation of new submissions was also discussed (proposal 3 in Table 1.5). The idea was to generate a database publicly available with the existing scientific data relevant for risk assessment. Different modules can be created focusing on different areas of biotechnology. One module will cover all the relevant information for environmental risk assessment (e.g. wild relatives and geographic distribution, invasive/weeds characteristics) for each of the specialty crops. Another module will focus on the basic tools and methods used in genetic engineering (e.g. promoters, terminators, marker genes, transformation technologies). The information of these modules will help to guide the tiered risk-based suggested regulatory system.

Table 1.5. Proposals of the Pew Initiative on Food and Biotechnology Workshop, 2007. Updated by personal communication with Sally L. McCammon, Ph.D., Science Advisor, Office of Science Biotechnology Regulatory Services, Animal and Plant Health Inspection Service APHIS-USDA, 2010, 2013.

Workshop's proposal		**Update of activities**
1	Develop a tiered, risk-based regulatory system	*APHIS developed one such system in proposed revisions of its biotechnology regulations, published in 2008 (http://www.aphis.usda.gov/brs/fedregister/BRS_20081009.pdf). The proposed revisions are still under internal discussion after receiving public comment on the proposed regulatory changes.*
2	Move away from event-by-event regulation	*APHIS is currently participating in the development of a project with Canada and Mexico to look at the topics related to retransformation as part of evaluating recent scientific advancements and analysis for their implications in regulatory assessment.*
3	Develop information modules and white papers for use in applications for regulatory clearance.	*APHIS continues to participate in the development of Organization for Economic Cooperation and Development (OECD) plant biology documents and other OECD biotechnology documents (http://www.oecd.org/department/0,3355,en_2649_34385_1_1_1_1_1,00.html). In addition, APHIS works with the scientific community to address regulatory questions through such avenues as the Biotechnology Risk Assessment Grants (BRARG) program and the Association of Official Seed Certifying Agencies (AOSCA).*
4	Increase transparency and condense the timeline	*Increase transparency and condense the timeline. APHIS is actively working to increase transparency and to streamline its review processes. In August, 2010 APHIS published updated guidance on notification process for field testing (http://www.aphis.usda.gov/biotechnology/notifications.shtml). In November, 2011 APHIS announced petition process improvements. After receiving comment, the plan for implementation was published in March, 2012. In May, 2012, APHIS provided and updated permit User's Guide (http://www.aphis.usda.gov/biotechnology/brs_main.shtml, USDA,APHIS-BRS 2012).*
5	Prepare for new types of products and technologies	*APHIS continues to actively assess technological developments in biotechnology for implications to its regulatory system.*
6	Legal concerns	*APHIS attempted to address some legal concerns with its proposed revision of the biotechnology regulations published in 2008 (link in item 1).*

One product of the Pew Initiative was the formation of the Specialty Crops Regulatory Assistance (SCRA) initiative (SCRA website, 2013). It is a public-private effort to assist developers from the public and private sector that work with specialty crops to complete the US regulatory process to achieve commercialization. SCRA was inspired by other public-private partnerships such as the IR4 pesticide management project to facilitate the registration with regulators of pesticides for minor uses (IR4 website, 2013) and the Food and Drug Administration (FDA) orphan drug approach (Office of Orphan Product Development OOPD-FDA website, 2013) (Personal Communication, Sally L. McCammon, 2010). The last workshop was in December 2011 entitled 'Nuts and Bolts of US Regulatory Dossiers for Genetically engineered Crops' (SCRA, 2013). In this workshop different case studies were presented as examples of specialty crops already developed, such as plum with virus resistance to plum pox, or in the process of commercial approval, such as peanut resistant to Sclerotinia Blight and Bt Potato resistant to potato tuber moth.

RISK ANALYSIS METHODOLOGY

Risk analysis is defined as a process for controlling situations where populations or ecological systems could be exposed to a hazard (EC, 2000). Risk analysis arises as a methodology to characterize, manage and communicate the risk by public policy decision-makers. The application of a formal risk analysis methodology has become routine in many activities including the use of chemical, biological and physical agents, industrial and general processes. Existing International Standard Guidelines refer to biological and environmental risk analysis (EC, 2002; International Organization for Standardization, 2005), as well as in other disciplines such as business, management and insurance (Hill and Sendashonga, 2003; International Organization for Standardization, 2009).

A measure against possible or perceived threats is something intuitive in routinely human activities considering mainly past experience, technical know-how and qualitative estimates of risk (EC, 2000). However, nowadays decisions of modern society increasingly require guarantees regarding food safety and environmental sustainability (Power and McCarty, 1998). Risk Analysis is a useful methodology to approach the scientific assessment. Additional factors such as societal, economic, ethic and political values may also be included in the decision-making process (EC, 2000). Governments have relied on Risk Analysis as a guideline for a decision making to be *as objective as possible* with regard to new technologies and processes including GE crops (US NRC, 1983, 1996; EC, 2002). General frameworks proposed for risk analysis and its components of other activities have been adjusted to agriculture biotechnology (Wolt and Peterson, 2000). Risk Analysis is the internationally accepted methodology that countries apply for the decision making process to determine whether or not to accept the introduction and use of GE organisms (OECD, 1993, 1995; US NRC, 1996; Secretariat of the Convention on Biological Diversity, 2000; Codex Alimentarius Commission, 2003; EC, 2002a,b).

Risk is the possibility of an unwanted harm to occur. Risk is defined in terms of likelihood of occurrence of the undesired harm as a measurable probability (Wolt and Peterson, 2000). Assessment of risk is a science-driven process in which probability of risk is quantitatively determined. However the product of the Risk Analysis process is a *judgement of an acceptable risk*, which corresponds with the concept of safety (Lowrance, 1976 cited in Wolt and Peterson, 2000). In the *judgement* of an acceptable risk other factors of public policy decision-making such as societal, economic, legal, ethic, cultural and political factors also are considered (Wolt and Peterson, 2000). Thus, the Risk Analysis methodology considers science and the other factors necessary for the judgement of an acceptable risk resulting in a process consisting of three interconnected components: risk assessment, risk

management and risk communication (Codex Alimentarius Commission, 2003; International Organization for Standardization, 2009).

Risk assessment follows a science-based methodology. Risk management considers the risk assessment results but includes other factors necessary for a public policy decision-making (societal, economic, ethic, legal, political). Risk communication has the objective that the risk analysis process deals transparently with scientific, social, cultural, economic and political issues for an effective final decision regarding the new technology risk (Wolt and Peterson, 2000). While risk analysis methodology is currently widely used internationally in decision-making as to whether or not to authorize the release of a GE organism, the risk analysis components could vary significantly among countries regarding legislation, administrative procedures and assessment protocols. Below follows a brief history of risk analysis in the international context and the description of its components adjusted to genetically engineered organisms.

The first formal guideline of risk analysis published in the United States by the National Research Council (NRC), contained the basis for risk assessment of human health in the so-called "Red Book" (US NRC, 1983). The 'Red Book' contains a general approach outline for the characterization of adverse effects on human health due to exposure to hazards from human activity. It recommends basing the framework on a science-driven risk assessment process to provide information to risk managers about human health (NRC, 1983). At the same time risk analysis was emerging as a formalized discipline and tool for policy decision-making (Wolt and Peterson, 2000) as reflected in international standards for risk analysis (OECD, 1986, 1993, FAO/WHO, 1995). Table 1.6 lists initial comprehensive guidelines published for risk analysis and/or its components.

Table 1.6. Guideline documents about risk analysis and/or its components, modified from Hill and Sendashonga (2003).

Year	Document	Description
1983	Red Book	Risk analysis framework on human health safety elaborated by the National Research Council (NRC), USA.
1986	Blue Book	Recombinant DNA Safety Considerations elaborated by the Organization for Economic Co-operation and Development (OECD).
1989	Framework on human health and ecological risk assessment	Integrated framework on human health (Red Book) and ecological risk assessment, elaborated by the Committee on Risk Assessment Methodology (CRAM) convened by NRC, USA.
1993	Updated of the Blue Book.	Updated of the Blue Book (OECD)
1996	Orange Book	Updated and expanded version of the Red Book (NRC)
1996	Framework for Ecological Risk Assessment	Framework for Ecological Risk Assessment elaborated by the Canadian Council of Ministers of the Environment (CCME) for application to contaminated sites.
	Technical guidance document on risk assessment for existing and new substances.	Technical guidance documents in support of the Commission Directive 93/67/EEC on risk assessment for new substances and the Commission Regulation No. 1488/94 on risk assessment for existing substances elaborated by the Commission of the European Communities (CEC), Brussels, Belgium
1998	Guideline for Ecological Risk Assessment	Guideline for Ecological Risk Assessment elaborated by the Environmental Protection Agency (EPA), USA.
1999	Guidance on principles for risk assessment and monitoring for the release of genetically modified organisms.	Guidance document of risk assessment elaborated by the Department of the Environment, Transport and Regions (DETR), London, UK

According to Barnthouse (1994), a Committee on Risk Assessment Methodology (CRAM) was convened by the NRC in 1989 to develop a conceptual framework, based on the 'Red Book', integrating human health with ecological risk assessment. The integrated framework reaffirmed the recommendation of the 'Red Book' that risk assessment and risk

management must be two separated phases but working together and included as four basic steps: Hazard identification, Exposure assessment, Exposure-response assessment, and Risk characterization. The CRAM guideline lists other recommendations including implementing a follow-up of the risk assessment with basic research and monitoring to verify accuracy of predictions and reduce uncertainties for future risk assessments.

In that time frame the US Environmental Protection Agency (EPA) published a guideline for ecological risk assessment of chemical substances (US EPA, 1998). The EPA guideline was based on the criteria established in the Red Book (NRC, 1983) and follows the methodology recommended by CRAM (Barnthouse, 1994). This EPA guideline introduces the approach of 'Problem Formulation' as a *'systematic planning step'* equivalent to the CRAM's first step of 'Hazard identification' (Barnthouse, 1994, US EPA, 1998).

The Organization for Economic Co-operation and Development (OECD) convened a Group of National Experts on Safety in Biotechnology that developed several guidance documents for GE organisms with a first document called the 'Blue Book' (OECD, 1986) containing safety considerations for working with recombinant DNA (Table 1.6). Since 1988 this OECD Group updated the Blue Book further developing scientifically sound principles for safety and practices for the application of GE organisms compiled on a guidance document published in 1993 (OECD, 1993). The general safety principles included in the OECD (1993) document were the identification of hazards, application of risk assessment for the determination of the likelihood and consequences of hazards, and risk management. This document also includes the criterion of the *principle of familiarity* used today (explained later in this chapter). It also considers the environmental safety of scale-up of GE plants which is a follow-up of the work "Good Development Principles (GDP): Guidance for the Design of Small-Scale Research with Genetically Modified Plants and Microorganisms" (OECD, 1993).

Risk Analysis is based on science but also requires dealing with probability and uncertainties. This explains the components of the risk analysis clearly separating the risk assessment phase where a strict science-based analysis is performed, from the risk management phase which its members assume a risk level and associated uncertainties. However, since the first frameworks in risk analysis (NRC, 1983; OECD, 1986), frameworks have evolved giving a greater involvement to stakeholders and higher social participation during the different process phases. The framework described in the 'Red Book' evolved to a framework that includes other policy considerations such as the societal, economic, ethic, legal, political considerations reflected in the 'Orange Book" (NRC, 1996). Thus, although frameworks maintain an important role for science, and the role assigned to science in risk assessment is the most agreed criterion among the existing frameworks, risk assessment is integrated with risk management and the risk analysis process is defined as an iterative cycle that links assessment with management (Power and McCarty, 1998; Hill and Sendashonga, 2003).

Risk assessment

Risk assessment is defined as the process of *evaluation, including the identification of uncertainties, of the likelihood and severity of an adverse effect (s) /event(s) occurring to man or the environment following exposure under defined conditions to a risk source(s)* (EC, 2000). The risk assessment phase follows a sequence of steps based on scientific criteria to predict possible harms (adverse effects) that may arise, in the case of GE technology as a consequence of hazards (risk sources) introduced by the GE crop phenotype (US NRC, 1983, 1996; US EPA, 1998; EC, 2000, 2001,2002). Another definition refers to risk assessment as a *process intended to calculate or estimate the risk for a given target system following exposure to a particular substance, taking into account the inherent characteristics of a*

substance of concern as well as the characteristics of the specific target system (EC, 2000). Hazards that could cause adverse effects on biosafety, including food and environmental safety, are evaluated. This thesis focuses on the risk assessment methodology for environmental issues. Socioeconomic aspects may also be included with a science-based methodology, but strictly speaking, biosafety covers human/animal health and environmental security.

One of the criteria applied to determine potential adverse effects of transgenic crops on the environment is to perform the risk assessment process on a *case-by-case* basis based on the trait, crop, and the receiving environment (Figure 1.1) (Snow and Palma, 1997; Grumet and Gifford, 1998; FAO/WHO, 1996, 2000; Secretariat of the Convention on Biological Diversity, 2000; Dale *et al*., 2002; Conner *et al*., 2003, Nap *et al*., 2003; Grumet *et al*., 2011). Another criterion is the concept of *familiarity* (OECD, 1993; Codex Alimentarius, 2003). The concept of familiarity considers whether the GM phenotype is novel for the ecosystem under study (NRC, 1989). It is based on a comparative risk assessment approach in which the GE plant is compared with the corresponding non-GE. The non GE-plant refers to the parent line or non-transformed near isogenic lines from which the transgenic crop derives, and other plant varieties developed by conventional breeding present already in the market with a history of safe use plant (Kuiper *et al*., 2002; Conner *et al*., 2003; EFSA, 2006, 2010, 2011). The commercial varieties that are generated by conventional breeding without being through rigorous laboratory tests before being marketed, have been consumed for decades and they have gained a history of safe use (Kok and Kuiper, 2003). This approach has been further elaborated by the OECD and formalized in the so-called *Principle of Substantial Equivalence* (OEDC, 1993).

Plants developed by traditional plant breeding are considered an integral and accepted part of agriculture (Conner *et al*., 2003; Nap *et al*., 2003). Which comparators are considered

acceptable for risk assessment has been recently adjusted by the European Union regulatory system (EFSA, 2011). The comparative safety assessment comprises a detailed analysis of the chemical composition of the GE plant and a detailed agronomic evaluation under field conditions performed to establish the *substantial equivalence* of the transgenic plant with the *familiar* nontransgenic conventional crop. Field trials are performed in multi-location field trials for several years. If no differences as a consequence of the transformation process are identified, the risk assessment analysis continues only focusing on the trait introduced following a step-wise (tiered) approach, with increased assessment complexity based on the knowledge gained in each specific step (US EPA, 1998; EFSA, 2010, 2011).

The initial framework proposed in the US by the National Academy of Sciences-National Research Council (NRC, 1983) in the 'Red Book', established four steps for risk assessment: 1) hazard identification, 2) hazard characterization, 3) exposure assessment, and 4) risk characterization (NRC, 1983). These steps have evolved and the risk assessment methodology established by Codex and Cartagena Protocol follows a sequence of six steps performed *case by case* for each GE crop-gene-trait-environment combination (Secretariat of the Convention on Biological Diversity, 2000; Codex Alimentarius Commission, 2003), as described below. In brief, risk assessment tries to answer the following three questions for each individual case: 1) '*What can go wrong?;* 2) *'How likely is that to happen?';* and 3) *What are the consequences if it happens?* (Conner *et al*., 2003). Independent of the number of steps and the terminology used to described the process, risk assessment frameworks for GE organisms share the following criteria: it begins with Problem Formulation approach for the identification and formulation of the problem; it is performed by an overall characterization of risks taking into account, the probability of an adverse effect to occur, and the consequence of those effects (Hill and Sendashonga, 2003).

The steps included by the Cartagena Protocol and the *Codex Alimentarius* (Secretariat of the Convention on Biological Diversity, 2000; Codex Alimentarius Commission, 2003) are the following:

1) Identification of potential hazards associated with the GE plant that would cause harm to the receiving environment. This will depend on the specific combination of crop-gene-trait and receiving environment.
2) Estimation of the probability of occurrence of the identified adverse effects, which is related to the exposure level.
3) Evaluation of the consequences (impact assessment) if those adverse effects occur, i.e. 'what are the consequences if it happens?
4) Characterization of the risk combining the probability of occurrence (2) by its consequences (3). Risk = probability X consequences.
5) Consideration of possible adequate strategies for the management of the risk characterized, which could reduce the probability of occurrence and/or its consequences and to meet contingencies.
6) Requesting further information, or implementing risk management strategies, and/or implementing a monitoring plan to manage uncertainty regarding the level of risk.

The term *hazard* refers to an *inherent property of an agent or situation capable of having adverse effects on something*. Hazard can also be defined as the *potential of a risk source to cause an adverse effect (s)/event(s)* understanding risk source as an *agent, medium, commercial/industrial process, procedure or site with the potential to cause an adverse effect(s)/event(s)* (EC, 2000). In the terminology used in Problem Formulation, hazard is referred as plant attribute with the possibility of causing harm. Table 1.7 lists terminology that is considered synonymous in the context of this thesis. Hazard is something that could cause *harm*, term that is defined below.

Table 1.7. Terminology used in the literature for risk analysis considered synonymous in the context of this thesis.

Risk terminology	**Synonymous**
Hazard	Risk scenario Plant attribute
Harm	Adverse effect
Exposure pathway	Risk scenario
Environmental values	Environmental entities

The term *harm* refers to *a negative outcome or effect of an action or event, used as synonymous of adverse effect* (Wolt *et al*., 2010). Thus, harm refers to the negative outcome of the hazard defined above.

Risk refers to the *probability and severity of an adverse effect* (=harm) */event occurring to man or the environment following exposure, under defined conditions, to* hazard(s) *(= risk source(s))* (EU, 2000). It is also defined as *the probability of adverse effects* (=harms) *caused under specified circumstances by an agent in an organism, a population or an ecological system* (EU, 2000). *Risk characterization* is one of the main products of the risk assessment process used by the risk management phase to analyze whether the risk level and uncertainty associated with its estimation, is acceptable to take a regulatory decision (Raybould, 2006).

Risk characterization is defined as *the quantitative or semi-quantitative estimate, including attendant uncertainties, of the probability of occurrence and severity of adverse effect(s)/event(s) in a given population under defined exposure conditions* (EC, 2000; Secretariat of the Convention on Biological Diversity, 2000; Codex Alimentarius Commission, 2003). Risk characterization is also referred as the *integration of evidence, reasoning and conclusions collected in hazard identification, dose-response assessment and*

exposure assessment and the estimation of the probability, including attendant uncertainties, of occurrence of an adverse effect if an agent is administered, taken or absorbed by a particular organism or population (EC, 2000).

In the first step of environmental risk assessment available information regarding the crop, gene, trait and the receiving environment is analyzed to identify potential *hazards* associated with the GE plant that could cause *harm* to the receiving environment. The *hazard identification* is the first stage of risk assessment consisting in the determination of a particular hazard(s) (risk source(s)) capable of causing adverse effect(s)/event(s) to humans or the environment species of a target system exposed to the hazard(s). Hazard identification is complemented with a *hazard characterization* that refers to a qualitative description and, whenever possible, quantitative or semi-quantitative evaluation of the nature of the hazard and the effect(s)/event(s) (EC, 2000). Hazard characterization could be considered in other risk assessment frameworks as a second step (NRC, 1983; EC, 2000). Hazard characterization may include a dose-response assessment or dose-effect relationships when the hazard is associated with a biological, chemical or physical agent, determination of mechanisms of action involved, biological extrapolations, and identification of respective uncertainties (EC, 2000).

Which information is requested in this step and how information is organized and analyzed is key to properly construct risk hypothesis later in the process that justify further analysis. The US Environmental Protection Agency proposes to begin risk assessment with Problem Formulation (US EPA, 1998). Problem Formulation is an approach that assists in the process of defining real and relevant risk hypothesis. The Problem Formulation approach will be explained later in this section.

Step two of risk assessment is to estimate the *probability of occurrence of harm* (Codex Alimentarius Commission, 2003; Secretariat of the Convention on Biological

Diversity, 2000), i.e. an adverse effect to the environment due to a hazard associated with the GE crop. The probability of occurrence of harm will depend on the likelihood of the hazard (=risk sources identified in step 1) to come into contact with entities that have been defined as valuable to protect (for example wild relatives as part of biodiversity). The process by which such contact occurs is the exposure (Wolt *et al*., 2010). Thus, as defined by Wolt *et al*., (2010) the *exposure pathway (or scenario)* "*is a particular set of circumstances describing the opportunity for harm to an environmental entity of value*". The quantitative or semi-quantitative evaluation of the likely exposure of the entity of value to hazards (= risk sources) is referred as *exposure assessment* (EC, 2000).

The estimation of the likelihood of occurrence of harm for a defined exposure pathway (exposure assessment) also takes into account the level and kind of exposure that the receiving environment will have with the GE plant (Nickson, 2008). A primary factor influencing exposure is category of use requested (e.g., field trial vs seed multiplication vs commercial release).

In some risk assessment frameworks *exposure assessment* is considered as a third step separated from the estimation of the probability of occurrence of hazard, not harm, as just described above (NRC, 1983; EC, 2000). In this risk assessment approach risk characterization is the next and last step considering it as function of a defined hazard and its likelihood of exposure (NRC, 1983). *Exposure assessment* may include the inference of possible *consequences* that harm may have for a given population of particular concern (EC, 2000). The evaluation of possible *consequences* if harm occurs is considered in the next step according to the framework established by *Codex Alimentarius* (Codex Alimentarius Commission, 2003) and Cartagena Protocol (Secretariat of the Convention on Biological Diversity, 2000).

Step three of risk assessment evaluates the possible consequences if harm occurs (Secretariat of the Convention on Biological Diversity, 2000; Codex Alimentarius Commission, 2003), i.e. the seriousness and magnitude of harmful effect when environmental values of the receiving environment are exposed to the identified hazards of the GE plant (Nickson, 2008; Wolt *et al*., 2010). Assessment of consequences also takes into account the level and kind of exposure that the receiving environment will have with the GE plant as was mentioned in exposure assessment that is related to the category of use requested (e.g., field trial vs seed multiplication vs commercial release). Table 1.8 lists examples of association between hazards, harms and possible consequences with regard to GE plants.

An example of *harm* to an environmental entity associated with a hazard derived from a GE plant would be that existing wild relatives in the receiving environment (environmental entity) become more persistent under water stress conditions (harm) due to the capacity of dehydration stress tolerance (*hazard* acquired from being *exposed* to a GE crop) (Table 1.8).

The fact that in the receiving environment there are wild relative plants does not necessarily indicate that the adverse effect (harm) will occur. Conversely, presence of the transgene alone also does not guarantee that harm will occur (Raybould, 2006). In the exposure pathway it is necessary to consider not only the existence of wild relatives in the receiving environment, but also whether it is a cross pollinated species, the level of fertility of infertility in such a cross, whether there are water stress conditions, and if the transgene effectively has the capacity to change the wild relative's phenotype in a way that will increase its persistence. If the exposure pathway is confirmed, a consequence to assess in this example would be the possibility of the wild relative to become an invasive species. Another example of hazard associated with a GE crop is the expression of a protein toxic to insects that can cause an environmental harm by decreasing the abundance of non-target beneficial insects present in the receiving environment.

Table 1.8 Examples of association between hazards, harms and possible consequences with regard to GE plants.

Hazard		**Harm**	**Consequence**
Category	**Example**		
Gene product/ Trait conferred by the introduced gene(s)	Insect resistance	Could be toxic to non-target insects	Decrease in the population of non-target insects changing the ecosystem functions.
	Drought tolerance	Could increase the persistence of the GE crop.	The GE crop becomes a weed in the agroecosystem or invades natural areas.
	Drought tolerance	Could be introgression of the transgene into wild relatives. The harm will depend on the resulting phenotype, for example increased persistence of wild relatives.	Wild relatives become an invasive species competing with other species affecting the ecosystem relationships.
Receiving environment	Center of origin of the GE crop species	Could be introgression of the transgene into wild relatives. The harm will depend on the resulting phenotype.	Could affect biodiversity depending on the competitiveness of the resulting phenotype.
Non-biosafety issues, for example, a commercial situation	The fact that Uruguay currently is exporting to Europe exclusively non-GE rice.	GE rice could affect sales to the EU's market if GE rice is found mixed.	The rice sector could lose one of the main markets.

In these examples, the probability of occurrence of harm (step 2), i.e. increased persistence of wild relatives and decreased abundance of non-target organisms, is estimated as function of the likelihood of risk scenarios, i.e. a wild relative is exposed to a GE crop that confers dehydration stress tolerance; and for the second example a susceptible non-target organism is exposed to a GE crop that expresses a protein in a level that is toxic to them. The possible consequences in these examples would be that wild relatives become an invasive species and a change in ecosystem relationships and function due to beneficial insects

decreased abundance or extinction (Table 1.8). Problem Formulation approach assists in this step of evaluating the consequences, also by narrowing the assessment of harm magnitude to relevant consequences that effectively can occur (Reybould, 2006; Nickson, 2008; Wolt *et al*., 2010).

In step four of risk assessment the risk is characterized based on the previous steps, as a function of the possibility of a defined harm to occur (step 2) and the consequences if the harm happens (step 3), according to the relationship: Risk = probability of harm to occur X consequences (Secretariat of the Convention on Biological Diversity, 2000; Codex Alimentarius Commission, 2003). In turn the possibility of harm to occur depends on the likelihood of a real exposure pathway between hazards of the GE plant with an environmental value (Figure 1.3).

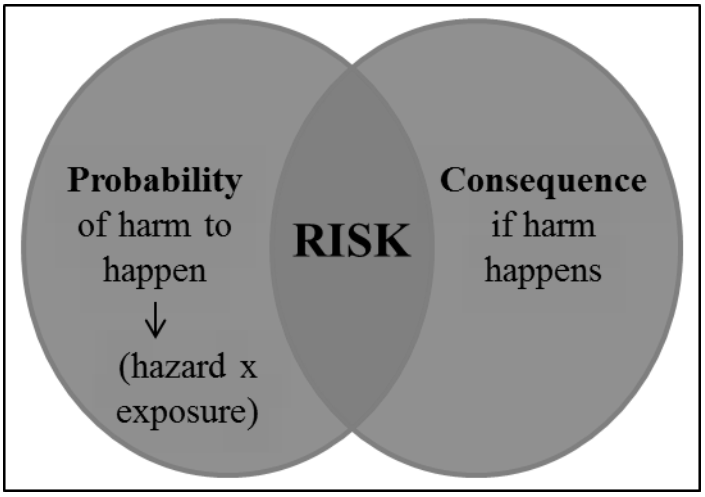


Figure.1.3. Schematic representation of risk estimation as a function of probability of harm X consequences if harm happens. The probability of harm to occur depends on the hazard X likelihood of exposure.

Sometimes risk characterization is expressed as function of a defined hazard by its likelihood of occurrence (exposure), i.e. Risk = hazard X exposure. However, risk assessment focuses more on the evaluation of the consequences of the harm itself rather than on increasing the precision to determine exposure (Raybould, 2010). A more comprehensive expression of risk characterization integrates this definition of risk (hazard X exposure),

referred as the probability of harm, with the magnitude of the consequences resulting in Risk = probability of harm X consequences (Figure 1.3). In the first approach (Risk = hazard X exposure), consequences of harm may be considered when the exposure assessment is performed (Nickson, 2008; Wolt *et al*., 2010). Independently of how risk is expressed, the important thing is not to confuse only hazard or only exposure with risk (Raybould, 2006), and to consider the magnitude of the harm in the risk characterization. As mentioned above, the presence of the transgene in wild relatives without causing a hazard phenotype constitutes a situation of exposure only. In the same way, the Bt protein could be only a hazard to non-target organisms, but to become a risk, the non-target organism has to be not only exposed to the Bt protein, but exposed to amounts of protein that could cause harm (Sears *et al*., 2001). Regarding the assessment of harm consequences, whether the increase in persistence is limited to the area of cultivation (agroecosystem), or if it expands to natural areas and the species become invasive, is also considered.

Assessment of consequences is related to the next steps of risk assessment, steps five and six, which consider risk management strategies (step five) and management of uncertainty through a monitoring plan (step six) (Secretariat of the Convention on Biological Diversity, 2000; Codex Alimentarius Commission, 2003).

The final report of the risk assessment should inform objectively and transparently the limitations and uncertainties found during the risk assessment process as well as if there is a lack of scientific consensus, indicating the different scientifically justified opinions. For each experiment performed the hypothesis tested and the results obtained should be clearly expressed. The conclusions of the risk assessment should include the uncertainty and variability of the data used to estimate the risk. It is not the assessor's responsibility but the manager's, to determine the importance of this uncertainty in the decision making process. If

managers consider the uncertainty is too high, risk managers will ask for extra information or experimental data in order to lower the uncertainty to an accepted level.

These last steps re-orient the risk assessment phase from a strictly science-based methodology to a more risk management oriented considerations (Wolt and Peterson, 2000). Other examples of aspects taken into account in risk assessment frameworks that show a more expanded interconnection with risk management include: consideration of socio-economic factors (NEW ZEALAND 1996); possible impacts of the associated technology that may determine changes in cultivation, management and/or harvesting techniques, and not only the impact of the event *per se* (EU EFSA, 2010; URUGUAY, 2011); consideration of the agroecosystem instead of the broad ecosystem (ARGENTINA, 2003, 2011); the feasibility of coexistence (Uruguay, 200); the acceptance of semi quantitative or qualitative conclusions to define a regulatory decision; and the level of integration of stakeholders in the risk assessment process (Power and McCarty, 1998).

A greater emphasis of risk management in the risk assessment phase is a request of a sector of the civil society observed in the Uruguayan regulatory system (see chapter 2 of this thesis). However the risk management commission that leads the risk analysis process maintains that the risk assessment should be exclusively science-based for biosafety issues with the exception of coexistence. The equilibrium between strictly science-based and incorporating risk management considerations also can be considered as moving from over-simplification to over complication of ecological principles (Power and Adams, 1997). Raybould (2006) indicates the necessity of better defining the ecological questions and the scope of the ecological risk assessment and advocates the Problem Formulation approach to aid in this matter. Power and McCarty (1998) indicate that a shift in the emphasis of the role of science in the different countries frameworks is due to the differences that arise when

defining problem formulation steps, specifically problem context and the consideration of stakeholders input (see later section regarding Problem Formulation approach).

Risk management

In the risk management phase the decision-making process takes into account the results of the risk assessment and may include other considerations such as economic, politic, legal, social and cultural factors relevant to a particular hazard in order to implement the optimal decisions and determine appropriate actions to ensure safety (Codex Alimentarius Commission, 2003). The final decision could be: accept the risk as it is, apply mitigation conditions to the risk, or avoid the risk. The risk management process is distinct from risk assessment by weighing policy alternatives according to the results of the risk assessment and consulting with all interested parties to take into account public (EC, 2002).

In order to preserve the structure and specific function of each of the components of the Risk Analysis methodology, the *Codex Alimentarius* recommends ensuring a functional separation between assessors and managers apart from the necessary interaction between them. This is a key issue in order to preserve the scientific integrity of the risk analysis process, to prevent confusion regarding the function that assessors and managers should accomplish, and to avoid any conflict of interests.

Managers should take into account the uncertainties identified in the risk assessment process and consider the recommendations suggested by assessors to manage the risk characterized. Managers are responsible for the implementation, surveillance, monitoring and review of the biosafety conditions established. As in the case of the risk assessment, the process of risk management has to be objective, transparent, coherent and completely documented. The challenge in risk management is to find equilibrium between the best

biosafety conditions to minimize the risk, and the real capacity (cost and feasibility) of their implementation.

Risk Communication

Risk communication refers to an interactive exchange of documented information and science based opinions throughout the risk analysis process. It concerns risk-related factors including the explanation of risk assessment findings, as well as risk perception factors and the basis of risk management decisions (Codex Alimentarius Commission, 2003). The risk communication process is performed among risk assessors and risk managers, with the participation of interested groups such as the industry, the academic community, consumers, news media and other actual or potential stakeholders as well as the general public.

The objective of the risk communication is to guarantee that all information and necessary opinion has been considered in the decision making process for an efficient management of the risk. Risk communication is not only the diffusion of information; it has to do with the transparency of the whole system transferring confidence to the public regarding the decision taken (Patton, 1998; Koch and Massey, 2011).

The risk communication process requires the implementation of mechanisms and the use of different consultation tools that invite and promote stakeholders participation in the decision making process (Koch and Massey, 2011). Managers choose the best mechanisms and tools for an efficient risk communication process and have the responsibility for its implementation.

PROBLEM FORMULATION

Problem Formulation has been proposed as a strategy to assist in the initial steps of the environmental risk assessment (US EPA, 1998). According to the risk assessment steps mentioned above, Problem Formulation provides a guideline identification of potential hazards that could cause an environmental harm. From the point of view of regulators, the objective of Problem Formulation is to identify real and relevant risk hypotheses that justify a science-based risk assessment to test them (US EPA, 1998, Hill and Sendashonga, 2003; Raybould, 2006; Nickson, 2008). It is not intended to broaden scientific knowledge in general but to contribute to decision-making to better understand and manage uncertainty. A coherent and logical sequence of steps is proposed to be followed in a Problem Formulation (US EPA, 1998; Raybould, 2006; Nickson, 2008, Wolt *et al*., 2010). While these steps are normally followed by regulators in some form, Problem Formulation offers a systematic order for the steps. To reach a risk hypothesis that warrants further analysis, Problem Formulation proposes to collect information regarding the a) Problem Context and b) Problem Definition (Wolt *et al*., 2010). Table 1.9 lists the steps of the risk assessment process (Secretariat of the Convention on Biological Diversity, 2000; Wolt *et al*., 2010) compared with the steps of the Problem Formulation strategy (Wolt *et al*., 2010) and the scientific method. The comparisons in Table 1.9 are adapted from Raybould (2006).

Problem Formulation begins with Problem Context and Problem Definition (US EPA, 1998). Problem Context and the majority of Problem Definition correspond with the first step in the risk assessment approach (Table 1.9) where *potential* agronomic and/or ecological risks are listed (see earlier section of this chapter about "Environmental risks for consideration when analyzing genetically engineered crops" page 15).

Table 1. 9. Sequential steps of risk assessment process, problem formulation approach and scientific method.

STEPS	RISK ASSESSMENT	PROBLEM FORMULATION	SCIENTIFIC METHOD
1	Identification of potential hazards	**a) Problem Context** i. Protection goals ii. Environmental scope iii. Baseline information	Collection of facts (observations/ experimental data). Definition of initial problem.
		b) Problem Definition i. Plant attribute (gene phenotype with the potential to cause harm) ii. Environmental value and its measurable assessment endpoints	Formulation of theories and hypotheses to explain the facts in terms of cause-effect.
2	Estimation of the probability of occurrence of potential harm identified	iii. Conceptual model with risk hypothesis	Inferences possible to be tested from theories and 'null' and 'alternate' hypotheses are formulated
3	Evaluation of the consequences identified harm occur		
4	Risk Characterization (step 2 X step 3)	iv. Analysis plan	Hypotheses testing by comparing new observations or experimental data with predictions.
5	Consideration of possible adequate strategies for the management of the risk characterized	---	---
6	Requesting further information, or implementing risk management strategies and/or implementing a monitoring plan to manage uncertainty	---	---

Problem Formulation strategy suggests framing the *potential* agronomic and/or ecological risks according to protection goals applied to a defined environmental scope for the assessment (US EPA, 1998; Raybould, 2006; Nickson, 2008, Wolt *et al*., 2010). Baseline information and measurable assessment endpoints should be used to identify *real* agronomic and/or ecological risks relevant for decision-making that are worthy of assessment.

The steps of Problem Formulation are also in agreement with the steps of the scientific method (Table 1.9) (Raybould, 2006), with the exception that in Problem Formulation the assessments are not always quantitative but the majority are semi quantitative and qualitative. However it is possible to increase knowledge of risk by a cycle of hypotheses formulation, testing, falsification and reformulation of hypotheses, as occurs in the scientific method (Raybould, 2006, 2010; Raybould *et al*., 2010).

Below are explained the different steps of the Problem Formulation approach. The process of Problem Formulation is divided into stages for explanatory purposes, but it is, in reality, a continuum where the steps overlap.

The parameters established in the Problem Context are defined according to protection goals and environmental scope of the country's environmental policies together with case-specific baseline information of the GE crop. Problem context is initiated by analysing *protection goals* that are defined in the country's law, statutes, regulations or guidance (US EPA, 1998; Raybould, 2006; Raybould *et al*., 2010; Wolt, *et al*., 2010). Protection goals in Uruguay for example, would be what is included in the General Environmental Protection Law, No. 17283 of 28/11/2000, such as conservation and sustainable use of the biological diversity and environment (air, soil, water), ecological protected areas and avoidance of invasive alien species. According to the US legislation, protection goals specifically for GE crops include no increased adverse effect to other organisms, no increased weediness of the crop plant, no gene flow to sexually compatible

plants leading to increased weediness or altered exposure scenarios leading to adverse effects, no increased disease and pest susceptibilities as well as no increase in adverse effects due to changes in cultivation practices (Cordts, 2011).

Environmental scope includes several other criteria needed to determine the parameters to be used in the risk assessment (US EPA, 1998; Wolt *et al*., 2010). One of the criteria to be defined is whether environmental impacts of the *event* itself will be analyzed, or whether impact of the "technology package" i.e. the associated technology will be considered; whether the event should be analyzed in the context of the agroecosystem or more broadly in the wild ecosystem; and whether coexistence will be considered and regulated. The other component of Problem Context is the analysis of case-specific *baseline information* used to identify relative risks that can be attributed to the genetic modification (Wolt *et al*., 2010). The baseline information includes information regarding the biology of the parent organism, the comparator and the GE plant, as well as characteristics of the receiving environment. With respect to the crop it is important to know centre of origin and taxonomy, plant biology and ecology, survival and persistence in agricultural and semi-natural environments, as well as interaction with other organisms (OGTR, 2009). Regarding the receiving environment, baseline information includes presence of sexually compatible relatives and/or weedy relatives, mode of pollination (cross vs self), closeness to protected areas, presence of similar genes in other GE plants, as well as general climatic conditions to determine gene flow to sexual compatible relatives. Production and uses of the crop as well as agricultural production practices and implications of coexistence are also considered. Information about previous releases in the receiving environment or in other countries and its risk assessment documents and experience is also valuable (OGTR, 2009).

Baseline information is analyzed with the objective to determine whether protection goals, i.e. biological diversity, ecological protected areas, the avoidance of invasive alien

species, could be affected by the GE plant. The analysis is performed according to the environmental scope defined, i.e. to analyse specifically the impact of the event and not of the associated technology, to analyse parameters of the agroecosytem and if necessary of the ecosystem, and to consider coexistence among different production systems.

The criteria defined and baseline information gathered in Problem Context is used for the next step, Problem Definition. This step is a critical challenge of Problem Formulation since the objective is to reach a relevant risk hypothesis worthwhile to test. First, *plant attributes* that could represent an environmental *hazard* are characterized (Wolt *et al*., 2010). The description of the *plant attribute* that may cause an environmental concern refers to the description of the genetic modification, characteristics of the trait that was introduced and the mechanism by which the gene/trait can cause harm to environmental concerns (Wolt *et al*., 2010).

Secondly, based on the plant attributes, the protection goals defined in Problem Context are translated to *environmental values* with observable and measurable attributes, named *assessment endpoints,* to assess the impact that the release of the specific GE plant would have on them (US EPA, 1998; Raybould *et al*., 2010; Wolt *et al*., 2010). In other words, the environmental values and assessment endpoints are used as indicators of the protection goals defined in Problem Context, which generally refer to broad concepts. It is then necessary to refine them into concrete and manageable parameters for the analysis (Raybould *et al*., 2010; Wolt *et al*., 2010), for example, an *environmental value* would be *'protect important beneficial insects such as pollinators'* and its *assessment endpoint* the 'abundance of pollinators' or for the environmental value *'to protect wild relatives from becoming invasive species'* the assessment endpoint would be *'persistence of wild relatives'.* A generic assessment such as *"Impact on beneficial insects"* is not an assessment endpoint. The assessment endpoint has to be an explicit expression of the environmental value to be

protected such as *"abundance of beneficial insects"* (Hokanson and Quemada, 2009). For each protection goal it is necessary to perform a specific problem formulation and risk characterization.

Third is the creation of a *conceptual model* in which potential *exposure pathways* are generated where the plant attribute (hazard) is linked to specific *environmental values* and *assessment endpoints* to determine if a particular *harm* could occur (US EPA, 1998; Raybould, 2006; Raybould *et al*., 2010; Wolt *et al*., 2010). Thus, the *conceptual model* combines: the plant attribute that has been modified with the mechanism to cause harm; the biology of the crop engineered; the characteristics of the receiving environment; and the complex interplay among these factors (Figure 1.1). The *conceptual model* is used to reach real environmental scenarios where potential harmful effects of the GE plant overlap with the environmental value to be protected.

Specifically, an *exposure pathway* or risk scenario represents the sequence of steps of the process for a particular harm to occur (Raybould, 2010). The objective is to reach testable risk hypothesis at each stage of the pathway that will derive from the general question of what is the probability of harm if the commercial cultivation of the GE crop is authorized. In this question it is important to specify the changes that could occur with respect to the environmental values, i.e. to define clearly the harm *a priori* that is being analyzed (Raybould, 2010). If the question is left open as to *what will happen if* a GE crop is released, it will result in weak broad hypothesis of '*no difference between transgenic and non-transgenic comparator*' (Raybould *et al*., 2010), or collection of data that test no hypothesis (Raybould, 2006, 2010). Thus, in the *exposure pathway* there should be an arguable and observable link between the hazard from the GE crop and potential harm to the environmental value (Nickson, 2008; Raybould *et al*., 2010; Raybould, 2010; Wolt *et al*.,

2010). A general scheme for a conceptual model considering exposure pathway and risk hypotheses is presented in Table 1.10 according to Raybould (2010).

Table 1.10. General scheme to state particular pathways with the steps for a harm to occur according to Raybould (2010).

Exposure pathway	**Hypothesis**
Cultivation of the GM crop	
↓	Event A will not occur
Event A	
↓	Event B will not occur
Event B	
↓	Event C will not occur
Event C	
↓	Event D will not occur
Event D (Harm)	

In each step of the exposure pathway can be postulated a testable risk hypothesis. Testing of these hypotheses is used in risk assessment to estimate the probability of a particular harm to occur. An important consideration in Problem Formulation is that it is possible to postulate numerous conceivable *exposure pathways that lead to a particular harm* (Raybould *et al*., 2010). This allows regulators to judge at an early stage of the analysis whether particular risk scenarios are sufficiently likely to occur to merit a detailed risk characterization (Raybould *et al*., 2010).

Each risk hypothesis is corroborated by providing evidence that show if a particular step of the pathway is possible or not. Evidence at an early stage of the analysis may come from analysing existing data and baseline information regarding plant attributes and mechanisms to cause harm, the biology of the crop and characteristics of the receiving

environment. Fully exploiting existing data allow ruling out unlikely harms early in the process, preventing unnecessary further assessment.

A useful guide that can be applied to GE plants to determine if already existing data can be used for hypotheses testing and risk characterization are the qualitative criteria described by WHO (2008) for chemical exposure assessment of: (1) *Appropriateness*: The degree to which data are relevant and applicable to a particular exposure pathway; (2) *Accuracy*: The degree to which measured, calculated, or modeled values correspond to the true values of what they are intended to represent; (3) *Integrity*: The degree to which the data collected and reported are what they purport to be; and (4) *Transparency*: The clarity and completeness with which all key data, methods, and processes, as well as the underlying assumptions and limitations, are documented and available (Romeis *et al*., 2011).

If a step in the process (Table 1.10) is reached that it is not possible, then the pathway is blocked and that particular harm can be eliminated from consideration. Nevertheless, for a good risk communication, even those exposure pathways that are readily ruled out by a first analysis of baseline information in Problem Context, it is necessary to state how that particular pathway was considered, and why it was rejected as implausible (Raybould, 2010).

In those exposure pathways for which steps are not blocked by analysing existing data, the harm warrants further evaluation to assess its likelihood of occurrence. The process of Problem Formulation will continue with regard to those particular harms developing an *analysis plan* to collect necessary data for hypothesis testing and its *risk characterization* (US EPA, 1998; Raybould, 2006; Raybould *et al*., 2010; Wolt *et al*., 2010). The analysis plan will include the experiments and measures for those considerations for which the information is incomplete. The analysis plan is discussed later in this section. In this stage of Problem Formulation the risk hypotheses postulated are key for the success of decision-making.

Raybould (2010) suggests when formulating hypotheses in risk assessment, to avoid formulating hypothesis that make precise predictions about an exact value of an assessment endpoint, unless this is required to make a decision. For example, a risk assessment hypothesis could be *'transgenic crop A does not hybridize with wild plant B'* rather than, *'the precise number of AxB hybrids formed are 'X''* (Raybould, 2010). Raybould (2010) indicates that it is more worthwhile to do research to defining thresholds of indicators than to improve the precision of prediction of the indicator.

A good risk hypothesis is one for which the result of testing is sufficient to make a decision whether to authorize the commercial release of a GE crop (Raybould, 2010). In risk assessment risk hypotheses are formulated considering pathways where the cultivation of a GE crop will cause harm to environmental values. Risk hypotheses at each step of the exposure pathway of a harm (Table 1.10) postulates that such particular step will not occur either because the process does not exist or it occurs below a defined threshold (Raybould, 2010). The corroboration or falsification of these risk hypotheses should be able to determine the risk level implied in the use of a GE crop. Therefore, in risk assessment the null hypothesis corresponds to a general or default position of no harm to specific endpoints. For example a null hypothesis might be: *'transgene will not confer a selective advantage to GE crop'.* The alternative hypothesis does not need to be the opposite of the null hypothesis, it predicts the results of the experiment if the alternative hypothesis is true. One example would be: *'transgene will confer a selective advantage to GE crop resulting in increased weediness'*.

As is the case for the scientific method, hypothesis testing in risk assessment does not provide an absolute truth. Experimental data and observations on a sample are used to make inferences regarding the population. The null hypotheses can never be proven, experimental evidence can only reject a null hypothesis or be insufficient to reject it (Raybould *et al*.,

2010). For example, if data on number of volunteer plants and persistence shows no statistically significant difference between transgenic and non-transgenic plants, it is not correct to assume that there is no difference in reality. The correct conclusion is that there is not enough evidence to reject the null hypothesis, i.e. the experiment failed to reject the null hypothesis.

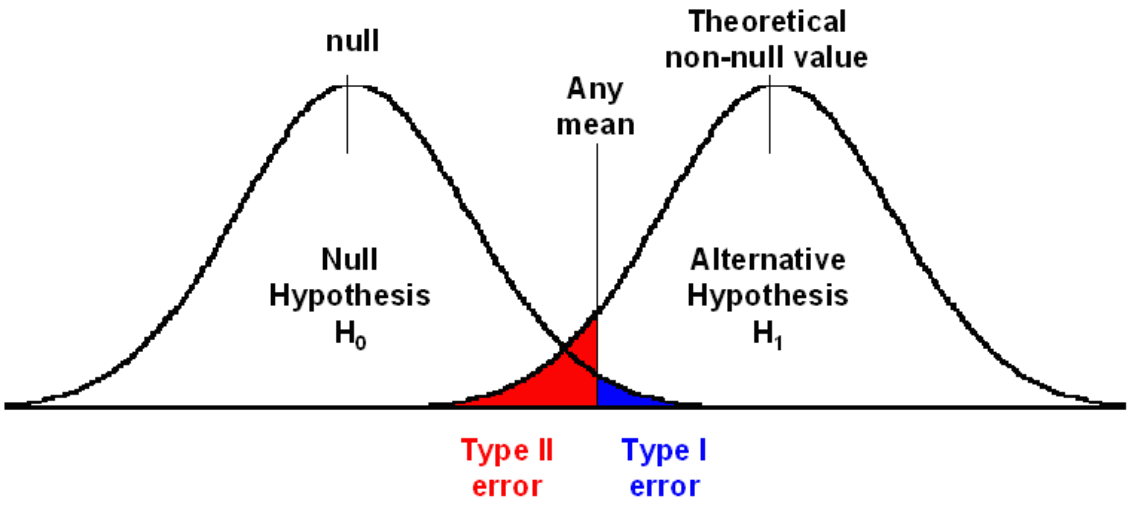
Null hypotheses cannot be proven true when are not rejected because there could be a Type II error (which probability is called β), i.e., when the null hypothesis is not rejected but in reality is false, therefore the transgene will in reality confer a selective advantage. Table 1.11 shows a schematic of the situation that corresponds to a Type I error or Type II error in risk assessment. A Type II error happens in risk assessment when a negligible risk level is predicted, but the actual level of risk is higher than negligible. The probability of a Type II error depends on the infinite possible values of the alternative hypothesis. It is difficult to estimate and therefore β is not controllable or fixed by the researcher. Thus, failing to reject the null hypothesis does not provide security that the transgene '*will not confer a selective advantage to GE crop'*.

Table 1.11. Schematic representation of statistical Type I error and Type II error applied in risk assessment.

		Estimated level of postulated risk	
		Greater than negligible	**Negligible**
Actual level of risk	Greater than negligible	True positive	False negative (Type II error)
	Negligible	False positive (Type I error)	True negative
Source: Modified from Keese, 2010.			

On the other hand, if experimental data give enough evidence to reject the null hypothesis, a Type I error could occur, i.e. the null hypothesis is rejected, but in reality it is true, and therefore the transgene will not confer a selective advantage. A Type I error

happens in risk assessment when a risk level greater than negligible is predicted but the actual level of risk is negligible. A Type I error is controllable and fixed by the researcher. Experiments to test the null hypothesis should be designed with the lowest probability of a Type I error (α) determining the highest confidence ((1-α)100%). The controversy lies in the fact that as shown in Figure 1.4 if α decreases, β increases, supporting the fact that a null hypothesis cannot be proven. However, the *weight-of-evidence* approach is useful in risk assessment. If the null hypothesis is supported time and time again, the weight-of-evidence becomes convincing that the relationship between the transgene conferring a selective advantage and the GE crop acquiring increased weediness does not exist (Raybould, 2010). This provides predictability reducing uncertainty.



Source: http://www.stattools.net/Probability_Exp.php

Figure 1.4. Summary diagram of Type I and II errors. "For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis".

For those exposure pathways that merit further consideration, an analysis plan is elaborated. The *analysis plan includes* the selection of parameters that can be used to measure the attributes of the environmental value as well as the experimental design for

hypothesis testing. High confidence in the risk assessment result is based on the thoroughness with which hypotheses are tested (Raybould, 2010).

A crucial step is to make appropriate selection of parameters of the environmental value that are going to be measured during hypothesis testing. This decreases the probability of having Type I and II errors.

Finally, once the analysis plan is carried forward, the data collected are used to perform the *Risk Characterization*. *Risk characterization* is formulated in terms of probability as a function of the probability of harm to occur by the consequences of the harm (Figure 1.3). *Risk characterization* defines the specific harm that can effectively happen to the environment (Raybould, 2006; Wolt *et al*., 2010). This harm will be a function of the *likelihood of exposure* of the environmental value to the hazard (exposure pathway) and the *consequences of the exposure* to the hazard.

CONCLUSIONS AND OBJECTIVES

The slow but steady tendency toward new genes, traits, crops and locations raise new challenges in environmental risk assessment. Regulation is considered to be one of the main limitations for the adoption of crop biotechnologies and several authors express the need for regulatory systems that are capable, appropriate to the needs, and time and cost effective (Nap *et al*., 2003; Bradford *et al*., 2005; Wolt *et al*., 2009; Bayer *et al*., 2010; James, 2010, 2011, 2012).

The objectives of my thesis are to:

1) Analyze the criteria and principles required for the implementation of a biosafety framework considering situations particular to developing countries, describe the experience implementing a new biosafety framework in Uruguay, and analyze the possibility of harmonization between regulatory systems, and

2) Examine possible secondary unexpected effects with respect to complex traits and to determine an approach for its analysis within the context of the risk assessment phase.

Chapter II is titled: '*Establishment and analysis of the Uruguayan regulatory system for genetically engineered crops'.* This chapter describes the Uruguayan experience implementing a new regulatory system after an 18- month moratorium (suspension) period imposed by a Decree since January 2007. This chapter analyzes the process of building a biosafety framework with emphasis on the criteria and principles required for the risk assessment phase.

Chapter III is titled: '*Comparison of the Uruguayan regulatory system for genetically engineered crops to other national and regional systems and potential for harmonization'.* In this chapter the Uruguayan approach for the establishment of a biosafety framework is compared with those of Argentina, Brazil and Paraguay, which together form the Southern Common Market (MERCOSUR for its acronym in Spanish). The possibility of harmonization in specific aspects of the regulatory systems is also discussed.

Chapter IV is titled: '*Application of the Problem Formulation approach when assessing environmental risks of crops engineered with complex traits - a case study of dehydration stress tolerance in cucumber'.* In this chapter the methodology of Problem Formulation is applied to the risk assessment process considering the Uruguayan biosafety framework and using the case study of cucumber plants engineered with the *Arabidopsis thaliana* transcription factor gene *CBF* to confer dehydration stress tolerance. Possible secondary unexpected effects are analyzed with respect to general complex traits.

The information provided in this thesis is based on scientific literature, published documents, unpublished public official documents, my personal experience working for the Uruguayan regulatory system and personal communication with national, regional and international regulators.

APPENDIX

APPENDIX

Table A1.1. List of crops with commercial approval, number of events and traits description.

CROP	Number of events with regulatory approval	Traits
Soybean (*Glycine max L.*)	13	(1) (2) (4) (5)
Corn (*Zea mays L.*)	58	(1) (2) (6) (7) (8) (9)
Cotton (*Gossypiumhirsutum L.*)	21	(1) (2)
Argentina Canola (*Brassicanapus*)	15	(1) (4) (5) (6) (10)
Polish Canola (*Brassica rapa*)	2	(1)
Wheat (*Triticumaestivum*)	1	(1)
Rice (*Oryzasativa*)	2	(1)
Alfalfa (*Medicago sativa L.*)	1	(1)
Creeping Bentgrass (*Agrostisstolonifera L.*)	1	(1)
Papaya (*Carica papaya*)	2	(3)
Squash (*Cucurbita pepo*)	1	(3)
Sugarbeet (*Beta vulgaris*)	3	(1)
Potato (*Solanumtuberosum* L.)	5	(2) (3)
Tomato (*Lycopersiconesculentum*)	7	(2) (3) (12) (13)

Table A1.1 (cont'd)

Melon (*Cucumismelo* L.)	1	(12)
Sweet pepper *(Capsicum annuum)*	1	(3)
Chicory (*Cichoriumintybus* L.)	1	(1) and (14)
Tobacco (*Nicotianatabacum*)	2	(1) (15)
Flax/Linseed (*Linumusitatissimum*L.L.)	1	(1)
Poplar (*PopulustremulaXPopulus alba*)	1	(2)
Plum (*Prunusdomestica* L.)	1	(3)
Petunia (*Petunia hybrida*)	1	(16)
Carnation (*Dianthus caryophyllus*)	3	(1) (16) (17)
Rose (*Rosa hybrida*)	2	(16)
Beans	1	(3)

(1) Herbicide tolerance
(2) Insect resistance
(3) Virus resistance
(4) High oleic acid
(5) Low linolenic acid
(6) Male sterility
(7) Heat stable alpha-amylase for dry grind ethanol process
(8) Enhanced lysine content
(9) Reduced yield loss under water deficit (cold shock protein B)
(10) High laurate and myristate acids
(11) Altered starch composition
(12) Delayed ripening
(13) Delayed softening
(14) Fertility restored
(15) Reduced nicotine content
(16) Modified flower color
(17) Delayed senescence

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CHAPTER II: ESTABLISHMENT AND ANALYSIS OF THE URUGUAYAN REGULATORY SYSTEM FOR GENETICALLY ENGINEERED CROPS

INTRODUCTION

Uruguay has recently adjusted its regulatory system that controls the introduction and use of genetically engineered crops. The objective of this chapter is to describe the Uruguayan experience over a period of approximately two years (2009-2011) implementing the new framework. The information presented in this chapter is based on my direct experience as the Technical Secretariat for the Interministries Working Group, coordinating the meetings, elaborating the group technical documents, providing bibliography and technical reports of the topics discussed, and monitoring approved events in the region and worldwide. I was hired and supervised by the National Division of the Environment (DINAMA) of the Ministry of Environment. Once the Decree 353/008 was approved I was hired by the National Agency of Research and Innovation as the coordinator for the analysis of the risk assessment. The President of the Risk Management Commission supervised me.

This overview includes a description of the regulatory system with special emphasis on the risk assessment phase. The general and underlying criteria used for the application of the risk analysis methodology to the genetically engineered crop technology are also presented to explain how the building process was guided.

HISTORICAL EVOLUTION OF THE BIOSAFETY REGULATORY FRAMEWORK ON GENETICALLY ENGINEERED ORGANISMS IN URUGUAY

Uruguay established specific procedures to regulate the introduction and use of genetic engineering for plants early in the development of these technologies worldwide. The first record is from 1995, when the General Direction of Agricultural Services (DGSA) of the

Ministry of Livestock, Agriculture and Fisheries (MGAP) issued a resolution establishing a procedure for risk analysis following transgenic corn and Round-up Ready (RR) soybean applications (Uruguay, 1995a,c, 1996a). An Advisory Committee for Risk Analysis (CAAR) was composed of representatives from the Direction of Agricultural Protection (DPA) of the MGAP, the Direction of Seeds of the MGAP – which later on was substituted by the National Institute of Seeds (INASE) (Uruguay, 1997, 2009) - and the National Agricultural Research Institute (INIA) (Uruguay, 1995a,c, 1996a). The authority with the final decision was the director of DGSA of MGAP. The advisory body CAAR was a fast solution to respond to the corn and soybean applications allowing for utilization of new technology regionally and worldwide.

At the beginning CAAR was convened by resolution of DGSA specifically for each application (Uruguay, 1995a, c). In January of 1996 CAAR was established permanently for the analysis of transgenic plants (Uruguay, 1996a). The advisory committee CAAR evaluated the applications applying the risk analysis methodology used by DPA for weeds and invasive species (Uruguay, 1995a, 1996a). Under this framework seven events in corn, one in soybean and two in eucalyptus were authorized for field trials. In addition, the herbicide tolerant RR soybean with the event 40-3-2 was soon deregulated under Resolution of the DGSA-MGAP in October 1996 (Uruguay, 1996d). A summary of all applications from the period of 1995-2012 including crop, event, trait, gene, year of application and authorizing agency has been compiled in Table A2.1 (see Appendix). These early applications and the following ones shown in Table A2.1 indicates the rapid advent of this technology to Uruguay and the need for policymakers to define a regulatory framework.

Since the establishment of CAAR, the regulatory framework has been adjusted and strengthened in two occasions as illustrated in Table 2.1. The first adjustment was in 2000 with the promulgation of the Decree No. 249/000 by the Ministries of Agriculture, Economy,

Health and Environment, that established a framework with specific procedures for genetically engineered plants (Uruguay, 2000a). The decree 249/000 created the Commission on Risk Assessment of Genetically Engineered Plants (CERV), expanding the institutions integrating the advisory commission and establishing new procedures (Uruguay, 2000a). CERV was composed of representatives from the Ministry of Agriculture, Environment, Health, INASE and INIA (Article 5 in Uruguay, 2000a). CERV based the procedure in the risk analysis methodology (Uruguay, 2000a). The competent authority was the Ministry of Agriculture and Economy for deregulations. DGSA was the competent authority to authorize events for "*contained use*", "*field trials*", "*national evaluation of cultivars*", and "*seed multiplication*" (see later the explanation of these proposed uses). The Ministries of Health and Environment had their respective authority over food safety and environment, respectively, according to other laws and rules existing at that time.

Under the framework established by the decree No. 249/000, two events in corn, MON810 and BT11, were deregulated for commercial use, and eight permits were issued for field trials (Tables A2.1 and A2.3). For each deregulated event two resolutions were issued, by MGAP-MEF and MVOTMA. The Resolution of MGAP-MEF deregulated the event itself (MON810 or BT11) (Uruguay, 2003a, 2004b). This resolution was without biosafety conditions, only with the requirement of an *insect resistance management plan* specific for corn Lepidoptera (numeral 5, Uruguay, 2003a, 2004b).

The Resolution issued by MVOTMA established conditions to the planting of the deregulated event (Uruguay, 2003b and Uruguay, 2004c respectively). MVOTMA's resolution established 250 meters as isolation distance and the planting of corn not resistant to lepidopteran insects in 10% of the planted area operating as refuge.

Table 2.1. Evolution of the Uruguayan regulatory framework on biosafety of genetically engineered crops.

<table>
<tr><th rowspan="2">Period</th><th rowspan="2">Framework</th><th colspan="2">Resolution issued by:</th><th colspan="2">Risk analysis done by:</th></tr>
<tr><th>Authority</th><th>Firms</th><th>Advisory body</th><th>Members</th></tr>
<tr><td>1995-2000</td><td>Resolution DGSA-MGAP</td><td>Director of DGSA</td><td>1</td><td>CAAR</td><td>3</td></tr>
<tr><td rowspan="2">2000-2007</td><td rowspan="2">Decree No. 249/000</td><td>Ministers of MGAP and MEF for deregulation.</td><td>2</td><td rowspan="2">CERV</td><td rowspan="2">5</td></tr>
<tr><td>Director of DGSA for uses under controlled biosafety conditions (field trials and seed multiplication).</td><td>1</td></tr>
<tr><td>2007-2008</td><td>Decree No. 037/007</td><td colspan="4">Suspension of the treatment of any new applications. GIM proposed a draft decree of a new regulatory framework, approved in July 2008 (Decree 353/008).</td></tr>
<tr><td rowspan="4">2008-present</td><td rowspan="4">Decree No. 353/008</td><td rowspan="4">GNBio composed of the Ministers of: MGAP, MEF, MVOTMA, MSP, MIEM and MRREE.</td><td rowspan="4">6</td><td>CGR</td><td>6</td></tr>
<tr><td>ERB</td><td>1</td></tr>
<tr><td>CAI</td><td>9</td></tr>
<tr><td>Ad Hoc Groups</td><td>25-30</td></tr>
</table>

Regarding the resolutions issued for field trials, six out of eight were for events that had been previously commercialized in other countries: BT11 (1), NK603 (2) and MON810XNK603 (2) in corn, and LLrice62 (1) (Table A2.1). The mentioned events already had an application in place requesting deregulation or were soon then presented. Thus, the field trials performed were either to test the efficacy or to assess agronomic traits for the national register of cultivars (Table A2.1). The other two resolutions authorizing field trials were for white clover also to test efficacy, but the event was in a less advanced state of development. This was also the situation for the events in eucalyptus authorized for field

trials under the responsibility of the former framework. None of the events in eucalyptus and white clover requested new permissions or reached the commercial phase.

The advisory body CERV had two periods while the decree 249/000 was in force. A first period is distinguished from 2000 to early 2005, and the second period since then until the promulgation of Decree 037/007 that disintegrated CERV. In the former period, CERV consolidated its procedures and analyzed the events described in the previous paragraph (Table A2.1). Between 2004 and 2005 it started a new wave of applications but the momentum slowly began to stop accompanying the political transition that the country went through in 2005.

In March 2005 a political transition period started between the guidelines and authorities from a government that traditionally alternated between the Colorado and National parties throughout most of Uruguay's history, to a new government elected from the Broad Front party - a coalition of socialists (Uruguay, 2005). Five applications entered in 2005, with events in corn (NK603, MON810XNK603, TC1507), soybean (FG74) and rice (LLrice62), which could not complete the risk analysis because members of CERV were replaced when the new administration took place and CERV, suspended its work *de facto* (in fact). These applications were rescinded waiting for new policy signals. Except for LLrice62 and FG74 soybean, the three events in corn re-applied under the currently regulatory system for the same proposed uses (Table A2.1).

At the same time stakeholders were actively participating in a project that had started in February 2004 to develop a proposal for a National Biosafety Framework (NBF) (Uruguay, 2007a). The NBF project was supported by the United Nation Program for Environment (UNEP) and it was led by the National Division of the Environment (DINAMA) of the Ministry of Environment (Uruguay, 2007a). The NBF project had broad participation, gathering stakeholders under the National Committee of Coordination (CNC). The CNC had

periodic meetings and were organized in working groups by issue: (1) research and development of LMO, (2) biotechnology in industry, (3) administrative issues, (4) environment, (5) socio-economic and (6) health (Uruguay, 2007a). The main criticisms to the existing regulatory system discussed at the CNC were the low participation of scientists at the risk analysis process and the lack of data provided by local environmental studies for risk assessment when the events in maize MON810 and Bt11 were authorized for commercial release (Uruguay, 2007a).

Therefore, besides the new government with a different political party, the NBF project put the genetically engineered organisms topic in the public agenda and a climate of tension and pressure started for a change in the existing regulatory system.

The first consequence of this transition period was a Resolution issued by the Ministry of Environment banning the import and planting of genetically engineered sweet corn, which has now been in force since August 2006 (Uruguay, 2006). This Resolution is based on the powers granted by the General Environmental Protection Act No. 17283 to the Ministry of environment in its Article 22 and 23 (Uruguay, 2000b). This Resolution was triggered by complaints received by environmental authorities from a nongovernmental organization of irregularities at the importation of sweet corn. The irregularities relate to an import that was divided for sale in small bags. The small bags were not properly identified with the required label indicating that was transgenic corn seed. Although the corn that was being sold contained the event BT11 that had been approved for commercial release in 2004 (Uruguay, 2004b), the resolution is for all kinds of sweet corn hybrids, including those containing authorized events such as MON810 and BT11. This resolution is discussed more thoroughly in this chapter when issues of the current agenda are discussed (page 145).

Soon after this Resolution, in January of 2007, the second adjustment period of the regulatory framework started with an 18 months moratorium (suspension) period imposed

with the promulgation of the Decree No. 037/007 by the Ministries of Agriculture, Economy, Health and Environment (Uruguay, 2007b). The Decree N° 037/2007 suspended the treatment of any *new* applications for authorization. The events already deregulated, round-up ready soybean (40-3-2 event), and the two events in corn, MON810 and Bt11, continued to be commercialized and planted according to the respective resolutions (Uruguay, 2003a, b; 2004 b, c). During that time CERV stopped functioning formally and an interministries working group (GIM) was convened to review and adjust the regulatory system established under the Decree No. 249/000.

Simultaneously, the discussions at the CNC of the NBF project were tense and nongovernmental organizations stopped participating (Uruguay, 2007a). In March 2007 the director of the National Division of the Environment (DINAMA) gave the project completion (stopped the project before its due date) and a final report was elaborated. The DINAMA's report organized the existing information at that time and compiled the recommendations elaborated at that point by the different working groups, as well as recommendations from the technical team of the project and the National Implementing Agency (Uruguay, 2007a). Although not all of the proposals described in the DINAMA's report were validated by the competent authorities, the background information gathered and the diagnoses of the genetically engineered organisms situation was used as starting point in the discussion at the GIM working group. The GIM group was composed of a representative from the Ministries of Agriculture, Environment, Health and Economy. The GIM group analyzed three scenarios for Uruguay, 1) a regulatory system for a country free of transgenic crops, 2) a regulatory system based on coexistence among different production systems, 3) no control over transgenic crops. These three scenarios were analyzed from the point of view of its legal, technical and economic feasibility (Uruguay, 2007b).

GIM recommended to the ministers the coexistence scenario, which was approved and they set a deadline for the submission of a regulatory system proposal. For the elaboration of the proposal of a biosafety framework, GIM created four inter-institutional *Ad Hoc* groups to analyze 1) the design of the operating structure for the regulatory framework (Institutional Working Group – GTI for its acronym in Spanish), 2) labeling (Labeling Working Group, GTE), 3) Public participation (Participation Working Group – GTP), and 4) legal issues (Juristic Working Group – GTJ). GIM developed a proposal of biosafety framework, which was approved under the Decree No. 353/008 (Uruguay, 2008). The Decree No. 353/008 revokes the Decrees No.037/007 and 249/000 and it contains the present regulatory framework that is in force since July 2008. Decree No. 353/008 has typos amended in Decree No. 535/008 of 11/03/08 and a modification to the addition of the possibility to charge the applicant for the risk analysis process performed.

CURRENT REGULATORY FRAMEWORK

Criteria and characteristics of its implementation

CGR led the building process of the current regulatory system guided by a set of criteria, some of which were taken from risk analysis methodology, and others defined by CGR. Those criteria, summarized in Table 2.2, frame the risk analysis process and were used to organize the description of the implementation of the current biosafety framework in Uruguay.

Table 2.2. General criteria of the Uruguayan biosafety framework.

Criteria	Risk Analysis	Risk Assessment
Criteria to guide the operation	Biosafety restricted to GE plants Process based Risk analysis methodology Case by case Step by step Iterative/independent Open/transparent/documented	Case by case Step by step Comparative Substantial equivalence Familiarity
Criteria to guide the decision making	Health safety Environmental security Socio-economic considerations Political considerations Legal considerations Commerce considerations Monitoring/surveillance Public confidence on framework	Assessment restricted to event Scientific justified statements. Weight-of-evidence approach

Scope. "Biosafety" restricted to GE plants

The current regulatory framework continues to include only genetically engineered plants and its parts. At this time there is no designation of a competent authority to regulate genetically engineered animals, microorganisms, and vaccines apart from what is established at the environmental Act 17283, article 23. The scope of the term "biosafety" in the decree 353/008 is less comprehensive than the definition used at the Convention on Biological Diversity (CBD) (CBD, 1992) and the Cartagena Protocol (CP) (Secretariat of the Convention on Biological Diversity, 2000) (see Chapter 1 of this thesis) since it covers only plants and their parts within the concept of GE organisms. The other aspects of biosafety are taken into account under a different Uruguayan legislation. For the Decree 353/008 "*biosafety*" refers to a) The goal of ensuring that the development and use of genetically engineered plants and products made from them do not negatively affect plant, animal, or human health; genetic resources; or the environment; b) Policies and procedures adopted to

avoid risk to human health and safety, and to the conservation of the environment, as a result of the use of genetically modified plants for research and commerce (Traynor *et al*., 2002).

The Decree No. 353/008 gives the responsibility to the authorities involved in the new framework to elaborate a biosafety bill for genetically engineered *organisms*. A national biosafety law is required to have the same legal hierarchy level as other related topics such as the national environmental protection law, land law, and Cartagena Protocol that was recently ratified (Uruguay, 2011). The fact of implementing a framework in parallel with the elaboration of a biosafety bill has advantages and disadvantages. The main advantage is that the experience generated allowed visualization of aspects necessary to be included in the law accordant with Uruguay's situation, as well as determining which the best mechanisms and tools for every step of the decision making process are. The main disadvantage is the delay in the consolidation of the system since it will mean inevitably another instance of adjustment.

Process based

The genetic engineering process utilizing recombinant DNA is what triggers the regulatory system (Uruguay, 200). The rationale for *process-based* regulation is that GE technology has a high potential to obtain new products that are morphologically and physiologically significantly altered. It appears that it is not the technology *per se*, but its potential impact that makes necessary to take a cautious attitude and determines that GE crops follow a regulatory channel different from conventional crops. All genetically engineered plants and their parts require an authorization to be produced and/or used in Uruguay. Other countries, like Canada, have a *product-based* system (Nap *et al*., 2003). A *product-based* approach gives coherence to the system in the sense of biosafety. For example, technologies applied in conventional breeding such as mutagenesis, which also may result in unexpected effects, may require a deeper analysis

Risk analysis methodology

The current regulatory system maintains the risk analysis (RA) as the methodology for the decision making process, as had been used under the previous framework (Uruguay, 2000a; 2008). The general principles and rationale behind the RA methodology are the basis for the operating structure of the new framework. The main changes compared with the previous framework (Decree 249/000) are found in the organization and institutionalization of the official bodies and steps involved in the decision making process (Tables 2.3 and 2.5).

As described in the literature review of this thesis, the RA is an internationally accepted methodology to determine whether to authorize the use of GE organisms (US NRC, 1996; OECD, 1993, 1995; Secretariat of the Convention on Biological Diversity, 2000; Codex Alimentarius Commission, 2001, 2003; FAO, 2009a; EC, 2002a,b). This methodology is broadly applied in the field of biosafety and in general for all new technology. This methodology allows for integration of the "*biosafety*" concept along with policy and regulatory frameworks (including instruments and activities) for analyzing and managing relevant risks to human, animal and plant life and health, and associated risks to the environment (FAO, 2007).

The RA has three independent, but highly connected components: risk assessment, risk management and risk communication (Codex Alimentarius Commission, 2003). The logic underneath the RA methodology explains the operating structure of the framework established in the Decree N° 353/2008 for genetically modified organisms. Uruguay has always based its framework for GE plants in the RA methodology. Throughout the past 15 years the number and variety of participating stakeholders has expanded (Tables 2.1 and 2.3).

The first advisory commission, CAAR, was created from the risk analysis commission for pests. CAAR had three members from the orbit of one ministry (agriculture) and applied the methodology for risk assessment of weeds and invasive species that was in place at that

time (Uruguay, 1995a,c, 1996a). The subsequent system under Decree 294/000 also applied the risk analysis methodology but created a specific commission for risk assessment, CERV, which was also in charge of the risk management and risk communication (Uruguay, 2000a). CERV had five members from the orbit of three ministries (agriculture, health and environment), and the ministry of economy participated when authorizing applications for deregulation.

The current system under Decree 353/008 created two figures for risk assessment, the Risk Assessment on Biosafety (ERB for its acronym in Spanish) and the Institutional Coordination Committee (CAI); and two figures for risk management, the Uruguayan National Biosafety Cabinet (GNBio) and the Risk Management Commission (CGR). The institutional design of the current framework tries to reflect the conceptual distinction that the risk analysis methodology applies between Risk Assessment, Risk Management and Risk Communication. The areas of assessment and management are separated in different specialized commissions and advisory bodies.

The National Biosafety Cabinet (GNBio) and the Risk Management Commission (CGR) are the risk "managers". The Risk Assessment on Biosafety (ERB) and the Institutional Coordination Committee (CAI), from where *Ad Hoc* groups on specific areas of concerns are formed, are the "evaluators". Multiple strategies can be employed for risk communication. For the time being, two mechanisms have been implemented, "inform" and "consult", as a requirement for each application in the decision-making process. The decree 353/008 also gives the possibility to form a Biotechnology Advisory Committee (CCB) for consultation regarding biosafety and biotechnology policies.

Table 2.3. Evolution in the establishment of bodies involved in the Uruguayan regulatory system from 1995 to present.

Framework	**Authority**	**Advisory body**	**Integration**
1995-2000 Resolution DGSA-MGAP	MGAP	CAAR	DGSA-MGAP INS-MGAP INIA
2000-2007 Decree No. 249/000	MGAP and MEF for deregulation. DGSA and INASE for contained applications under biosafety conditions.	CERV	MGAP INASE INIA MVOTMA MSP
2008-present Decree No. 353/008	GNBIO: MGAP, MEF, MVOTMA, MSP, MIEM, MRREE	CGR	MGAP MEF MVOTMA MSP MIEM MRREE
		ERB	Specialists on biosafety of GE plants in environment, health and animal issues, acting as coordinator of the risk assessment process.
		CAI	MGAP MVOTMA MSP INIA INASE UDELAR LATU IIBCE IP
		Ad Hoc groups GAHCIM GAHFG GAHONOB GAHSHA	Conformed from specialists from CAI's institutions.

The new framework involves twenty-two members, ten for risk assessment and twelve for risk management, from the orbit of seven ministries (agriculture, health, environment, economy, industry, foreign and culture), academia and research institutions (Table 2.3). Figure 2.1 summarizes the structure in a diagram. In the diagram the risk management phase is shown to the left and in a higher position compared with the risk assessment phase. This is not the standard arrangement described in the risk analysis methodology where assessment come before management, but was adopted in Uruguay to interpret the hierarchical relationship between "managers" and "evaluators" where the formers lead the process (CGR) and take the final decision (GNBio). This is also the reason that the application process begins at the CGR, since CGR determines the terms of references and deadlines for the risk assessment phase (ERB, CAI and *Ad Hoc* groups).

There is a clear example in rice that illustrates the rationale for beginning the application at the CGR. After the problem experienced by the US with low-level presence of transgenic rice in a shipment to the EU, Uruguay was able to establish a new market (EC RASFF, 2006, 2007). The rice growers association asked the government for support to keep the EU market, by prohibiting production of transgenic rice. This was a political decision due to the current market. Thus, if an application for GE rice was received, the CGR may decide not to pass it to evaluators and may directly answer that it will not be authorized due to trade reasons. Or, the CGR may give it to evaluators even though will not be authorized, but to save time if the market changes in the future.

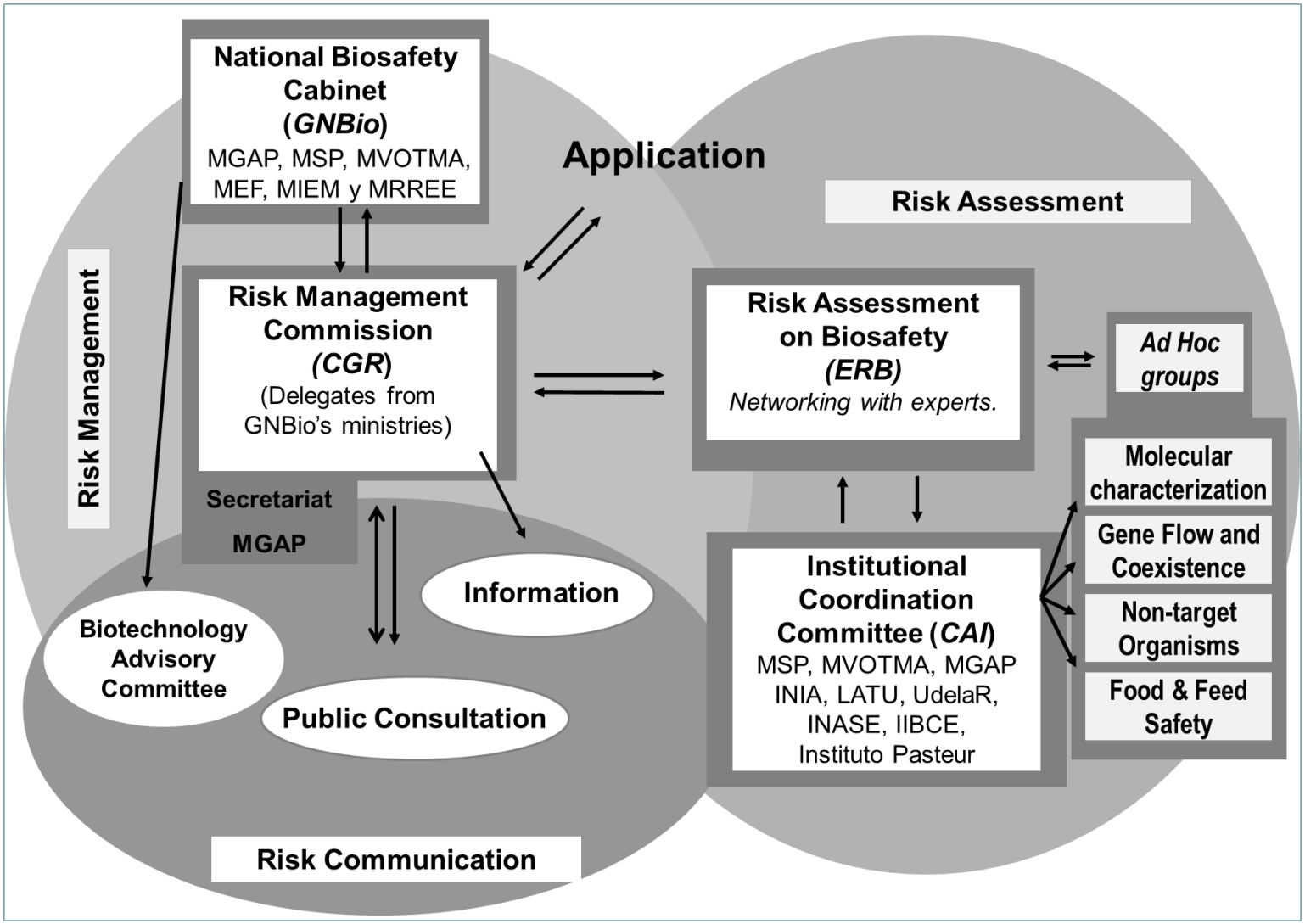

Figure 2.1. Diagram of the operating structure of the Uruguayan biosafety framework according to Decree No. 353/008.

The National Biosafety Cabinet (GNBio) is the ultimate authority composed of six Ministers: the Ministers of Agriculture, Economy, Environment, Health, Industry, and Foreign affairs. The Ministry of Agriculture presides over the GNBio. The GNBio responsibilities include:

- Make the final decision of the RA process to authorize or not the requested permissions. An authorization for any type of use requires the signature of the six ministers.
- Define the guidelines of the national biosafety policy of GE plants.

The Risk Management Commission (CGR) has the responsibility of implementing the regulatory system acting as the task force and advisory body of the GNBio. CGR is composed by a representative of each ministry of GNBio with a total of six members plus their respective alternates. The representatives are elected directly by each minister having their trust and professional knowledge in the subject. CGR meets twice a month at the Ministry of Agriculture (MGAP).

The CGR responsibilities include:

- Advise the Executive Power in terms of biosafety
- Define the terms of references to guide the risk assessment
- Establish a time-frame for each step of the RA process, once expired CGR should take a decision
- Inform GNBio of each action taken at the RA process
- Advise the competent authority regarding risk management conditions to be enforced
- Manage the public participation process
- Followed up the authorized GE plants and biosafety conditions to ensure that they are being implemented

The Risk Assessment on Biosafety figure (ERB) is an advisory body of CGR that address the assessment of the potential risk to human, animal and plant health, as well as that of the environment, based on technical and scientifically sound arguments. ERB leads the environmental and health risk assessment in a systematic way with all the public and non-state public institutions with competence on the subject respectively involved at the Institutional Coordination Committee (CAI).

ERB can be composed up to a small number (1-3) of independent scientific experts that lead the risk assessment of genetically modified crops for health and the environment working within a scientific network. The ERB has support for the implementation of the risk assessment network from CAI, which is composed of head staff from nine public and non-state public institutions respectively involved in the scientific network. ERB's scientific opinion is forwarded to the Risk Management Commission (CGR) that leads the risk analysis process.

Since the implementation of this framework, I have been hired by the National Agency of Research and Innovation (ANII) to work for GNBio-CGR at ERB. The ERB responsibilities include:

- Ensure a *case-by-case* analysis of the risk assessment performed on an objective scientific basis.
- Identify national and regional capacities for the multidisciplinary analysis required for risk assessment.
- Design protocols for environmental and health risk assessment to be adjusted case by case,
- Elaborate an action plan according to the terms of references received from CGR for each application, to be used with CAI.

- Promote the networking in a multidisciplinary and inter-institutional coordination for the complementary development of skills allowing to focus on different aspects of the risk assessment.
- Ensure the right functioning of the network, systematically assembling the information and interchanging it among evaluators.
- Elaborate a final report advising CGR on the results obtained by ERB and CAI after analyzing the risk assessment received by the applicant and/or requesting additional experiments and additional scientific information to be reviewed during the process.
- Budget for performance of additional experiments, if required, and to manage the resources assigned by CGR to ERB for the evaluation. Provide information to the biosafety network and to the public consultation process.
- Ensure the guarantees of the right process.

The nine institutions that form CAI are: the Ministries of Health, Environment, Agriculture, and Education (Institute of Biological Research Clemente Estable, IIBCE), academia (UDELAR, public University), Technological Laboratory of Uruguay (LATU), National Institute of Agricultural Research (INIA), National Institute of Seeds (INASE), Institute Pasteur of Montevideo (IP). The delegates of the CAI have the responsibility to provide evaluators for the biosafety network according to the institutions' competencies and to maintain the flow of communication and information between ERB and evaluators. The relationship between ERB and CAI is preceptive (mandatory) but non-binding. Thus, ERB has to convene CAI, receive their opinions and transfer them to CGR in its final global report. But ERB's opinion does not necessarily have to agree with each institution of CAI. Also, ERB does not need to wait for CAI delegate's reports if they are not issued on time.

Regarding risk communication, there are two formal instances in place during the risk analysis process: "*information*" and "*public consultation*" (Figure 1.1) (Uruguay, 200). In Annex 1 of the application form is requested a summary of the submission that should not include confidential information. As soon as the application enters the system Annex 1 is made available on the web as a mechanism to "inform" the public about the events that entered the system and are being evaluated. In the "*information*" mechanism comments are not received.

The second mechanism, "*public consultation*", is implemented once CGR has performed a preliminary analysis of ERB's final report regarding food safety and/or environmental security. The tool used for the mechanism "*public consultation*" is "*to manifest*" to the public the final report of the risk assessment phase by making available at the web site the ERB-CAI's final report. The consultation is communicated to the public through publishing a press in two-high circulation national newspapers during two days of high newspaper demand. CGR assigns a period of time in which written comments are received by the biosafety office at MGAP via electronic or paper. The duration of the public consultation varies according to the proposed use. CGR has defined one week for applications requesting contained uses and two weeks for deregulation.

Given the structure of the risk assessment phase with a wide participation of related institutions in the field, it is expected that no comments are received from scientific experts but their opinions are channeled through the *Ad Hoc* groups and CAI to ERB during the analysis process. The role that the delegate of CAI plays is key to achieve that experts interested in the matter get involved in the risk analysis. Thus, the mechanism of public consultation is targeted to interested parties that do not participate in the risk assessment or management phases.

Case by case

Applications are analyzed on a *case-by-case* basis (Uruguay, 2008). *"Case"* in this context is defined by the: 1) event (crop-gene), 2) applicant and 3) proposed use. If any of these three changes, it is considered a different *"case"* and it requires its own specific administrative file and final GNBio resolution. Note, that transfer of a given approved transgenic event from one variety to another by conventional breeding is not subject to re-evaluation by the regulatory process. Nevertheless, during the risk analysis process there are general discussions regarding environmental risk assessment that move away from a strict *case-by-case* approach and consider categories of crop-trait combinations. For example, events in maize that confer herbicide tolerance, or events in maize that confer resistance to Lepidopteran insects are analyzed all together at the end of the process in the risk/benefit analysis.

An event is defined as an independent genotype derived from a transformation experiment with a specific/unique gene integration pattern in the genome according to: the number of copies, the location in the chromosomes and the level of integrity of the transferred sequence. An event refers to the unique recombinant DNA inserted in one plant cell, which was then used to generate entire transgenic plants. Every cell that successfully incorporates the gene of interest represents a unique "event". Every plant line derived from a transgenic event is considered a biotech crop. The Event Names correspond to the identifiers commonly used by regulatory authorities and international organizations, such as the Organization for Economic Cooperation and Development (OECD).

Different proposed uses are defined from contained and controlled use, to commercial release that means deregulation and can be used without biosafety conditions except if they are required due to the biology of the crop or special environmental conditions. The proposed uses considered in the Decree No. 353/008 are:

- "Contained" use, which include laboratory, growth chamber, greenhouse or any other physical structure that allow to "contain" the event in it.
- "Controlled" use, which include environmental release for:
    - field trials for research purposes
    - field trials for the National Register of Cultivars (NRC)
    - pre-commercial seed production or seed multiplication which includes counter season or winter seed production
- Deregulated use which includes release for production and commercial use for direct consumption or processing.
- Import of GE food, feed (grains) or industrial raw material.

For the proposed use under the category "contained" CGR has planned to request the formation of a biosafety committee at each institution or company that develops and/or uses GE plants. The biosafety committee will be responsible for controlling the use of the GE plant under biosafety conditions and will need to communicate to CGR each event that is being used sending a report with its information.

The proposed use under the category "controlled" refers to the release into the environment under biosafety conditions. The purpose of research field trials is to be able to do risk assessment especially in the case of recently developed or events currently under development. However under this category the applications submitted so far have been for events already deregulated in some countries to test efficacy and/or agronomic performance. Efficacy tests are not mandatory and it is the choice of the developer whether to apply for testing the event under the Uruguayan specific environmental conditions. The applications submitted under the current framework for research field trials with efficacy purposes are all from the same company (Table A2.1).

Field trials for NRC are field trials to evaluate agronomic characteristics of new cultivars to be included in the National Register of Cultivars. The category "evaluation for the National Register of Cultivars" is similar in area to an experimental field trial but it is done by an official organization, INASE. The Act No. 16811 determines that each new cultivar, GE or not, has to be evaluated by the official authority which will determine if it could be incorporated to the National Register of Cultivars (INASE, 1997, 2009). This is not a risk assessment, it is an agronomic evaluation to ensure that it is a new cultivar. Any cultivar needs to be included in this Register in order to be commercialized in Uruguay. INASE has standard protocols for the evaluation of agronomic traits for each crop. The field trials are performed by INASE at two locations for two years. The data obtained is published yearly in the web. This is a condition in order to commercialize the seed, whether GE or not, and whether or not the GE crop was deregulated. Recently INASE has allowed commercialising the seed after the first year of the two-year agronomic field trials (INASE, 2007). The category "evaluation for the National Register of Cultivars" allows anticipating this agronomic evaluation before the event is deregulated. Otherwise, a GE crop after being deregulated will require one additional year of agronomic performance to be included in the national register of cultivars. This category of use saves time on the one hand, but on the other it forces INASE to perform field trials under confined conditions for all cultivars with non-authorized events. This has required INASE an statistic redesign of the field trials in order to have together "non-yet authorized" events[1] in order to save money and being able to compare the data with new cultivars of the same crop with authorized events or non-transgenic. For example new corn hybrids with the event MON810 are required to be evaluated by INASE, but field trials do not need to be done under confined conditions

[1] "non-yet authorized" refers to events that have not yet being authorized for commercial use, only for the field trials performed by INASE.

because the event MON810 was deregulated in 2003 (Uruguay, 2003a). On the other hand, corn hybrids with any event that has not yet been deregulated are required to be evaluated under biosafety conditions until the event is deregulated.

There is another clarification necessary to include in this proposed use, related to the process for commercial release of a new event. That an event is authorized for NRC field trials does not necessarily mean that in one year it will be commercialized. In order to be commercialized the event needs the specific authorization of deregulation. As it is intended to allow agronomic evaluation of new cultivars with non-yet authorized events for commercial use, the applicant usually applies first for NRC field trials. But the applicant may or may not apply for deregulation. During the two years at NRC field trials the market could change and there may be no interest of the company to sell it or the breeder may discontinue the line. On the other hand, an event used in counter-season seed production may request deregulation in order to avoid seed production with biosafety conditions. As long as this event is only planted for counter-season seed production and not commercialised in Uruguay, it does not need to be in the National Register of Cultivars. Table 2.4 lists the events submitted for review under the current framework for the two categories, NRC and deregulation, or one of the two.

Table 2.4. Applications submitted between 2009 and 2011 by event for the two categories, National Register of Cultivars (NRC) and deregulation, or one of the two.

	Crop/Event	Field trial NRC (c)	Deregulation (e)
1	Corn GA21	√	√
2	Corn GA21XBT11	√	√
3	Corn TC1507	√	√
4	Corn NK603	√	√
5	Corn MON810XNK603	√	√
6	Corn GA21XMIR162XBT11	√	√
7	Corn MON89034XMON88017	√	√
8	Corn TC1507XNIK603	√	X
9	Soybean MON89788	√	X
10	Soybean MON89788XMON87701	√	√
11	Soybean A2704-12	X	√
12	Soybean A5547-127	X	√

Since the beginning of a biosafety framework in Uruguay, 16 events have been authorized for NRC field trials, from which 11 have also requested deregulation (eight are at the present deregulated and the other three are under evaluation) (Table A2.1). Regarding the five events that requested NRC field trials but not deregulation, it is known that three of them have been discontinued by the breeder (events in corn 176, T25 and CBH351) and for one event in soybean (MON89788) the applicant lost interest to commercialise it in Uruguay because there is a new stacked event that combines MON89788 with insect resistance (MON87701). The other event (TC1507XNK603 in corn) may be submitted for deregulation in the future; the application has not been pursued due to a business decision and not trade-related reasons.

The other use included in the category "controlled" is pre-commercial seed production or seed multiplication. A kind of seed multiplication that has been successfully initiated in Uruguay under the current regulatory system is counter seed or winter seed production. Under the previous frameworks in the 90's there were small areas of winter seed production of GE-corn for France and Italy authorized under biosafety conditions (Personal Communication, Bayce D, Manager of the Uruguayan Seed Chamber, 2011). Under the current regulatory system winter seed production was initiated with GE-soybean. The first year 1500 ha was planted, which was increased to about 2500 ha this past season. Winter seed production of soybean provides the grower 4.5 times greater income than grain production (Souto, 2010). This category necessitated the implementation of a system to monitor the compliance of the biosafety conditions established and the traceability of the seed from the time of import until it is exported or destroyed.

A problem within the "controlled" category has arisen from the fact that an upper limit in area for each of the proposed uses, especially seed multiplication, is not defined. Even though counter season seed production is done under biosafety controlled conditions, it

implies large areas. Environmental NGOs, the Ministry of Environment and a sector of the public university claim that counter season seed production should be considered as commercial release due to the big area. The risk analysis process is focused as a "field trial" but it is argued that it should be considered under the approach used for a deregulation. This is the case for Brazil that does not allow winter seed production unless with an event that has been deregulated.

The inclusion of counter production under the "controlled" category is a clear political decision related to economic and commercial opportunities. Winter seed production is a way to realize two cycles of the crop in one year thereby obtaining more seed in less time. When this production is required for the launch of a new event, there would not be time for a risk analysis of the type applied for a deregulation. Instead the risk analysis performed for "controlled" use applications is shorter since it does not include food safety analysis because the seed will not be used for human or animal consumption in the country. Besides, the authorization is issued season by season which allow more flexibility in case a risk was underestimated. The official view that underlies this category is that as long as there are enough human resources and infrastructure to monitor the compliance of the biosafety conditions, the area allowed to plant should depend on the estimated risk level according to the gene/trait/crop combination as well as the information and weight of evidence available at the time of the risk analysis.

Applications for counter season seed production could apply also for deregulation. Once deregulation is issued the expensive procedures to comply with the biosafety protocol and its control are not required. Except for the event MON89788, the rest of the events authorized for this category of use (MON89788XMON87701, A2704-12 and A5547-127) had later applied for deregulation. If the events are deregulated they will neither require applying for permission nor biosafety conditions for winter seed production. However, it will

be necessary to seriously consider the markets where Uruguay exports soybean grain to determine the necessity to maintain a traceability system in order to avoid either low level presence or adventitious presence above the import country's thresholds. At the moment Uruguay exports the majority of the soybean production to China and there has not yet been a problem of this kind (Souto, 2010).

Finally, the category of use "Import of GE food, feed (grains) or industrial raw material" refers to products of crops containing transgenic events that are imported to be consumed as food, feed (grains) or industrial raw material. It is established in the Decree as mandatory to apply for authorization for the import of food, feed and raw material for processing as food or feed for events not yet deregulated in the country. This category is important because there may not be an interest for all events to be cultivated in the country, but they can be imported as food or feed. This use can be authorized for consumption but not for planting. Germination capacity also can be eliminated, for example by drying the grain with a heat treatment or breaking it in half as a way to avoid its dissemination in the environment. Thus, it requires mainly food safety assessment while cultivation requires also a deep environmental assessment.

This category should contemplate consumption in its broad sense including human and animals. A split use approval for feed use but not for human consumption is not recommended due to infrastructure limitations and difficulties for assuring that "feed only" products remain exclusively in "feed only" marketing channels. This difficult situation was demonstrated in the US with the corn line StarLink™[2] (U.S. EPA, 2001; Uchtmann, 2002) which was approved for cultivation and feed use only but was found in the human food supply. Although there is no document evidence that the presence of StarLink™ in human

[2] OECD unique identifier: ACS-ZM004-3 (CBH-531), trade name StarLink. This corn contains a modified cry9C gene and bar gene conferring insect resistance and herbicide tolerance respectively.

food caused harm to any person, it had significant economic consequences (U.S. EPA, 2001). In this category of use that implies consumption as food and feed but not cultivation, it would be necessary to control if there is no deviation of use mainly from feed to cultivation. This control would imply a traceability system between the seed and grain importation and their commercialisation.

The possibility of authorizing an event for food and/or feed but not for cultivation implies a shorter assessment, and offers a solution to the potential problems resulting from asynchrony in the events approved by different countries in a region. This situation can lead to intentional or non-intentional illegal introduction from neighbour countries that already have approved the event for cultivation. However, there are no antecedents of these kinds of applications and at the same time there is not yet a control of the imports in this regard. In general it is the seed company, which has the interest[3] to have an event approved for the cultivation of their varieties/hybrid containing the event. An option while the control of imported events as food/feed is adjusted, is for the government itself (*"from the official sector")* to apply for food safety assessment of events that could be imported as food/feed. The goal would be to achieve approval for human and animal consumption. This could be coordinated with the regulatory authorities of the other countries of the region and with the companies that own the event and had performed the food safety analysis in order to obtain the necessary information.

Step by step

Figure 2.2 outlines the steps followed from 1 to 9 by an application for the RA process. (1) The application form (see Table A2.2 in the Appendix) is formal delivery to the

[3] For an event to be cultivated not only requires environmental studies but also the agreement of the owner of the event for its planting in relation to the intellectual property.

Biosafety Office at the Ministry of Agriculture. My assignments included drafting of the form to be in compliance with the new guidelines. As inputs I used the final document of a workshop organized by FAO which had the objective to develop regional consensual criteria for the basis of decision-making on biosafety. In the agenda was included the discussion of the criteria and parameters to be included in the application form. This workshop was part of a regional project regarding the development of technical tools to be used as reference in biosafety by regulators of the expanded MERCOSUR countries i.e. Argentina, Brazil, Paraguay, Uruguay, Chile, Ecuador and Bolivia (FAO, 2009b). I participated in this Workshop as one of the Uruguayan regulatory system representatives. I also reviewed other systems currently in place in the US, Canada, EU, New Zealand, Australia, Mexico and Japan. The draft was made available on the website for public consultation. Uruguay emphasized inclusion of the ecosystem for the risk assessment and not only the agro ecosystem. It also requires information and data specifically to evaluate the feasibility of coexistence, and includes a section in the form requesting information for a risk/benefit analysis which would contribute in part to socioeconomic consideration. Particular attention was put in each area of concern to request information and data needed by a regulator to characterize potential risks and to be able to formulate a hypothesis risk if additional tests are required.

As the system became operational, adjustments to the application form were necessary. For instance, adjustments were made to the required information for the different categories of uses and for the information required for each area of concern. The dynamic nature of the biotech crop sector determines adjustments to be a continuous process.

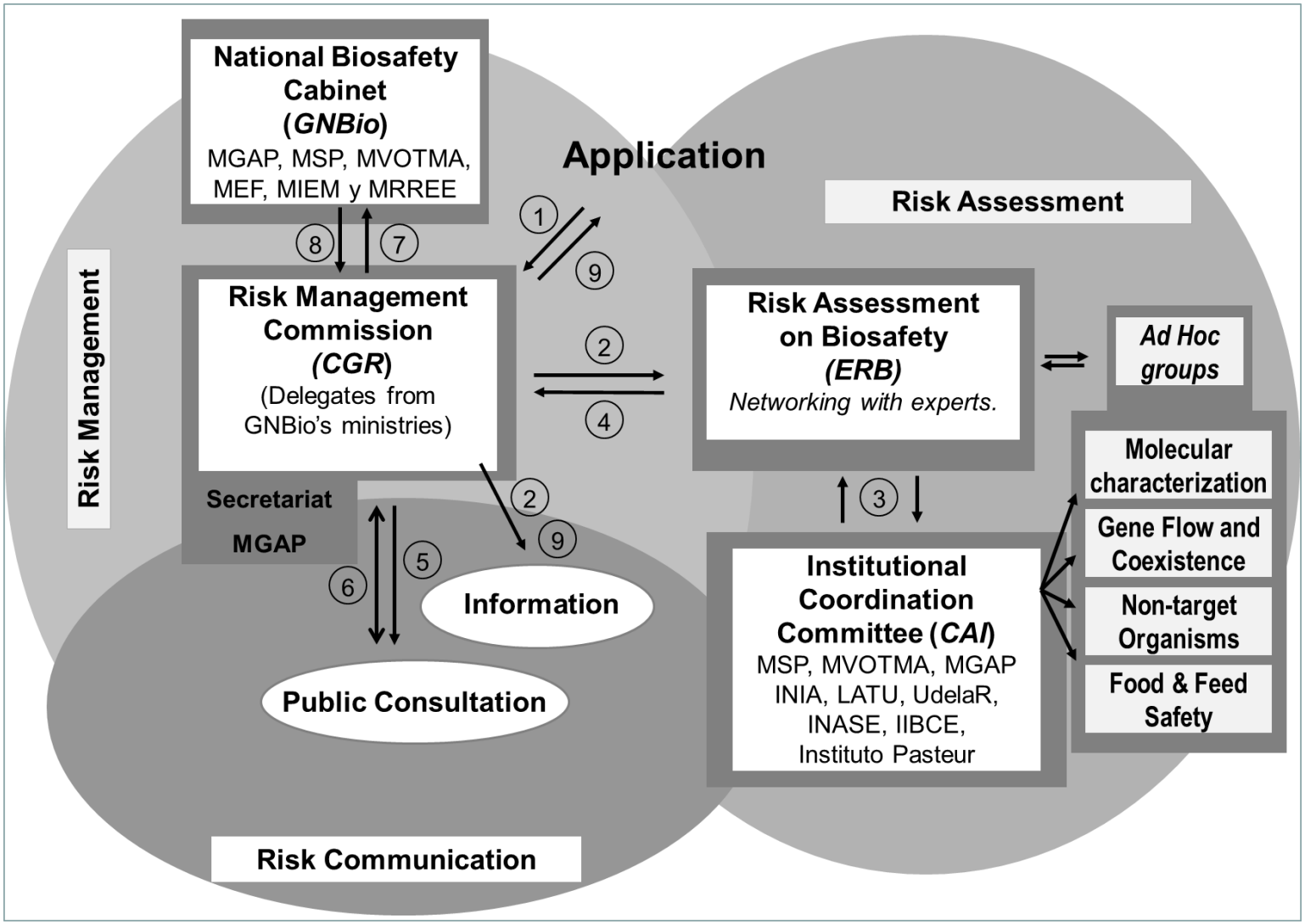

Figure 2.2. Diagram of the operating structure of the Uruguayan biosafety framework according to Decree No. 353/008 with steps followed by an application for the risk analysis process.

ERB does a first analysis of the application to summarize to CGR the information provided in section I of the form. Section I includes general information of the applicant, the event, the trait it confers, the mechanism of action of the gene, the proposed use and countries where the event has been already authorized (Uruguay, 2009).

(2) CGR defines the terms of references case by case for the risk assessment. The terms of references provide a guide for the analysis performed by evaluators. The evaluators are then responsible to perform a strict analysis of biosafety aspects directly related to the event under consideration. The terms of references also include any concerns that CGR feels are required for special emphasis in the study. For example in the case of events in corn, CGR called for special emphasis on the study of management practices and measures to ensure coexistence. In the case of winter seed production, based on the results or changes in area planted compared with a previous season, CGR may request the adjustment of specific conditions of the protocol that are required for technical recommendation from evaluators. In the terms of references, CGR also sets deadlines for CAI and ERB submission of final reports and deadlines for each process. The terms of references are communicated to ERB. At the same time CGR publishes on its homepage (http://www.mgap.gub.uy (enter at "Biosafety Cabinet") a summary of each application so that citizens know what is being analyzed in the system.

(3) ERB coordinates with the biosafety network through CAI the analysis of the risk assessment provided by the applicant in the dossier.

(4) ERB compiles the *Ad Hoc* groups and CAI reports in a global final report regarding food and environment safety that is submitted to CGR.

(5) CGR performs a first analysis taking into account the technical/scientific ERB/CAI report, a socio-economic report requested to the Office of Agricultural Planning and Policy (OPYPA) of MGAP and introduces other political and commerce factors.

(6) CGR implements a period for public consultation during which the ERB/CAI report is available on the Web and comments are received at the Biosafety Office. CGR analyses and responds to comments. Technical or scientific considerations are transferred to ERB for its analysis.

(7) CGR elaborates a recommendation report to GNBio taking into account the report by ERB/CAI on environmental security and food safety, the report by OPYPA on socio-economic aspects, the comments received during the public consultation period, as well as other factors that include, political, legal and commercial considerations, whichever is applicable.

(8) GNBio takes the final decision. If it is an application for commercial release, the Ministry of agriculture convenes the GNBio for the analysis of the events under consideration. Applications for uses under biosafety controlled conditions are analyzed without a face to face meeting.

(9) Once GNBio has taken a final decision, CGR communicates it to the applicant and to the civil society. The resolution is notified to the applicant and then it becomes available at the website for the civil society.

Iterative but independent

Risk analysis is performed in an iterative process that implies a permanent interaction between risk managers and risk assessors. ERB serves as a link between the two phases. On the other hand, while this interaction is necessary for a practical application of the risk analysis, it is essential to keep a functional separation of risk assessment and risk management to ensure the scientific integrity of the risk assessment analysis recommended by the Codex Alimentarius (2007). CGR has put forth a strong emphasis to avoid contaminating risk assessors with issues of risk managers and conversely. Members of CGR

can neither be members of CAI or *Ad Hoc* groups nor participate in the meetings of risk assessors. In the same way, ERB participates at CGR's meetings by the iterative condition of the process but ERB leaves the room when CGR discuses a final recommendation. This has been a practical mechanism applied by CGR to prevent confusion over the functions to be performed by risk assessors and risk managers reducing any conflict of interest.

Open, transparent and documented

An open, transparent and documented process is the baseline set forth by the risk analysis methodology. Everything is required by the risk analysis process to be documented fully in a transparent manner indicating different opinions with their respective justifications and how a final decision was reached. Information and reports are available to interested parties at the Biosafety Office, as well as the applicant. Legitimate justified confidentiality is strictly preserved. This criterion has to do with the members of the different commissions with regard to the independence of interests involved.

Risk Assessment phase

Strictly speaking evaluators do not perform a "risk assessment". The ideal situation to instill confidence by the public would be that governments individually or regionally perform the risk assessment experiments. However this is too expensive and this role has been transferred to the applicant, which usually is a large company with economic resources to face them. Applicants in turn, send much of their evaluations to standardized laboratories that are established as meeting requirements for good regulatory practices with referencing. Applicants also often make agreement with research institutes to perform field trials as a way to build confidence in the data generated. Thus, evaluators from the government analyze the information and data that the applicant submits. In the case of Uruguay, as a "technology

taker" country, evaluators also analyze data collected on the GE plant in the country of origin and/or other countries where the event was already authorized. A common confusion regarding the role of experts as regulators is to believe that tests and analyses provided by the applicant have to be repeated by the regulatory system. However, the function of the evaluator is, by using his expertise, to verify the results in order to validate or not the analyses.

The criteria discussed later in this section frame the analysis that evaluators perform on the risk assessment presented by the applicant. The risk assessment phase was organized to facilitate efficient use of human and infrastructure resources, and to prevent duplication of efforts while working in a multidisciplinary and inter-institutional scientific network.

ERB leads the risk assessment phase coordinating the analysis performed for each event with the biosafety network through CAI and *Ad Hoc* groups. The number of evaluators that comprise the biosafety network varies between 25 and 30. ERB has generated *Ad Hoc* groups to organize the biosafety networking. The *Ad Hoc* Groups are formed with evaluators from the institutions that form CAI. The aspects addressed by the *Ad Hoc* groups are 1) molecular characterization and identification of the event, 2) gene flow and coexistence, 3) non-target organisms and 4) human and animal health. Their acronyms in Spanish are respectively GAHCIM, GAHFG, GAHONOB and GAHSHA.

Functions undertaken by ERB include:

- Promote broad and active participation at CAI and *Ad Hoc* groups to avoid conflicts after a decision is taken
- Provide clear explanations to each institutional authority regarding the key function of the delegate at CAI, who must be qualified and have some authority.

- Promote an open environment for discussion at the *Ad Hoc* Groups where evaluators can offer their independent opinion. CAI can include in its report the institutional opinion.
- Elaborate consensus documents on specific topics by the *Ad Hoc* groups. in order to move forward and avoid wasting time disusing the same topic.
- Enforce deadlines for meetings and reports.
- Keep all discussion on a scientific basis.
- Ask for clear terms of references from CGR.
- Keep a transparent and documented interaction with CGR and CAI
- Explain clearly to the evaluator its function. As regulators do not perform the research, they need to verify information, determine if there are studies that need to be repeated locally due to a different receiving environments, or if new studies are required. The experimental design must be based on a risk hypothesis to aid the decision making process, not for the purpose of increasing scientific knowledge per se.

As mentioned before, some of the criteria applied to analyze the risk assessment, are principles of the risk analysis methodology itself, such as using a case-by-case, step-by-step and comparative approach based on the concepts of familiarity and substantial equivalence (Table 2.2). Other criteria are defined by CGR, such as the requirement to center the analysis on the event and not on issues related to the associated technology package (e.g., agronomic practices), or to require the analysis of stacked events, even though the individual events have been already deregulated, or that local efficacy studies are not a requirement. The next paragraphs expand on aspects of the operating structure of the Uruguayan biosafety framework according to Decree No. 353/008 relative to these criteria.

Scope

The analysis performed by ERB, CAI and *Ah Hoc* groups has clear boundaries set by CGR to focus on the event and not on issues related to the associated technology package. These issues refer to aspects such as no tillage technology, the impact on soil conservation due to monoculture of soybean, the impact of agrochemicals use, or generation of herbicide resistance. Specific divisions at the Ministry of Agriculture and Environment have responsibility for these aspects. Notwithstanding what is mentioned above, evaluators have recommended to CGR to create an additional *Ad Ho*c group on issues associated with the technology package.

Instead of a permanent *Ad Hoc* group, the recommendation was channeled by CGR through a pilot mechanism consisted of a meeting with the directors of the Natural Renewable Resources (RENARE) of MGAP (with responsibility on soil conservation and Good Agricultural Practices (GAP) plans), and the director of DGSA of MGAP (with responsibility on the registration and use of agrochemicals), with the President of GNBio (Minister of Agriculture who convenes the meeting), the president of CGR, and ERB's coordinator. The meeting holds at MGAP among RENARE, DGSA, GNBio and CGR presidents and ERB, was conducted as part of the risk analysis process of five events in corn that were recently deregulated with insect and herbicide resistance. The main focus was on the fact that an eventual deregulation of additional crops with glyphosate tolerance would necessarily determine an increase in the use of glyphosate, with the possible consequence of generating resistance. As a result it was determined, that in addition to recommendations for management practices for soil conservation that RENARE will include in its GPA policy, consideration of the appropriate crop rotations to avoid generation of resistance to the herbicides glyphosate and glufosinate ammonium. This mechanism must be formalized in the CGR statutes adding to be able to include directors from the Ministry of Environment as

appropriate. This meeting among authorities from MGAP and the regulatory system would be made when CGR considers it necessary, with the main objective to keep a fluid horizontal communication among the divisions of the Ministries involved in the matter to articulate and coordinate the agricultural policies.

Case by case

In the Decree 353/008 it is determined that the complex interplay of factors to consider determines the necessity to organize the assessment of potential impacts on a *case-by-case* basis according to the specific intended conditions of use. In this process "a case" is defined by: 1) the type of the plant (species/crop), 2) the gene, 3) the trait and 4) the environment where is going to be released, interacting as shown in Figure 2.3. While CGR can move away from a strict *case-by-case* approach and consider categories of crop-trait combinations, ERB, CAI and *Ad Hoc* groups require a strict *case-by-case* analysis for each defined area of concern.

For example, evaluators require, in the context of gene flow risk, verifying whether the reproductive biology of GE plants has been altered as a result of the introduction of a genetically engineered-derived trait compared to conventional corn. The review of the reproductive biology information provided by the applicant has to be done for each particular event separately, although the gene and the conferred trait under consideration could be the same, such as the case of MON810 and BT11 in corn or A2704-12 and A5547-127 in soybean. An event is an independent genotype derived from a transformation experiment with a specific/unique gene integration pattern in the genome. The situation is different for "*managers"* in that once "*evaluators*" have characterized on a *case-by-case* basis a non-significant risk related to gene flow, CGR can consider categories of crop-trait combinations

such as corn with insect resistance or soybean with herbicide tolerance, to define, for example, measures for feasibility of coexistence and for measures to avoid insect or herbicide resistance.

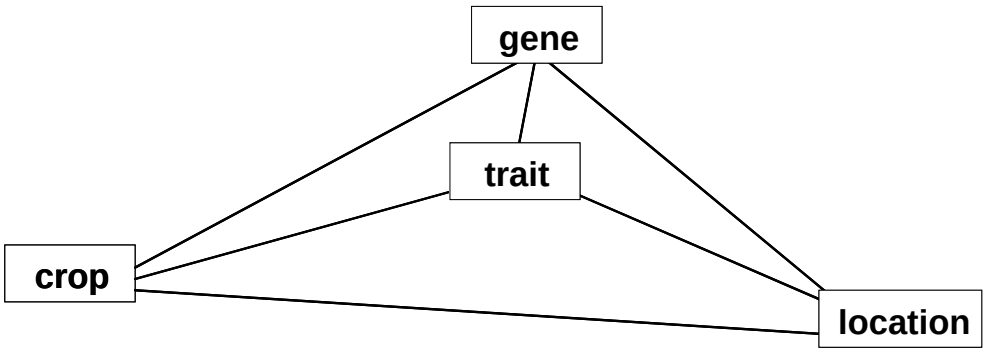

Figure 2.3. Environmental safety considerations for GE crops result from a complex interplay of factors that vary with crop, gene, trait, and location. (Figure from Grumet *et al*., 2011).

In the case of GE plants containing stacked events, i.e. where more than one event is introduced by conventional crossing of lines containing the single events, a risk assessment is required even though the individual events have been already deregulated. Possible effects of the combination on the biology and/or reproductive characteristics of the GE plant are specifically compared with the single events, following the European Union approach (EFSA 2007; 2011a). The analysis focuses on the stability of the stacked inserts and potential synergistic or antagonistic effects between the gene products of the stacked events that could raise safety concerns. The first stacked event submitted for permission in Uruguay was in 2003. Corn with the events MON810XNK603 was authorized for field trials (Table A2.1). Under the current regulatory framework, six out of nine applications of events in corn are stacked while one out of four in soybean are stacked (Table A2.1).

In addition to stacking traits by conventional crossing of two or more transgenic lines, genes can also be combined in the original transformation construct. These are single events but with two or more genes in the inserted cassette that confers different traits, such as insect resistance and herbicide tolerance. There are also cases of single events containing more than

one gene for the same trait (e.g., multiple insect resistance genes) transformed with a multiple-gene cassette. Three of nine events in corn reviewed by the current regulatory system have a single event with a multiple-gene cassette (TC1507, MON89034 and MON88017) (Table A2.1). In some cases, the gene that confers herbicide tolerance is also used as a marker gene. Interestingly two of these stacked events, BT11 and TC1507, were submitted to the previous regulatory system in 2003 and 2005, respectively (Table A2.1), but were treated as insect resistance single events (Uruguay, 2004b,c). Only now in their recent application, the applicant promotes the herbicide tolerance gene, which was indicated in the previous application as a marker gene, as a second trait because that herbicide is now registered in Uruguay.

Under the current regulatory system the more complex cases so far are the stacked events GA21XMIR162XBT11 and MON89034XMON88017. The former has three single events stacked by conventional breeding with a total of four genes (*mepsps X Vip3Aa20 X Cry1Ab, pat*) conferring two traits (resistance to certain insects of the same family conferred by two different genes (*Vip3Aa20* from MIR162 and *Cry1Ab* from BT11), and tolerance to two different herbicides conferred by the other two genes (*mepsps* from GA21 and *pat* from BT11). In the case of the event MON89034XMON88017, it has two single events stacked by conventional breeding with a total of four genes (*Cry1A.105, Cry2Ab2 X Cry3Bb1, cp4 epsps*) that confer two traits (resistance to certain insects, in this case from different families conferred by three different genes (*Cry1A.105* and *Cry2Ab2* from MON89034 for Lepidoptera and *Cry3Bb1* from MON88017 for Coleoptera), and tolerance to one herbicide conferred by the gene *cp4 epsps* from MON88017) (Table A2.1).

Step by step

The RA methodology proposes to follow a series of steps to be applied in risk assessment for each area of concern (Hill, 2005; Johnson *et al*., 2006; Craig *et al*., 2008; EFSA, 2010a) The application form requires information and data that is used by evaluators to follow the following sequence of steps.

7) Identification of potential adverse effects, which will depend on the specific combination of crop-gene-trait and receiving environment.
8) Estimation of the probability of occurrence of those adverse effects identified, which is related to the exposure level (e.g., field trial vs commercial release).
9) Evaluation of the consequences (impact assessment) if those adverse effects occur.
10) Characterization of the risk combining the probability of occurrence (2) by its consequences (3). Risk = probability X consequences
11) Consideration of possible adequate strategies for the management of the risk characterized, which could reduce the probability of occurrence and/or its consequences and to meet contingencies.
12) Estimation of the global impact including possible positive effects, namely risk/benefit balance.

Comparative analysis based on familiarity and substantial equivalence

The current regulatory system accepts the approach for the risk assessment that includes the use of the concepts of *familiarity* (FAO, 2009a) and *substantial equivalence* (FAO, 1996, 2000; Codex Alimentaruis, 2003). A comparative approach is employed, since substantial equivalence is determined by comparative analysis of the GE plant with its conventional counterpart, which has a long and well-established history of safe use, i.e. it is "familiar". One of the most important aspects of risk assessment is the identification of

intended and unintended differences and equivalences between the GE plant and its comparators. This comparison requires an appropriate comparator and robust baseline information to take into account the range of natural variation (Codex Alimentaruis, 2003; EFSA, 2011a).

The current regulatory framework has not yet defined a specific requirement for comparators. There is an ongoing discussion by ERB with evaluators regarding which lines should be the conventional counterpart for comparison due to the difficulty in obtaining non-GE lines with comparable genetic background. So far, the comparators are either isogenic lines in the case of vegetative propagated crops, or non-GE lines as close as possible genetically to the GE plant under assessment in the case of sexually propagated crops (EU Directive 2001/18/EC; EU Regulation (EC) No. 1829/2003). However, these comparators are not always available due to the increasing complexity of events when traits are stacked or those in which the trait implies significant compositional changes.

The current regulatory system needs to incorporate a flexible approach according to the situation. The GMO Panel of EFSA has recently published a guidance with options for the selection of comparators recognizing also a different requirement for comparators for the different aspects analyzed at the risk assessment phase (molecular characterization, food/feed safety and environmental security) (EFSA, 2011a).

It is also necessary to take into account the range of natural variation among current cultivars, and whether changes in the transgenic line fall within or outside that range (EFSA, 2011a). For this reason, non-GE commercial varieties are also included in the comparative assessment to provide a reference range for phenotypic and ecological characteristic values common to the crop under consideration.

Analysis guided by areas of concerns

Evaluators identify possible adverse effects (step 1) from general areas of concerns. The areas of concerns that Uruguay includes in the application form are aligned with the ones proposed by the US, EU, countries of the region (FAO, 2009b) and in general by all countries with a regulatory system in place. The areas of concern included in the Uruguayan application form are summarized in Table A2.2 located at the end of this chapter. The identification of the general areas of concern is discussed more thoroughly in chapter 4 of this thesis.

The major topic areas to be evaluated are molecular characterization, food and feed safety and environmental security, referred in the first column as "terms of references" like it is expressed in CGR's convocation. Until now food/feed safety has been evaluated only in applications for deregulation. In the short term it is expected to start being evaluated for events under the category of use "import of GE food, feed (grains) or industrial raw material" in which case food/feed safety would be the main assessment and environmental security would be included to be covered as a preventive measure in case of release into the environment due to deviation of use. In the other columns are shown the specific information needed for each topic area. The application form requests the information in a way to allow evaluators to characterize the risk and complete the steps mentioned above regarding each area of concern.

Molecular characterization includes evaluation of the genetic elements inserted, the products from those elements expressed in the GE plant and genetic stability of the insertion (Uruguay, 2009). The description of the inserted genetic elements is considered important because it provides the number of integration sites and number of copies of the gene as well as whether there also was insertion of portions of genes. The regulator looks for stable events preferentially with only one insertion site, one copy of the gene and without portions of genes

inserted in other parts of the genome or rearrangements inside the insert as well as absence of new open reading frames.

In the case these things happen, the regulator reviews molecular and bioinformatic analyses to confirm that additional copies or portion of the gene are not functional, or there is no transcription and translation from new ORF, or the protein expressed is not functional and does not have homology with allergenic or toxic molecules. If a scientifically justified concern (risk hypothesis) arises, more information or additional studies are requested to the applicant.

Food safety evaluation includes mainly history of use and familiarity of the GE plant, substantial equivalence of the GE plant compared with its conventional counterpart in terms of nutritional composition, digestibility of the GE protein, pathogenicity, allergenicity and toxicity in mammals, as well as carcinogenic and teratology studies if required (Uruguay, 2009). The criterion defined and being used by CGR is to base the decision-making regarding food safety on EFSA's risk assessment and risk analysis performed at the European Union (see as example GNBio resolution 27, Uruguay, 2011). The main constraint in this regard is the lack of experts with capabilities and training as evaluators to analyze the GE food safety assessment provided by the applicant. There are specialists on nutritional aspects but they do not have the training of what to look for as regulator. Specialists from the area of health in allergenicity and toxicology have clinical knowledge based on observation of the patient, while for risk analysis are needed specialists on the molecular biology aspects of the protein. Institutional support in this regard is a requisite before the criteria applied by CGR could change and for Uruguay to be able to complete the analysis of food safety information provided by the applicant.

Environmental assessment includes the analysis of the biology of the receiving organism, the resulting GE plant, and the receiving environment. The characterization of the

receiving organism (homolog) is important for the regulator to determine if there could be compatible wild relatives, if the crop species present features characteristic of invasive species or weed as well as to determine the feasibility of coexistence according to its reproductive biology (Hancock, 2003). While coexistence is not a biosafety issue, as explained later in this chapter, it could be included in the terms of references indicated by CGR to evaluators, in order to recommend management measures and production conditions to ensure coexistence. The characterization of the genetically engineered plant is important to determine if there are any changes in the biology of the plant as a consequence of the genetic modification compared with its homolog, that could allow the crop to be produced in new environments and if so, if the crop could become invasive or a weed and/or could cause a change in gene flow pattern that could affect coexistence conditions (Hancock, 2003).

Finally, the characterization of the receiving environment is important to determine whether there are compatible wild relatives in the region where the crop is going to be planted (Ellstrand, 2003; Hancock, 2003). However, the existence of compatible relatives does not mean that the event would not be authorized. The situation of corn in Mexico is a clear example in which although the characterization of the receiving organism determined that there are compatible relatives, the characterization of the receiving environment allowed to define areas free of compatible relatives where research field trials are being conducted (Ortíz, 2011). The characterization of the receiving environment is also important to determine if the gene/trait introduced could confer a selective advantage that could influence wild populations as well as if could occur non-target impacts of the new trait (Ellstrand and Hoffman, 1990; Snow and Palma, 1997; Hancock, 2003). In the characterization of the receiving environment is where the nuances of a more ecological vs agroecological approach are emphasized.

Hierarchical decision tree and Weight of evidence

Field-generated data, molecular characterization data, compositional analysis and ecotoxicological testings already generated are gathered in the dossier according to the Uruguayan form. Uruguay indicates areas of concerns that the applicant has to provide data in the form, but does not propose how to assess them. In this process, evaluators led by ERB, analyze *case-by-case* the events going *step-by-step*, following a hierarchical decision tree in which the areas of concerns discussed above are the starting point.

A weight-of-evidence approach (EFSA, 2010a,b, 2011b; Codex Alimentarius, 2009) is validated by the current regulatory system to support assessment conclusions. For each defined potential area of concern, evaluators perform a review of the dossier to analyze *how* the studies were performed by the applicant with respect to their statistical and study design, comparator, treatments and receiving environment in order to validate the results. Thus, it is important for the applicant to send much of their evaluations to be performed by standardized laboratories that meet good regulatory practices.

Additional studies performed locally

Additional studies and/or experiments could be asked of the applicant if the data are not valid and sufficient for the Uruguayan situation/condition. This could be the case for environmental areas of concerns if there are uncertainties with respect to a characterized risk that require locally field-generated data to answer them. This is usually not the case for human and animal health where molecular characterization and compositional studies are more standardized and natural variation is lower. The criteria to require additional studies in the context of risk assessment is to aid decision making, not to increase scientific knowledge per se (Hill and Sendashonga, 2003; Romeis *et al*, 2009). Additional studies need a risk hypothesis justifying the requirement. It is ERB's responsibility to orient the analysis not to completely understand a natural process but to help in the decision making process. The

baseline argumentation is used by evaluators to define the necessity of additional local studies is discussed below.

Persistence and invasiveness of the GM plant or its compatible relatives

The environmental areas of concern of *persistence and invasiveness of the GM plant or its compatible relatives* could raise uncertainty if there are in Uruguay compatible relatives that are not present in the environment where the studies were performed. If no compatible relatives are present and data from comparative analyses show no differences assuming an environment similar to the receiving environment, the probability of the event to increase the persistence and invasiveness of the GM plant itself in our environment will be low, and will not require additional local field studies. It is also possible to monitor this concern in a monitoring plan.

Interaction of the GE plant with non-target organisms.

In the case of *interaction of the GE plant with non-target organisms,* the species and relevant functional groups selected for this risk assessment *usually* are valid for our agro ecosystem, if not, additional experiments would be required. A higher chance is the requirement of new data for risk assessment of non-target organisms characteristic of our ecosystem/biodiversity.

A problem that is faced by Uruguay, is that it does not have the laboratory infrastructure that is required (Personal Communication Ing. Agr. PhD Castiglioni E. and Ing. Agr. PhD Scattoni B, Plant Protection Department, School of Agronomy -UDELAR, 2011) for a tiered framework approach, such as the one used to assess the environmental impact of conventional chemical plant protection products (García-Alonso *et al*., 2006; Rose, 2007; Romeis *et al*., 2008, 2011). The rationale behind the "early-tier" studies is to conduct experiments under worst-case exposure conditions increasing the likelihood of detecting

adverse effects on non-target organisms. Indicator species of non-target organisms are exposed to concentrations of the protein under evaluation in large excess of exposure that would be experienced in the field. If under this extreme conditions there is no adverse effects, the risk can be characterized as being acceptable, since in the field the non-target organisms would be exposed to much lower concentration of the toxin (Romeis *et al.*, 2010).

A possibility is to test the impact directly in field trials which is considered as "higher-tier" studies in the tiered framework discussed before that include semi-open field tests under controlled conditions (contained) and open field tests. Romeis *et al*., (2010) indicate that higher-tier tests demand higher skills and greater resources for their design, execution, and analysis. Results from open field tests may be more difficult to interpret in order to contribute with confidence in the conclusion of the risk assessment (Romeis *et al*., 2010). This discussion is just starting in Uruguay among entomologists. In the short term it is proposed to include possible indicator species and functional groups in a monitoring plan, while adjusting the methodology recommended by the regulatory system to the applicant in those cases where risk assessment is required for different species than the ones the applicant already has tested.

Efficacy studies

It has been recommended by Uruguayan evaluators that the regulatory system should require two seasons of efficacy studies (e.g., impact of the event on target organisms for insect resistance or herbicide tolerance traits) performed locally before its commercial release (Uruguay, 2009). Variation in environmental conditions could determine that although an insect species is present in the tested agro-ecosystem, it may not occur at a sufficient level to be economically important. On the contrary, an event may have low impact on a species because it has low density in the agro-ecosystem of one country, but may provide efficient control when the species is at high density in another agro-ecosystem.

This latter situation was the case of the event MON810 and BT11 in corn for Uruguay (Zerbino and Castiglioni, 2005-2010). Those events produce the Cry1Ab protein that controls *Diatraea saccharalis*. *D. saccaralis* was not as important a pest as were *Spodoptera frugiperda* and *Heliotis* for the Uruguayan conditions. Monitoring results shows that the Cry1Ac protein controlled efficiently *Spodoptera* and *Heliotis,* both species not originally indicated as the main target pests. In recent years drier conditions resulted in increased population density of *D. saccaralis,* the initial intended target species. Thus efficacy will depend on the prevailing pest in the receiving environment (Castiglioni and Zerbino, 2005-2009). If efficacy studies had performed, there would have been available suitable data in which to base the regulatory decision avoiding tension at the time of its authorization.

Efficacy studies do not answer biosafety uncertainties. Their purpose is to verify the impact of the event on the intended target organisms. The requirement of local efficacy studies is defined politically. In the case of Uruguay there is a political definition to promote this technology as long as there is "*an acceptable risk level"* and it solves a problem for the Uruguayan production system, environment, health and/or socioeconomic situation. This intention implies consideration of the efficacy of the regulated product. However efficacy studies are not mandatory under the Uruguayan regulatory system.

The rationale of the evaluator's recommendation to require efficacy testing is based on the prevention of the introduction of unimportant or inefficacious technologies to society (Falck-Zepeda and Zambrano, 2011). This would necessitate that efficacy studies be performed locally to verify three things: 1) that the declared target organisms are economically important pests in the agro-ecosystem of Uruguay, 2) that the event has a real effect on the declared target organism in the agro-ecosystem of Uruguay, and 3) if the event has impact on other target organisms not declared but with economic importance in the agro-ecosystem of Uruguay.

On the other hand, Uruguay does not require demonstration of improved agronomic qualities prior to introduction of new cultivars, whether they are transgenic or non-transgenic. This is in accordance with the World Trade Organization (WTO) directives, to which Uruguay is member since 1995. According to the policy trade, the only reasons to prevent the commercialization would be due to phytosanitary (WTO) reasons or environmental risk (Environmental Protection Law). This implies that the introduction of new cultivars/hybrids with a lower agronomic performance than presently used is not forbidden. The agronomic performance is publicly documented and it is available at the INASE website for new cultivars/hybrids of the majority of the crops (INASE website, 2013). The information and data published derive from the field trials for the National Register of Cultivars mentioned above. In the context of efficacy studies for transgenic crops, this would imply that a negative result would not be a condition to prevent its introduction if there is no risk for the environment and health.

In the case efficacy studies become required a third party such as academia, the National Agriculture Research Institute (INIA) or the National Institute of Seeds (INASE), is recommended to perform the efficacy studies. One possibility could be to adjust the statistic design of INASE's field trials for NRC in a way that includes efficacy data.

Even though efficacy studies are not included in the terms of references when CGR convenes ERB and CAI to analyze the risk assessment, evaluators have repeatedly recommended requiring their performance locally. CGR has channeled this recommendation through the inclusion of efficacy studies as one of the projects of the plan that CGR has of implementing biosafety research lines co-financed between CGR and the National Agency of Research and Innovation. The intention of CGR is to contribute to the trial costs with the fee it receives from applicants for the submissions. This proposition has been accepted by GNBio and is currently being negotiated with the research agency. Besides, applicants have

requested permission for field trials to determine agronomic performance in the receiving environment and efficacy studies. GNBio has authorized these field trials performed by INIA and a private company. The resolution issued requires including the comparator in order to obtain useful data for comparison analysis. Applicants have interest in agronomic and efficacy field trials to determine whether it is worthwhile to apply for deregulation and if so, to save time by providing local data.

Underlying criteria applied in the establishment of the Uruguayan biosafety framework

While the general criteria, concepts and issues discussed so far explain the operation of the Uruguayan biosafety framework and the risk analysis process, other underlying principles, some of them implied, though not directly expressed or written, were essential in the building process. Here I examine some of the factors that I believe, based on my observations and participation in the development process, were important for establishment of the current regulatory system. It is my opinion that the criteria discussed below allowed regulators to take advantage of the opportunity to amend the former framework where necessary and consolidate the current regulatory system contributing to the progressive evolution of the Uruguayan regulatory policy toward biotechnology.

The primary programmatic guideline of the Uruguayan government to construct the current regulatory framework was to start from integration, articulation and participation among the different stakeholders and institutions with capacities and responsibilities relevant to genetically engineered plants. The new structure is being built using already established and functioning institutions. Instead of creating new official bodies there has been a real effort being promoted by the authorities leading the implementation process, to use the institutions, agencies and offices, that already exist in an efficient way (Uruguay, 2007b). Under the Decree 037/007 GIM discussed within the institutional working group (GTI),

which of the institutions were needed and justified their participation in the system (Uruguay, 2007c).

At the risk management level, the Ministries of Agriculture, Environment, Economy and Health have a direct impact in the decision making process for the cultivation and consumption of GE plants and/or their parts. These four ministries were the ones that participated in the elaboration of the decree 353/008. The Ministry of Foreign relationships was included due to the international agreements for which Uruguay is a member. The Ministry of Industry was also included thinking forward to the inclusion in the future National Biosafety Law of all GE organisms apart from plants and possible development of Uruguay's own GE products. At the risk assessment level, the nine institutions involved in CAI were identified for having technical competence to assist in the analysis.

In the same way that integration and participation is sought, it is intended to avoid overlap of responsibilities. An example is the control of the biosafety conditions imposed by GNBio for seed multiplication. CGR is responsible for the monitoring and compliance control for which coordinates with INASE and DGSA of MGAP. In this coordination there is a permanent interagency effort of articulation and coordinated approach in order to avoid duplications and overlapping responsibilities among different authorities. DGSA is the authority at the points of entrance and exit of the seed because there is a DGSA's office at every possible port of entrance and exit of the seed either by air, sea or land.

A strong emphasis is being put forth by the president and members of CGR to assure harmonious interaction to submit coordinated work to the ministers they represent at the National Biosafety Cabinet (GNBio). The six ministries at the GNBio represent the involvement in the GE technology of agriculture, environment, health, economy, industry and foreign relationships. The goal is not to think individually regarding the competence of each ministry but globally for the interests of the country. A fluent communication has to be

maintained among the President of CGR with the rest of the delegates at the commission, and they in turn have a close relationship with the Minister that they represent. It is expected that the commission will reach a consolidated opinion including all considerations in a unique resolution.

The previous consideration of ensuring a good relationship among system members is connected to the necessity to have written, legally correct, procedures at each step of the process. Working groups are composed of members who can be frequently substituted. Clearly defined procedures will allow for a more durable regulatory system by providing continuity and reducing the possible influence of subjectivism. The objective is to define a regulatory system adjusted to the general programmatic objectives of Uruguay in such a way that *its operation* is independent of political parties.

Regarding the risk communication process, the objective is not a change in position but the mutual understanding between regulators and stakeholders (FAO, 2007). To achieve this, it is necessary to explain the reasons behind the decision making and not simply provide announcements from authorities or a description of acts. At the same time it is important that everyone understands the mechanism of public consultation, including the role of each part, and what public consultation does *not* imply. A common misconception is to believe that authorities will take the actions suggested in comments. But, "*risk managers*", which have the responsibility for the decision-making, will consider the opinions by analysing and reviewing the concepts implicit in the comments, decide whether or not they agree with suggestions received, and then decide which actions will be taken. Under the current regulatory system it is CGR that analyses the comments and responds, explaining the reasons for the decisions taken in each aspect.

The previous framework also had a mechanism of public consultation for risk communication. Apart from the tool of "*made manifest*" (documents are released for public

consultation on biosafety office homepage for a period of time in which stakeholders are invited to submit written comments), there was also used the tool of "*public hearing*" in which interested parties meet with regulators. However the previous framework experienced acts of physical and verbal aggression directed by the public to the applicant and regulators during the public hearing set for the risk analysis process of MON810 deregulation (Uruguay, 2003a). The "public hearing" process carried out was not successful partly due to the transition period the country was going through on this matter. But also, the public was not warned in advance that the purpose of the public hearing was to collect stakeholders' views on the matter, and not an instance for discussion, reproach or decision making. Under the current framework CGR has given special attention to the process of risk communication.

A feature of the Uruguayan risk communication process is that only the result of the risk assessment analysis (ERB-CAI's final report) is presented to the public requesting comments. CGR's report is confidential until GNBio's resolution is published; it is then included as part of GNBio's resolution. If both documents were presented to the public more focused comments were expected on specific aspects of the CGR's recommendation to the Ministers. The reason of this approach is not explicitly established. While holding CGR's recommendation report as confidential could appear as insecurity by the "*managers*", in so far that this approach has been consolidated, it is more efficient for CGR to have all the opinions for elaborating the final recommendation included in the CGR´s report to the Ministers.

With regard to transparency, this concept goes beyond the goal that everyone has access to information and is informed about the systematic process as it was done. Transparency also refers to a mechanism to reach a final decision without conflicts among stakeholders. In order to achieve an effective and efficient final resolution, transparency in this sense has to be applied from the beginning of the process. CGR promoted awareness and

was clear in its explanation to interested parties regarding the function and responsibilities of risk assessors and risk managers in the Uruguayan regulatory policy and specifically in the risk analysis process. CGR has been clear in explaining that "risk zero" does not exist and risk managers make the final decision assuming a risk level. ERB and CGR has taken special time to explain to interested parties about the procedures followed to reach each of the decisions taken along the process that led to the final recommendation to GNBio. To the degree practicable, CGR and ERB met both with companies and with NGO explaining factually the uncertainties and constraints as well as the premises and assumptions on which the assumed levels of uncertainty and risk were based.

Another strong guideline, set forth from the early stages of elaboration of Decree 353/008 by the authorities under decree 037/007, was to decide norms according to what is justified in aspects strictly pertaining to biosafety and what is possible to control. There has been a real effort to avoid unreasonable rules and ideal procedures only possible to comply with on paper. A clear example is the decision taken regarding labeling. Permission to use voluntary labeling indicating whether a product is GE or not was established in Decree 353/008 for those foods in which presence or absence of the relevant DNA or protein can be proven by analysis of the final product (Uruguay, 2008). The Working Group on labeling (GTE) under GIM, recommended voluntary labeling instead of mandatory labeling, and product based labeling instead of process based labeling, because it was determined that it would not be affordable by the government to comply with the control implied by mandatory labeling and even less for process based labeling (Uruguay, 2007d). Mandatory labeling requires controlling the whole row of products that are *not* GE to verify that they are not GE. Voluntary labeling puts the onus of controlling all products that are labeled either as "GE" product or as "not GE" product on the producer and marketer of the product.

The same criterion is applied when an event is approved subjected to conditions imposed by GNBio. CGR took the precaution to be sure that the system has the capacity to assure that the conditions are actually being met post-approval. In the case of counter season seed production, that is a new category of use in Uruguay and there was not certainty of its permanence. CGR had to define a legal framework for the control of the biosafety conditions to be imposed by GNBio. CGR used INASE's legal backing to control certified quality seed production in Uruguay to be the official inspection agency. INASE had already a traceability system in place for the seed from the time of import until its harvest and commercialization. CGR added to the traceability system the responsibility to ensure compliance of the Biosafety protocol. This explains the additional condition of certified quality seed production applied in counter season seed production category although customers do not require certified quality. By requiring certified quality, CGR found the legal way that INASE is the official inspection agency to oversee biosafety conditions. Additionally GNBio requires the applicant to present to CGR the reports of an external audit.

This last example is also applicable to the criteria discussed above regarding interagency efforts for a coordinated approach by integrating and articulating responsibilities. It is the objective to build a system with predictability so the applicant can organize its work. Due dates are established for each step by CGR and after completing the risk analysis process an answer is returned to the applicant in a reasonable timeframe. The definition of deadlines had a process of adjustments to ensure enough time for each step as appropriate. However, to achieve a predictable system CGR strictly requires that deadlines are met. This is possible in part by the fact that the relationship of dependency between ERB and CAI is prescriptive but not binding. This means that ERB has to convene and ask CAI's opinion (this is mandatory); however, ERB not only can have a different opinion from CAI, but also cannot wait for CAI's report if the deadline expired (ERB has its own deadline to meet).

At the risk assessment level, from the beginning of the formation of the *Ad Hoc* working groups that were composed with specialists from different institutions, ERB explained the importance of preserving the technical and scientific baseline for the discussion. Every opinion must be technically and/or scientifically justified in order to be taken into account by ERB with the final opinion that is elevated to CGR. This criterion is essential when defining additional information to request to the applicant as well as defining additional local studies. It is ERB's responsibility to elevate a report to CGR in those terms.

The specialists selected from each institution need to have the right skills and competencies. Where a gap of human resources exists, external specialists can be consulted by ERB. In each institution it is desired to establish the right structure and hierarchy by having a delegate at CAI that are expert in the topic and has a high position to be able to name experts for the *Ad Hoc* working groups.

The participation in the working groups is a non-paid activity. Thus it is important to institutionalize the task of risk assessment in the institution in order for the evaluators to find the time for it and be recognized for this work. To obtain a prioritization of this task at each institution is a current weakness of the system. If this is not obtained, there is no motivation for participation, no generation of time for this task, and it will lose relevance. The option to pay a fee for each dossier analyzed would be a motivation to increase participation. Another suggestion was to raise awareness at the National Agency of Research and Innovation (ANII) where scientists are voluntarily ranked at the National System of Researches (SNI) and receive a supplement of the salary according to the level reached. For being at SNI the participation at the risk assessment process would be a moral duty.

Soon after the entry into force of the decree 353/008, CGR decided to charge a fee for the risk analysis in order to obtain money to co-finance national research on biosafety. This adjustment involved changes in the original text that were approved in the Decree 535/008

(Uruguay, 2008b). The creation of research projects on biosafety of GE crops is being negotiated with the National Agency of Research and Innovation (ANII) for its implementation (Uruguay, 2011).

Issues of the current agenda

Approach for the regulation of coexistence

The recently defined policy expressed in Decree 353/008 promotes the coexistence between the production system that uses GE crops and those that do not use GE crops (conventional, organic, landraces) (Uruguay, 200). There is not yet a regulation with norms establishing specifically the nature of the relationship between the authority and growers to guarantee coexistence. CGR, however, which is responsible for the elaboration of a recommendation to GNBio in this respect, has been discussing a norm for coexistence in the context of the risk analysis of the events in corn recently deregulated (Table A2.1). The main ideas that are being discussed by technicians and politicians are discussed below.

It is important to clarify that "coexistence" is not a biosafety issue, but instead refers to the fact that different production systems (conventional, organic and transgenic) should be able to exist together at the same time. Coexistence should not be treated as if it were an environmental or health-related risk. The product itself, whether it be organic, conventional or transgenic, must be produced in correspondence with appropriate food safety requirements and environmental safety.

Environmental biosafety issues include the potential loss of biodiversity, the probability of the GE plant to become an invasive species or weed, the potential negative impact on other non-target organisms (Table A2.2). The existence of compatible relatives as part of the country's biodiversity and a GE plant mechanism for gene flow that determines transgenic introgression would be a biosafety issue assessed during the risk assessment phase.

According to the Uruguayan legislation, introgression would be considered a potential loss of biodiversity (Environmental Protection Act No. 17283, 2000) and therefore would be analyzed during the biosafety risk assessment phase. The feasibility of coexistence is a second, independent analysis, subjected to the finding of an acceptable environmental and food safety risk level.

Coexistence concerns agronomic, commercial and economic risks. It refers to the potential economic loss and impact of the admixture of non-GM with GM crops or the opposite. Thus, "regulated coexistence" refers to a regulatory system in which coexistence is guaranteed by implementing management measures and production conditions in order to avoid the unintended presence of GMO in conventional and/or organic crops by gene flow or admixtures. These measures are determined according to a list of agronomic, natural and crop-specific factors in the context of specific local, national and regional conditions (ECoB, 2010). Specific regulation for coexistence would avoid lawsuits and judges determining biosafety measures by themselves instead of baseing the decision on appropriate legislation.

Coexistence is a complex issue that has been discussed since the beginning of the Uruguayan regulatory policy passing from one commission to another without a specific policy. When the two events in corn, MON810 and BT11, were deregulated under the previous framework (Uruguay, 2003a, 2004b), the Ministry of Environment issued separate Resolutions for each event establishing conditions for their planting ("planting conditions" hereinafter) (Uruguay, 2003b, 2004c). Among those conditions was the requirement of 250 meters as isolation distance from other non-GE corn plantations that could be considered as a measure to ensure coexistence between different production systems. However, these resolutions were not justified in terms of coexistence but rather in terms of biosafety issues (Uruguay, 2003b, 2004c). Later on a draft of coexistence policy was elaborated by CERV

(Uruguay, 2005), but not enacted, as the commission stopped working by that time as explained in first section of this chapter.

Coexistence gained strength in the current regulatory system where it is explicitly stated in the second preliminary statement of the Decree 353/008 "*that is of* (political) *interest to promote a policy of coexistence between GE plants and unmodified ones*" (Uruguay, 200).

The discussion on coexistence restarted in the framework of the risk analysis of the five recently deregulated GE corn events (Table A2.1) (Uruguay, 2011a-e). Two of them are stacked events that include the single event MON810 or BT11, both deregulated under the previous framework (Uruguay 2003a, 2004b). A conflict was generated by the fact that MON810 and BT11 had additionally a specific resolution with conditions for their planting as explained above (Uruguay 2003b, 2004c). The arguments and procedures established in those conditions were put forward by CGR for analysis resulting in two Resolutions issued by GNBio (Uruguay, 2011 f, g) that modifies old resolutions in order to harmonize regulations. The arguments of the planting conditions and the present approach are discussed more thoroughly below.

The conditions set forth for planting MON810 and BT11 corn included: a) the requirement of 250 meters as isolation distance from other non-GE corn plantations; b) the planting of corn susceptible to lepidopteran insects in 10% of the area planted operating as refuge; c) affidavits of commercial transactions with the seed; d) affidavits of planting with information regarding area planted and a sketch of the location of the refuge (Uruguay 2003b, 2004c). The arguments provided for these conditions were based on biosafety issues generating confusion regarding the distinction between biosafety/biodiversity issues and agronomic issues such as coexistence and resistance management. The Ministry of Environment issued the resolutions supporting them on the General Environmental Protection

Act No. 17283 (Uruguay, 2000). This Act has a special provision for biosafety[4] establishing that the Ministry of Environment is competent *if no authority has been designated*, for the regulation of GMO or when *the introduction could be risky for the biological diversity or the environment*. The confusion arises from the fact that there was an authority designed for the regulation of GE plants, CERV (Uruguay, 2000); it has been debated that there is no significant risk for the biological diversity or the environment with the introduction of these events in corn (Uruguay, 2011).

The resolutions refer to the responsibility of the Ministry of Environment of "*conservation and sustainable use of biological diversity*". However, in the case of Uruguay the risk of cross-pollination between GE corn with compatible relatives is negligible since Uruguay is not the center of origin of maize and there are no compatible relatives (Burkarta, 1969; Rosengurtt *et al*., 1970; Zuloaga *et al*., 1994; Speroni, 2010). Landraces instead could be an issue in the framework of conservation of biological diversity due to their adaptation to the local environment and valuable genetic diversity. Local varieties were not mentioned in those resolutions and there has never been a norm to protect them neither from conventional hybrids nor from GE hybrids. Landraces will be discussed later in this section. On the other hand, if as a result of the environmental risk assessment a biodiversity risk is determined, isolation distances could be a measure to mitigate the risk. But in the absence of such risk, the isolation distance becomes a measure to address coexistence.

[4] Article 23 of the General Law of Environmental Protection Act No. 17283 gives powers to the Ministry of environment *"to apply the necessary measures to prevent and control environmental risks derived from the creation, handling, use or release of genetically engineered organisms as a result of biotechnology applications, as might affect the conservation and sustainable use of the biological diversity and the environment".* It also gives powers to the Ministry of environment to act in case of risks "*derived from these activities but related to human health, industrial and occupational safety, laboratory good practices and pharmaceutical and food use".*

The coexistence approach that is starting to be applied under the current regulatory system is based in the following baseline information: a) The majority of the agricultural production in soybean and corn is currently transgenic. It is estimated that 100% of the soybean and between 60-90% of the corn planted is transgenic (MGAP-OPYPA, 2011); b) There is no registration at the DGSA-MGAP database of certified organic producers; c) It is estimated about 3-5% of the production systems would require coexistence accommodations (MGAP- OPYPA, 2011); d) Since the first authorized event in corn for commercial release in 2003 there have not been complaints regarding contamination with GE corn; e) there are alternative/complementary measures to isolation distance to attend coexistence.

Based on the above statements, instead of requiring isolation distance as a coexistence measure to all growers that use GE corn, the current policy seeks to apply a *case-by-case* approach focusing on situations that justify the necessity of coexistence accommodation. It is expected to be less the requirement of official control in a *case-by-case* approach than if imposing general coexistence measures in all cases as planting conditions established. Under the current approach any request for regulation of coexistence has to be put into consideration by CGR which will determine *case-by-case* the conditions and management required.

By this, Uruguay is applying a subsidiarity-based approach on coexistence as Europe has (EU, 2009), but while in Europe the minority is transgenic production system, in Uruguay the minority is non-transgenic production system and so the control is limited to specific cases that demonstrates the necessity of isolation. Possible scenarios requesting regulation of coexistence include: commercial issues (organic or conventional producers that needs to certify their production as free of GE), biodiversity issues (growers that use landraces), political reasons (supported by the Land Law). Philosophic reasons (freedom to choose) will not be considered as no health, environmental, or economic risks are associated.

Landraces

Local varieties could be considered as part of the biological diversity of Uruguay due to their adaptation to the local environment and valuable genetic diversity. It is being debated in the Ad Hoc Group of gene flow and coexistence (GHAFG) whether the impact of GE crops must be evaluated apart from non-GE conventional corn varieties and hybrids as a biosafety issue of the environmental risk assessment, or either GE or non-GE commercial varieties must be considered together in the risk assessment and in the national protection system of plant genetic resources. The later should be the situation if the risk from GE crops on landraces were the same as would be from non-GE conventional corn hybrids (Bellon and Berthaud, 2004). The environmental risk assessment would define if the trait conferred by the transgene requires different, complementary or additional measures to the ones applied for conventional corn hybrids. On the other hand, it is the possibility that non-GE conventional corn hybrids and varieties would not cause a negative impact in the development of the evolution of a local variety (Bellon and Brush, 1994; Bellon *et al*., 2003; Louette *et al*., 1997; Perales *et al*., 2003). In this case the issue would be addressed from the regulatory system focused on the trait conferred by the transgene.

The information regarding landraces in Uruguay is scarce and partial. There are few records with location of local varieties with confirmed identity (Vidal, 2011). Lately the value of local varieties is being re-considered by the Uruguayan society. NGOs and specific family farmers are involved in promoting the conservation of landraces. But local varieties from other countries have also been introduced recently, generating confusion regarding their identity as landraces (Personal Communication, Ing. Agr. Vidal R., Assistant in Plant Breeding, School of Agronomy, 2011). The regulatory system recognizes the value of landraces as a genetic resource of Uruguay, but considers it is necessary to gather information from credible and reliable sources. While the baseline information is being generated to

determine if local varieties should have the same precautions with conventional corn hybrids than with GE corn hybrids, the current regulatory system has defined to consider landraces as another production system that need coexistence accommodation.

Adventitious presence

Adventitious presence for transgenic crops refers to the accidental, unintended, presence by gene flow or admixtures of low amounts of the product under consideration with an event approved in the country. *Low-level presence* refers to the special case of adventitious presence in which the event has not yet been authorized in the country (Codex Alimentarius, 2003; Demeke *et al*., 2006; Brookes, 2008; McCammon, 2010). Management measures and production conditions to ensure coexistence are correlated to adventitious presence threshold. Uruguay has just started the discussion of adventitious presence to define thresholds. In the case of grains, Uruguay would need to set threshold values if it had a mandatory labeling and/or if countries to which Uruguay exports grain define a threshold as a requirement for the import of grain. Uruguay should seek to harmonize thresholds at the regional level (Uruguay, 2010).

Prohibition of sweet corn

MVOTMA's resolution banning the use of sweet corn, even if it has deregulated events, is justified in accordance with Article 22 of the General Law of Environmental Protection Act No. 17283 referring to biodiversity. As mentioned above, under the Uruguayan environment the risk to biodiversity of the deregulated GE corn is negligible. However, the sweet corn resolution also indicates that the environmental authority (at that time) considered that the planting conditions for MON810 and BT11 of 250 m "*are not applicable to the technological and productive situation of the family farmers at the*

horticultural sector" characterized by small properties with plots planted adjacent one from each other (Uruguay, 2006).

This argument relates to coexistence but it has been debated that there are other measures to avoid contamination at the horticultural sector if plots are close that do not allow 250 m as absolute distance between crops. Such other measures include intermediate barriers with another crop (e.g. sorghum) or rows of the non-transgenic crop to trap pollen, as well as mismatch in the timing of planting or the use of hybrids with different crop cycle to avoid overlap in the pollination stage (EC, 2009).

The sweet corn ban was appealed by the Uruguayan Chamber of Seeds (CUS, 2006), but rescinded after receiving no answer from the Ministry of Environment. In case the resolution were repealed, under the current coexistence approach GE sweet corn could be produced and cases of non-GE sweet corn would be considered to require coexistence accommodation. In the present agenda of the regulatory system this topic is pending a specific revision of arguments and procedures.

Insect resistance management

Insect resistance management is another issue of the agenda linked to the planting conditions discussed above. The condition of planting a 10% of the area as refuge was included in the main resolution issued by MEF and MGAP for deregulation of MON810 and BT11 in the Insect Management Plan requirement. Repeating the requirement of a "refuge" by an environmental authority with the argument of conservation of biological diversity generated the misconception that in the "*refuge*" insects will be kept in an undisrupted ecosystem. However, the refuge area is controlled chemically against pests, theoretically except to the target insects (CUS, 2003). The refuge addresses the agronomic concern of insect resistance for which the main objective is to avoid generation of resistance to maintain

the technology. The benefit of the "*refuge*" on non-target insect population will depend on the target insect trophic chain.

Under the current framework it was discussed whether the control of the refuge compliance that is being done by INASE and MVOTMA, should be transferred to the stakeholder as is done in Brazil. However, at this time CGR has maintained the requirement for an Insect Management Plan that includes the refuge while considering analysis of the benefit that refuges provide for non-target species.

Monitoring/Surveillance

Another condition established for the planting of MON810 and BT11 corn (Uruguay 2003b, 2004c) was the necessity to monitoring after commercial release. This could have been a valid biosafety assumption at that time as these were relatively new events worldwide, and there was a lack of environmental data from plantings at a bigger scale than field trials. It was discussed under the current regulatory framework that to address the unknown impact on the environment at a larger scale should be applied a monitoring plan (Uruguay, 2011).

Distinction of Uruguay as "Natural Country"

The planting conditions discussed above also refer to the objective of maintaining Uruguay with the distinction as a *"Natural Country"*. The use of this distinction as an argument is not clear, since there is no legal definition of what is a *"Natural Country"*. This distinction was promoted in the context of beef production and exports related to the natural way beef production is developed in Uruguay. However, it does not say that animals cannot eat transgenic feed. It also makes no allusion to GE crops.

SUMMARY OF THE PROGRESSIVE EVOLUTION OF THE URUGUAYAN REGULATORY SYSTEM

Evolution of the basic components of a biosafety framework

In this section is summarized the evolution of the Uruguayan regulatory system regarding two aspects, the main structural components required for a biosafety framework and the dynamic respecting the number of application forms received, the categories of uses, crops, traits, genes and events analyzed.

According to the program of the United Nations for the building of National Biosafety Frameworks in the context of the Cartagena Protocol, there are five basic components required for the structure of a biosafety framework (Ortíz, 2011). These components include:

1) Politics on biotechnology security;

2) Legal support for a regulatory regime;

3) Operational system to handle applications;

4) Operational system for follow-up actions of monitoring, surveillance and inspection;

5) Public awareness and participation.

The components listed above have been addressed in this chapter from different points of view and will be used in this section to summarize where the Uruguay system stands and what is needed to continue moving forward.

Looking at the evolution of the Uruguayan regulatory system on GE plants, a clear consolidation has occurred regarding the first component listed, politics in support of biotechnology. Biotechnology is considered by the Planning and Budget Office of the Presidency as a key sector for the economy of the country (OPP, 2009). In addition to the creation of the National Biosafety Cabinet specifically for GE plants (Uruguay, 200), the Production Cabinet launched a Tripartite Sectoral Council in Bio and Nanotechnology as a tool for articulating and generating inputs for this area (Production Cabinet, 2010). This

Council has recently published the Biotechnology Sectoral Plan for the period 2011-2020 (Production Cabinet, 2011).

The second component listed above, legal support, is solidly based on Decree 353/008 and is slowly progressing to a law that covers not only plants but also GE animals and microorganisms. The main constraint in this regard is the lack of experts with capabilities and training as evaluators to analyze the GE food safety assessment provided by the applicant. A draft bill has been discussed by CGR parallel to the risk analysis process of the corn events that have been recently deregulated. Aware of the situation regarding capabilities for food safety assessment, the bill discussion process has proceeded at a laggard pace and is pending a solution to the food safety issue. A possibility is to extend the criterion being used by CGR for GE plants as explained earlier, by basing the decision-making regarding food safety, on EFSA's risk assessment and risk analysis performed at the European Union (Uruguay, 2011).

The third component, the operational system to handle applications, includes an administrative system which supports the risk analysis phases of risk assessment and management for decision-making and information handling. The Uruguayan operational system has been strengthened in terms of capacities involved in the decision making process since its origin in 1995 (Table 2.1). The main difference between the current regulatory system compared to the previous frameworks, is the greater participation both in the political commission of risk management and the risk assessment phase. In the period from 1995 to 2000 authorizations required the signature of 1 Minister (Agriculture), either for field trials or deregulation. In the second phase of the regulatory system from 2000 to 2007, the advisory body CERV issued permissions for contained use and the signature of two Ministers was required for deregulation (Agriculture and Economy). From 2008 to present the signature of six Ministers (Agriculture, Economy, Environment, Health, Industry and Foreign Affairs) is required for all uses except the "Contained" category that include any physical structure. In

the same way the advisory bodies increased in number of members, from 3 in the first period (1995-2000), to 5 members in the previous system to 16 (plus 25-30 evaluators at the Ad Hoc groups) in the present system in which there is a broad participation of institutions with competence in the subject.

This strengthening in capacities meant an increase in complexity regarding coordination. However the current regulatory system was instituted to allow broad participation and with a structure that includes public awareness, the fifth component of the list mentioned above. The higher complexity was needed in order to guarantee the maximum participation in the risk analysis phases to accomplish a decision making that is solid scientifically and considers all the concerns of interested parties.

Another important difference from previous frameworks is the clear separation established between the technical/scientific phase of the risk analysis from the risk management phase in which the discussion is at a technical/political level. This consideration and the above mentioned contribute to a transparent process and in turn facilitate non-controversial decision making.

On the other hand, signatures from the six GNBio ministers are necessary for all uses. A review of procedures for confined field trials is required. . Field trials for research and cultivar registration are small (less than one hectare), and confined with biosafety controlled conditions. For these cases would be desirable to enable CGR to issue the permits. In addition to this, it is necessary to adjust the application form to the different categories (i.e. confined field trial vs. commercial release) because with newer events, the applicant would likely be able to provide less information in the application form. In the cases where trials are performed to test efficacy or agronomic performance with events that have already been deregulated in other countries, the applicant will likely have the required risk assessment information. However, for new events under development, a key point of the confined field

trials is to obtain the information needed to perform a risk assessment. Regarding the fourth component, an operational system for follow-up actions of monitoring, surveillance and inspection, there have been actions for specific categories of uses on demand, such as counter season seed production and research field trials. These actions have been isolated but oriented toward an integrated and systematic scheme. The system is being based in INASE as the official agency for inspection and external audit. It is necessary to consolidate this operational system by formalizing in CGR the procedures and member's responsibilities in the monitoring of approved events and conditions imposed by GNBio.

As mentioned above the current regulatory system has a clear space for risk communication. This phase of the risk analysis is still under development and adjustments of procedures but there are already two systematic mechanisms in place, information and public consultation, as an inclusive action done from the government. Aspects for improvement include the mechanism by which it is reported the beginning of a public consultation period and the duration of the consultation. The instances generated in the current regulatory system between CGR-ERB and NGOs, helped to understand the answer to the comments received during the public consultation, previous to its publication on the web. Similar instances between CGR-ERB and applicants helped to understand information provided in the application form, additional information required, as well as decisions taken. These instances of face-to-face communication arose under the current framework as isolated and specific actions but would be desirable to incorporate the possibility of generating these instances when considered necessary into the risk analysis process in a systematic way. Additionally the framework needs to make the system known to citizens and reinforce risk communication with staff with the capacity to maintain an updated web site, to classify the comments received, to elaborate the response, and to clarify as needed when confusing issues are published in the national, regional and international media.

Analysis of applications received by the evolving Uruguayan regulatory system

The evolution of the regulatory system can also be observed with respect to the number of application forms received, the categories of uses, crops, traits, genes and events analyzed. These factors are summarized in Table A2.3 by the periods that the Uruguayan regulatory system went through since its origin in 1995 until present. The tendencies that stand out from the information provided in each column of Table A2.3 are discussed below.

In recent years there has been a decrease in the variability of plant species submitted for evaluation. Although the number of years in the two periods mentioned is not equal (12 vs 3), in the early stages of the Uruguayan framework there were applications with less traditional GE plant species, i.e. eucalyptus and rice submitted by the private sector , and white clover by the public sector. Under the current regulatory system the applications have been for the more common GE crops, corn and soybean. This tendency is also observed in the traits analyzed. In the previous frameworks both traditional and non-traditional traits, such as low lignin content and delayed senescence, were handled. It would be expected that as the new framework becomes more established that a greater variety of crops and traits will be submitted for review.

The decrease in the variability of plant species and traits submitted between 2005 and 2008 could be attributed to the transition period between different administrations and the 18-month moratorium that ended in 2008. The fact that the new government was from a different political party resulted in a change in the politics on biotechnology. Companies would not risk the investment without policy support and a biosafety framework. Also, the 18-month moratorium delayed the evaluation of events that were approved in other countries in the region. Thus, on one hand the significant change in the government led companies to take a cautious attitude toward the new regulatory system, resulting in the submission of

events with common crop-trait combinations that were already approved in neighbor countries.

However, in the number of stacked traits, the trend is in the opposite direction when comparing the previous periods with the current framework. It is observed an increase in the number of applications with stacked events from 3 applications to 20 applications submitted in a period of three years. This increase in the number of stacked events coincides with the global trend (ISAAA, 2011) of pyramiding genes in a same plant to confer more than one trait and/or to provide more than one mechanisms of action to avoid the generation of resistance.

With regard to insect resistance there has been an increase in the variability of genes used to confer the resistance, i.e., from 3 genes (*cry1Ab, cry9c* and *cry1Fa2*) used during the first ten years of the regulatory system, between 1995 and 2005, to 7 genes (*cry1Ab, cry1Fa2, cry1A105, cry1Ac, Vip3Ab20, cry2Ab2* and *cry3Bb1)* in two years period since 2009. This variability is not observed for herbicide tolerance trait in which the number of genes used remains constant at 3 (*epsps, bar* and *pat*).

Regarding the number of events, corn continues to be the crop with the largest number of applications per event. In the entire period of the regulatory system from 1995 to present there have been 16 events submitted in corn out of a total of 27 (59%), while in soybean there have been 6 events applied out of 27 (22%).

Respecting the proposed uses, the categories remained the same along the evolution of the regulatory system, although the current regulatory system has added the category of use "*counter season seed production*". The establishment of such production in Uruguay, which implies biosafety and traceability conditions applied in large areas and big volumes of seed, is a successful experience of the framework in regard to coordination and teamwork between the public and private sector.

At the moment soybean is the only crop that has been submitted for winter seed production although there is also interest in corn. The first applications are for soybean because it is easier to handle as it is primarily self-pollinating, and there are no compatible relatives or local varieties in the Uruguayan environment. Submission of "easier" events may assist implementation and consolidation.

A detail of the number of application forms entered by category of use since the beginning of the current regulatory system is shown in Figure 2.4. Counter season seed production is the only category that increased the number of applications per year. The number of applications for the other categories, either have decreased after a peak in the first year, such as field trials for cultivar registration (c) and deregulation (e), or had a peak in the second year of the system and then decreased as occurred with research field trials (b).

The fact that in the first year of the new framework there were no applications for the category of use research field trial (b), could be also attributed to the situation explained above, including a new administration and prior 18-month moratorium. The applications submitted to date do not include risk assessment tests, but only efficacy and agronomic performance trials for events in advanced development for whichrisk assessments data have already been generated. Efficacy tests under the Uruguayan environment are not mandatory and could be the reason there have not been applications for (b) category. The applications were submitted entirely by one company (Table A2.1).

The fact that there was no application submitted for cultivar registration (c) in 2011 could indicate that all available events with potential interest to be grown in Uruguay were already submitted.

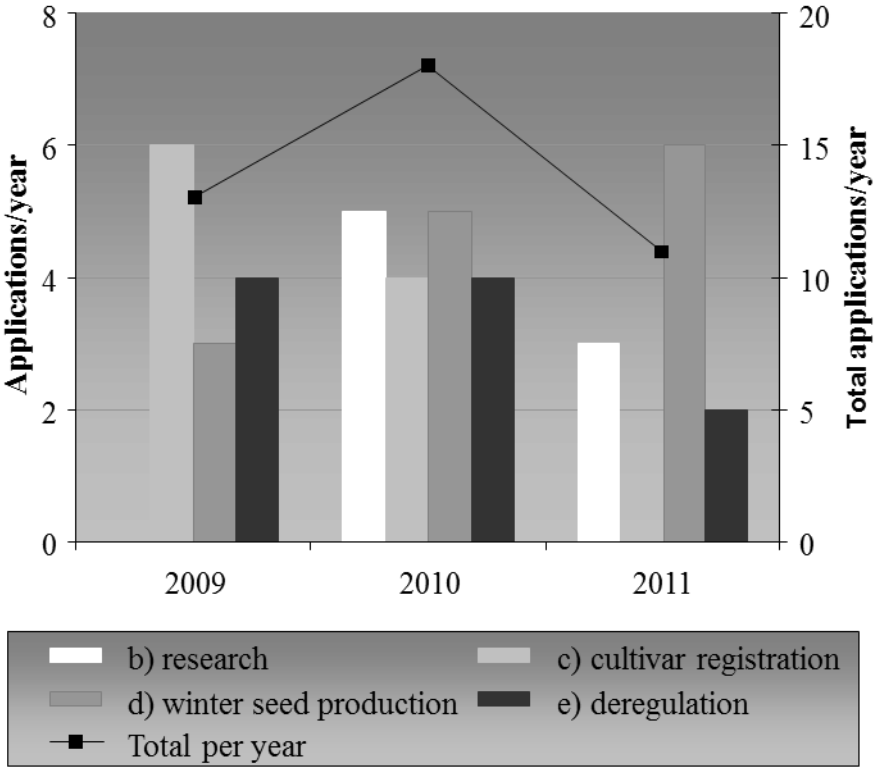

Figure 2.4. Number of application forms entered into the system by category of use between 2009 and august of 2011 (bars) and total number of applications per year (black line).

The dynamic observed in the total number of submissions per year, 13 applications in 2009, 18 applications in 2010 and 11 applications in 2011, is translated globally in a peak of application forms. This dynamic of submissions is also observed in previous periods of the regulatory system (Table A2.3) characterizing it by "waves" that went through the regulatory system since its origin. In Figure 2.5 are represented these "waves" of submissions. The curve in gray shows the actual number of applications entered into the system by year. The curve in black is the rolling-3 year average. The Uruguayan regulatory system has evolved over the past 17 years accompanied by fluctuations in the number of applications submitted.

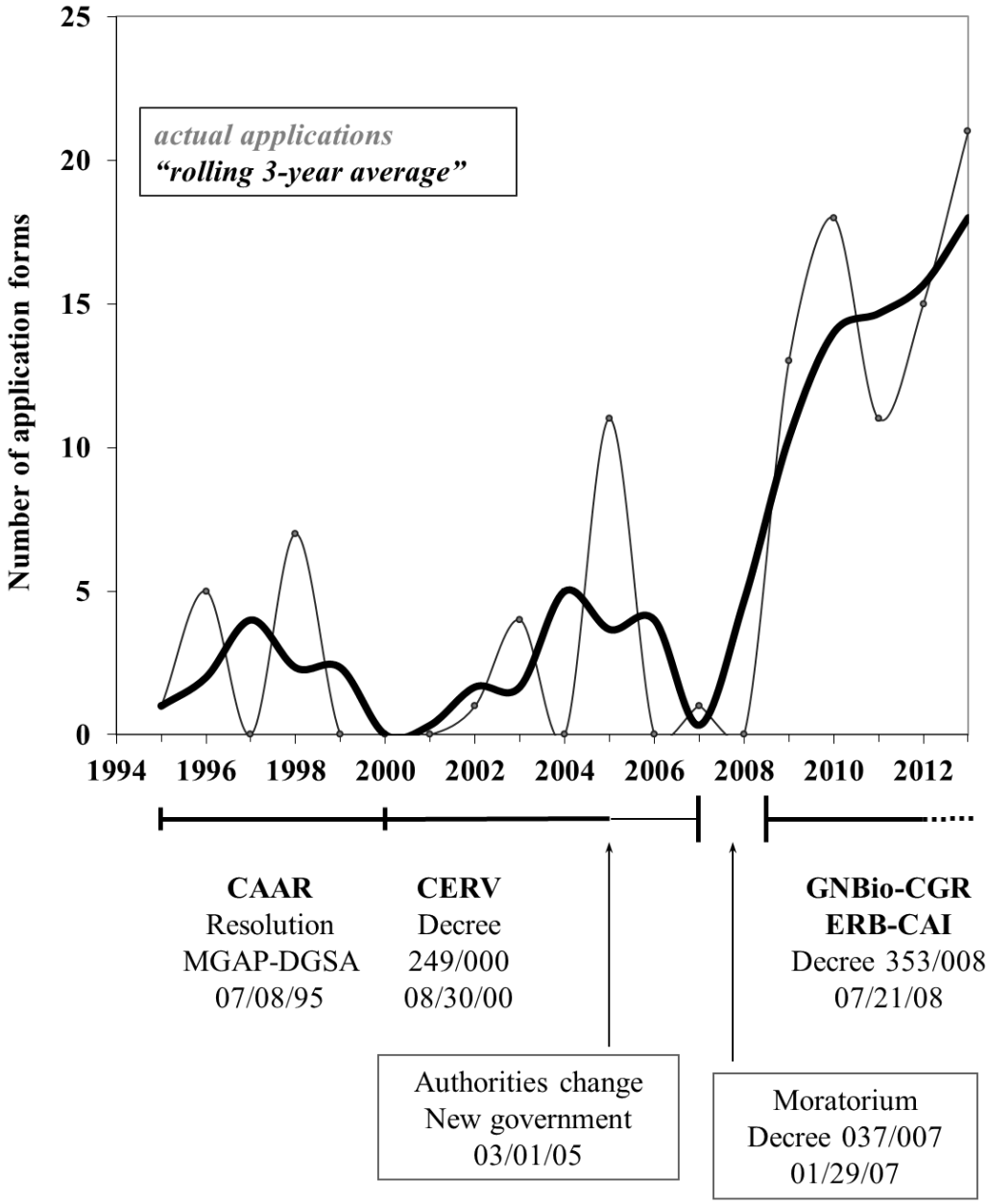

Figure 2.5. Scheme of the evolution of the number of application forms entered by year and by period of the regulatory system.

The first "wave" of events concentrated between 1996 and 1998. This tendency was followed by a period from 1999 to 2001 with no submissions (Tables 2.2 and 2.8). The reason for this variation could be the normal tendency in the flux of submissions that varies for different factors, including commercial competition, availability of new products, time required to meet official requirements previous to the commercialization of an approved

event, and efforts to facilitate regional coordination. That time period also included an adjustment in the regulatory system with the promulgation of the Decree 249/000 that created the advisory body CERV. Adjustments to the regulatory systems could imply a period of inactivity for the applicant to analyze the changes and reevaluate the interest in applying. However in the case of Decree 249/000 changes gave structure to the system and consolidation of procedures.

The second "wave" of applications, was concentrated between the years 2002 and 2005, (Tables A2.1 andA 2.3). This time the abrupt decrease in the number of applications resulted from election of a new government in 2005 that was from a different political party. There was a complete change in the members of the review committee. Applications that were submitted in 2005 either were not completed or were not forwarded to enter the risk analysis process. Then the Decree 037/007 of the 18-month moratorium formalized the inactivity of the regulatory system.

Since current regulatory system entered into force in July 2008 under Decree 353/008, once again there has been an increase in applications (Figure 2.4), reaching the highest number since its origin. In the first five years of the regulatory system, between 1995 and 2000, there were a total of 13 applications (2.6 applications per year). In the following seven years (2000-2007) there were a total of 17 applications (2.4 applications per year) and under the current regulatory system covering only three years (2008-present), there has been a total of 46 applications (15.3 applications per year) (Table A2.3).

Summary and future directions

In summary, the current regulatory system needs to continue implementing the legal aspects of the framework, moving from a decree for GE plants to a law for GE organisms including animals, microorganisms and vaccines. The framework needs to further consolidate its operational structure for risk analysis and post-release surveillance. In this regard it is necessary to keep working toward a systematic and methodical administration with order and planning in a systemic way considering the entire system's components. It is necessary to strengthen the technology applied and capabilities for the risk assessment phase in order to handle the greater number of events and higher complexity in the combination of genes/traits-crops-environments that are expected in the near future.

From a longer term perspective, Uruguay may also generate national transgenic products. The Biotechnology Sectoral Plan for the period 2011-2020 recently published, provides criteria on the promotion of the inclusion of biotechnology, including strengthening of the academia-industry bridge, support for public-private partnerships, and identifications of niches and emerging markets for Uruguayan bio-business. This will require field trials for risk assessment, which design and parameters evaluated (chapter 4 of this thesis) would be defined from the regulatory system in agreement with the applicant.

Finally, whether from abroad or in country, an increase in the number of applications is expected. The trend displayed in Figure 2.5 for the overall evolution of the regulatory system with regard to the number of applications submitted to the system, may be attributed to a combination of the normal dynamic of submissions with the changes and adjustments that the framework underwent since its origin. As to the third wave observed under the current regulatory system, it may be caused by a normal dynamic of submissions. A well-designed and implemented biosafety framework is necessary to guarantee quality, consistency, clarity and transparency for the success of the decision-making process.

APPENDIX

APPENDIX

Table A2.1. Summary of applications from the period of 1995-2011 by crop, event, trait, gene, year of application and authorizing agency. Event name between brackets corresponds to the OECD ID.

Crop	Event	Trait/Gene(s)	Use	Year of application/ authorization		Authorizing agency
Corn	Unspecified in record available	Unspecified in record available[1]	b)	---	1995	DGSA (Uruguay, 1995a)
Corn	Unspecified in record available	Unspecified in record available	b)	---	1996	DGSA (Uruguay, 1996e)
Corn	176 (SYN-EV176-9)	Insect resistance (lepidopteran) and herbicide tolerance (glufosinate ammonium)/ *Cry1Ab,bar*	c)	---	1998	DGSA (Uruguay, 1998)
			b),d)[2]	---	1998	DGSA (Uruguay, 1998)
Corn	T25/Liberty Link™ (ACS-ZM003-2)	Herbicide tolerance (glufosinate ammonium)/ *pat*	b), **c**), d)	---	1998	DGSA (Uruguay, 1998d)
Corn	CBH351/Starlink™ ACS-ZM004-3	Insect resistance (lepidopteran) and herbicide tolerance (glufosinate ammonium)/ *Cry9C, bar*	b), **c**), d)	---	1998	DGSA (Uruguay, 1998)
Corn	GA21/Roundup Ready™ (MON-00021-9)	Herbicide tolerance (glyphosate)/ *mepsps*	b), **c**), d)	---	1998	DGSA (Uruguay, 1998e)
			c)	2009	2009	GNBio (Uruguay, 2009)
			e)	2009	2011	GNBio (Uruguay, 2011)

Table A2.1 (cont'd).

Corn	MON810 (MON-00810-6)	Insect resistance (lepidopteran)/ *Cry1Ab*	a)	---	1998	DGSA (Uruguay, 1998b)
			b), **c**), d)	---	1998	DGSA (Uruguay, 1998c)
			e)	2002	2003	MEF-MGAP (Uruguay, 2003a) MVOTMA (Uruguay, 2003b)
			e)	2011		Harmonization with resolutions for new events in corn - GNBio (Uruguay, 2011f)
Corn	BT11 (SYN-BT011-1)	Insect resistance (lepidopteran) and herbicide tolerance (glufosinate ammonium)/ *Cry1Ab, pat*	c)	---	---	---
			e)	---	2004	MEF-MGAP (Uruguay, 2004b) MVOTMA (Uruguay, 2004c)
			e)	2011		Harmonization with resolutions for new events in corn - GNBio (Uruguay, 2011g)
Corn	NK603/Roundup Ready® (MON-00603-6)	Herbicide tolerance (glyphosate)/ CP4 *epsps*	b)	2003	2004	DGSA (Uruguay, 2004a)
			b)	---	2005	DGSA (Uruguay,2005b)
			c)	2005	---	*Application rescinded, File MGAP 2005/7/4/1/1055 of 06/10/05.*
			b)	2010	2010	GNBio (Uruguay, 2010)
			c)	2009	2009	GNBio (Uruguay, 2009)
			e)	2010	2011	GNBio (Uruguay, 2011d)

Table A2.1 (cont'd).

Corn	MON810XNK603/ Roundup Ready® YieldGard (MON-00810-6X MON-00603-6)	Insect resistance (lepidopteran) X herbicide tolerance (glyphosate)/*Cry1Ab* X *cp4 epsps*	b)	2003	2004	DGSA (Uruguay, 2004d)
			b)	---	2005	DGSA (Uruguay, 2005c)
			c)	2005	---	*Application rescinded, File MGAP 2005/7/4/1/1056 of 06/10/05.*
			b)	2010	2010	GNBio (Uruguay, 2010)
			c)	2010	2010	GNBio (Uruguay, 2010)
			e)	2010	2011	GNBio (Uruguay, 2011e)
Corn	GA21XBT11 (MON00021-9X SYN-BT011-1)	Herbicide tolerance (glyphosate) X Insect resistance (lepidopteran) and herbicide tolerance (glufosinate ammonium)/ *mepsps X Cry1Ab, pat*/	c)	2009	2009	GNBio (Uruguay, 2009)
			e)	2009	2011	GNBio (Uruguay, 2011b)
Corn	TC1507 (DAS-01507-1)	Insect resistance (lepidopteran) and herbicide tolerance (glufosinate ammonium)/ *Cry1Fa2, pat*	c)	2005	---	*Application rescinded, File MGAP 2005/7/4/1/0575 of 04/04/05.*
			e)	2005	---	*Application rescinded, File MGAP 2005/7/4/1/0575 of 04/04/05.*
			c)	2009	2009	GNBio (Uruguay, 2009)
			e)	2009	2011	GNBio (Uruguay, 2011a)
Corn	TC1507XNK603 (DAS-01507-1 X MON-00603-6)	Insect resistance (lepidopteran) and herbicide tolerance (glufosinate ammonium) X Herbicide tolerance (glyphosate) / *Cry1Fa2, pat X cp4 epsps*	c)	2009	2009	GNBio (Uruguay, 2009)

Table A2.1 (cont'd).

Corn	GA21XMIR162XBT11 (MON00021-9X SYN-IR162-4X SYN-BT011-1)	Herbicide tolerance (glyphosate) X Insect resistance (lepidopteran) X Insect resistance (lepidopteran) and herbicide tolerance (glufosinate ammonium)/ *mepsps X Vip3Aa20 X Cry1Ab, pat*	c)	2009	2009	GNBio (Uruguay, 2010)
			e)	2009	---	*Under analysis*
Corn	MON89034XMON88017 (MON89034-3X MON88017-3)	Insect resistance (lepidopteran) X Insect resistance (coleopterans) and Herbicide tolerance (glyphosate)/*Cry1A.105, Cry2Ab2 X Cry3Bb1, cp4 epsps*	b)	2010	2010	GNBio (Uruguay, 2010)
			c)	2010	2010	GNBio (Uruguay, 2010)
			e)	2010	---	*Under analysis*
			b)	2011	---	*Under analysis*
Corn	MON89034XNK603 (MON89034-3X MON-00603-6)	Insect resistance (lepidopteran) X Herbicide tolerance (glyphosate)/*Cry1A.105, Cry2Ab2 X cp4 epsps*	b)	2011	---	*Under analysis*
Soybean	GTS 40-3-2/ (Roundup Ready®) (MON-04032-6)	Herbicide tolerance (glyphosate)/ *cp4 epsps*	c)	---	1996	DGSA (Uruguay, 1996b)
			e)	---	1996	DGSA (Uruguay, 1996d)
Soybean	FG74 (CPU) (none OECD ID)	Herbicide tolerance (isoxaflutole and glyphosate)/*hppd, epsps*	b)	2005	---	*Application rescinded, File MGAP 2005/7/4/1/2153 of 10/31/05*

Table A2.1 (cont'd).

Soybean	MON89788 (MON-89788-1)	Herbicide tolerance (glyphosate)/ *cp4 epsps*	d)	2009	2009	GNBio (Uruguay, 2009a)
			(d)	2009	2009	GNBio (Uruguay, 2009b)
			(d)	2009	2009	GNBio (Uruguay, 2009c)
			(d)	2010	2010	GNBio (Uruguay, 2010a)
			(d)	2010	2010	GNBio (Uruguay, 2010b)
			(d)	2010	2010	GNBio (Uruguay, 2010c)
			b)	2010	2010	GNBio (Uruguay, 2010)
			c)	2010	2010	GNBio (Uruguay, 2010)
			(d)	2011	---	*Under analysis*
			(d)	2011	---	*Under analysis*
			(d)	2011	---	*Under analysis*
Soybean	MON89788XMON87701 (MON-89788-1 XMON87701-2)	Herbicide tolerance (glyphosate) X Insect resistance (lepidopteran)/ *cp4 epsps X Cry1Ac*	b)	2010	2010	GNBio (Uruguay, 2010)
			c)	2010	2010	GNBio (Uruguay, 2010)
			d)	2010	2010	GNBio (Uruguay, 2010)
			(d)	2010	2010	GNBio (Uruguay, 2010)
			b)	2011	---	*Under analysis*
			(d)	2011	---	*Under analysis*
			e)	2011	---	*Under analysis*

Table A2.1 (cont'd).

Soybean	A2704-12 (ACS-GM005-3)	Herbicide tolerance (glufosinate ammonium)/*pat*	d)	2009	2009	GNBio (Uruguay, 2009)
			(d)	2009	2009	GNBio (Uruguay, 2009)
			(d)	2010	2010	GNBio (Uruguay, 2010)
			e)	2010	---	*Under analysis*
			(d)	2011	---	*Under analysis*
Soybean	A5547-127 (ACS-GM006-4)	Herbicide tolerance (glufosinate ammonium)/*pat*	d)	2011	---	*Under analysis*
			(d)	2011	---	*Under analysis*
			e)	2011	---	*Under analysis*
Eucalyptus	CP4 (none OECD ID)	herbicide tolerance (glufosinate ammonium)/ *cp4 epsps*	b)	---	1996	DGSA (Uruguay, 1996c)
Eucalyptus	11/25 (none OECD ID)	Low lignin content/ ---	b)	---	1996	DGSA (Uruguay, 1996f)
Rice	LLRice06 (ACS-OS001-4)	Herbicide tolerance (glufosinate ammonium)/ *(bar)*	b)			*Authorized but trials not done.*
Rice	LLRice62 (ACS-OS002-5)	Herbicide tolerance (glufosinate ammonium)/ *(bar)*	c)	2005	---	*Application rescinded, File MGAP 2005/7/4/1/0565 of 04/01/05*
			e)	2005	---	*Application rescinded, File MGAP 2005/7/4/1/0565 of 04/01/05*

Table A2.1 (cont'd).

White clover	ATMYB32-IPT (none OECD ID)	Delayed flowering and delayed foliar and peduncle senescence by increased cytokinins biosynthesis/ *(ipt)* (Capdevielle and García, 2005)	b)	---	2005	DGSA (Uruguay, 2005a)
			b)	---	2007	DGSA (Uruguay, 2007)

Categories of uses:	Color code according to the framework period:	
a) Contained experiment. b) Field trial for: research, agronomic assessment, demonstration for propaganda. c) Field trial for National Register of Cultivars. d) Counter season seed multiplication under biosafety controlled conditions – authorization for the use of the event is issued one time, the owner company applies; (d) authorization for seed production is issued each season, local companies apply. e) Deregulation (commercial planting, food and feed)	1995-2000	
	2000-2007	
	2008- present	

1. The first two authorizations in 1995 and 1996 do not specified the event. Probably these two unspecified resolutions corresponds to any of the first events submitted, 176, T25, or CBH351 (Bayce D, Manager of the Uruguayan Seed Chamber, 2011 Personal Communication).
2. In the cases where the resolution was issued for more than one use, it is indicated in bold the use performed.

Table A2.2. Specific information required in the Uruguayan application form for the areas of concerns of molecular characterization, food safety and environmental security (Parts B, C and D of the form). The numbering corresponds to the form. Missing numbering correspond to information requested for the evaluation of other aspects different from the areas of concerns.

MOLECULAR CHARACTERIZATION (PART B)
Specific information required by area of concern
Description of the genetic elements inserted in the GE plant
B2 Molecular biology of the *donor-vector-recipient* system. A detailed description including: … B2.4 Final construction map (cassette) with the restriction sites that allow to reproduce the determination of the number of copies inserted. B2.5 Description of all the elements included in the cassette/s used for the transformation, including for each element: its position in the vector and in the insert, size (pb), donor organism, whether it was modified, function (in the donor and receiving organisms), probe and/or its sequence. B3 Detailed description of the genetic elements inserted in the receiving organisms (GE plant), indicating: B3.1 Subcelular location of the event B3.2 Molecular analysis of the insertion (event characterization) including: B3.2.1 number of integration sites B3.2.2 number of copies of each gene B3.2.3 whether occurred insertion of portions of genes. B3.3 Genome sequences flanking the insert (event) B3.4 Description of all the elements inserted in the GE plant genome, including for each element: size (pb), function, whether it is a sequence transcribed but not translated in the GE plant, molecular mechanism of its expression, whether the element confers a non required function for the expected phenotype. B3.5 To indicate the regions of the vector that have been incorporated in GE plant. B3.6 Presence/absence of fragments of the insert in regions of the GE plant genome outside the region of the functional insertion B3.7 Capacity of the construction to transfer genes by transposition, recombination, conjugation, integration or other mechanism that could occur in the GE plant or in relation to other organisms. B3.8 Information regarding the possibility of transpositions and/or rearrangements inside the insert once in the GE plant (respect

Table A2.2.(cont´d).

to the position the elements had in the vector), and/or of/with portions of the GE plant genome and in the flanking regions. B3.9 Probability that the transcription process continues outside the insert to the GE plant genome ignoring termination signals, as well as the probability of transcription and translation of fusion proteins or the generation of new open reading frames as consequence of the insertion.
Description of the products from the genetic elements expressed in the GE plant.
B4 Detailed description of the products from the genetic elements expressed in the GE plant B4.1 Description including for each genetic element: kind of the expression product (protein, RNA), molecular characterization of the product, amino acid sequence, biological activity of the product in the GE plant indicating specific function and molecular mechanism for its function, tissue/organ where is present, level of expression and its temporal evolution regarding the GE plant cycle. B4.2 Submit structural and functional analysis if there were performed using high-throughput methods of genomic, transcriptomic, proteomic or metabolomic. B4.3 Analysis to determine homology of the expressed products sequences with known sequences of expression products of pathogens, toxins or allergens. B5 Specific information for stacked events B5.1 To inform whether different phenotypic characteristics than the ones expected occur from the expression of the individual events. B5.2 To inform whether occur interactions (or there are reasons to expect them to occur) among the genes from the individual events, and its consequences.
Genetic stability
B8 Genetic stability B8.1 Segregation and transfer to the progeny B8.2 Molecular analysis to confirm the genetic stability (Southern blots, PCR).

Table A2.2. (cont'd).

FOOD SAFETY (PART C)
History of use and familiarity
C1 History of use and familiarity C1.1 History of use of the conventional counterpart C1.2 History of use of the protein expressed by the donor organism C1.3 Aptitude of the GE plant or its derivatives to be used as food, in case that the use of the GE plant as food were new
Substantial equivalence
C2 Food and feed safety analysis C2.1 To indicate if the nutritional quality of the food could be altered by the event C2.2 To indicate if the GE plant is capable of produce metabolites that can cause adverse effects in the consumer (human or animal) C2.3 Quali-quantitative chemical composition of the GE plant and its derivatives if corresponds and its comparison with conventional counterpart regarding: macro and micronutrients and anti-nutrients.
Digestibility of the GE protein
C2.4 Absorption, distribution and biotransformation of food components (nutrients and non nutrients) "in vitro" or "in vivo" C2.5 Nutrients bioavailability in case of a the GE plant non substantially equivalent
Pathogenicity in mammals
C2.6 Pathogenicity in mammals

Table A2.2. (cont'd).

Allergenicity
C2.7 Allergenicity C2.7.1 Bioinformatic analysis of the expressed proteins C2.7.2 Identification of allergens in donor and receiving species. C2.7.3 Bioinformatic analysis looking for homology of the expression products with known allergens C2.7.4 To provide other features used as indicators of allergens such as: molecular weight, food levels, protein resistance to food processing (heat or other), in vitro digestibility.
Toxicity
C2.8 Toxicity C2.8.1 Toxins and antinutrient factors naturally present in donor and receiving organism. C2.8.2 Bioinformatic analysis looking for homology of the expression products with known toxins C2.8.3 Acute toxicological tests of pure proteins with no history of use C2.8.4 Subchronic or chronic tests of pure protein, where appropriate C2.8.5 Subchronic or chronic tests of food, where appropriate.
Carcinogenic and teratology studies
C2.9 Carcinogenic and teratology studies in the short and medium term, where appropriate.
Other considerations
C3 Other considerations C3.1 Functional characterization of the GE plant and food derived from it, compared with its homolog. C3.2 Whether there are changes in the way of the food use, processing or cooking, compared with its homolog. C3.3 When using gene markers that confer antibiotic resistance, see considerations of Codex Alimentarius (CAC GL 45-2003, pág 8-9, paragraph 55-58.

Table A2.2. (cont'd).

ENVIRONMENTAL SECURITY (PART D)
Characterization of the receiving organism (homolog) used as comparator.
D1 Information of the GE plant D1.1 Characterization of the receiving organism/parental organism/homolog D1.1.1 Centre of origin and genetic diversification D1.1.2 Geographic distribution D1.1.3 Description of the natural habitat D1.1.4 Biological function of the specie in the ecosystem D1.1.5 Pathogenicity, toxicity and allergenicity of the species D1.1.6 Phenotypic characteristics of the species D1.1.7 Phenological phases and growth rate or duration of each phase D1.1.8 Flower and reproductive biology, factors that affect reproduction D1.1.9 Pollen viability and longevity D1.1.10 Pollen dispersal mechanisms D1.1.11 Potential pollinator agents and its distribution in Uruguay D1.1.12 Kind of fruit dehiscence D1.1.13 Natural seed dispersal mechanisms D1.1.14 Dormancy and seed capability of surviving after a long period of dormancy D1.1.15 Description of periods of life latent conditions or inactivity of the plant. D1.1.16 Survival structures and their capacity of persistence in the growing area and natural ecosystem. Factor that affect this capacity. D1.1.17 Ability and mechanism of competence and dispersion in the growing area and natural ecosystem. Factors that affect this capacity. D1.1.18 Whether the species has features that classify it as an invasive species or as potential invasive species. D1.1.19 Whether the species has features that classify it as a weed or potential weed.

Table A2.2. (cont'd).

Characterization of the GE plant.
D1.2 Characterization of the GE plant D1.2.1 To determine if there are any change in the biology of the plant as a consequence of the genetic modification compared with its homolog, for the aspects described in item D1.1 (D1.1.1 to D1.1.19) D1.2.2 Effects on the GE plant of the genetic elements introduced and their expression. D1.2.2.1 Physiologic and/or metabolic effects on the GE plant D1.2.2.2 Pleiotropic effects that could lead in the expression of allergic or toxic proteins or an increase or the production of new secondary metabolites. D1.2.2.3 In the case of stacked events, to inform whether different phenotypic characteristics than the ones expected occur from the expression of the individual events. D1.2.2.4 To indicate other risk factors derived from the presence of the introduced genetic elements or its expression products. D1.2.3 Phenotypic stability of the GE plant D1.2.3.1 Inheritance pattern of the introduced trait. D1.2.3.2 Phenotypic stability indicating the number of generations in which it was verified. D1.2.3.3 Frequency of reversion or loss of the genetic material. D1.3 Detection methods. Extend the information submitted in item B7.1 by including whether there are phenotypic markers or others that allow the GE plant identification in the field.

Table A2.2. (cont'd).

Characterization of the receiving environment
D2 Information of the receiving environment D2.1 Ecological characteristics of the area where the GE plant would be released, including for example wind direction, existence of a water table, proximity to watercourses and protection areas. D2.2 Biological function of the GE plant in the receiving environment. D2.3 How the GE plant responds to the biotic and abiotic environment of the area where the GE plant would be released. D2.4 To indicate the GE plant taxonomic related species including related and closed-related cultivated and wild species indicating if they are weeds or invasive species. D2.5 Geographical distribution of taxonomic related species if indicated in D2.4 D2.6 Crossing mechanisms and frequency, if related species were indicated in item D2.4. Rate and stability of selfing and/or cross pollination D2.7 Probability of hybridization and introgression if compatible species were indicated in D2.4 and whether the hybrid will have any selective advantage or to compete that would determine changes or losses in populations of the species. D2.8 Possible interactions of the GE plant with other non vegetables organisms of the ecosystem that could lead in a change of the number in natural predators, parasites, competitors, symbionts and hosts D2.9 Impact on non-target organisms in the case of the GE plant with insect resistance traits D2.10 Whether the GE plant is able to add or remove soil substances (nutrients or toxic substances) compared with its homolog, and its effect on the soil microbial population. D2.11 Impact on the receiving environment as a consequence of a change in the agronomic practices according to the technological package associated to the trait introduced. D2.12 Management measures to avoid possible unwanted effects (for example resistance development in insects originally susceptible to Bt proteins) D2.13 Management measures for monitoring possible adverse effects on the receiving environment in the long term. D2.14 Coexistence feasibility. To indicate management measures to avoid adventitious presence of other production systems present in the receiving environment.

Table A2.3. Summary of the number of applications in the period 1995-2011 by uses, crop, trait, gene, event, total applications and permits issued.

Framework by period	**1995-2000 Resolution DGSA-CAAR**		**2000-2007 Decree No. 249/000 CERV**		**2008-present[(1)] Decree No. 353/008 GNBIO/CGR/ERB/CAI**		**TOTAL 1995-2011**	
Uses	4	a) (1) b) (5) c) (6) e) (1)	3[1]	b) (7) c) (6) e) (4)	4	b) (8) c) (10) d) (18) e) (10)	**5**	**a)** **b)** **c)** **d)** **e)**
Crops	3	Corn (9) Soybean (2) Eucalyptus (2)	4[2]	Corn (11) Soybean (1) Rice (3) White clove (2)	2	Corn (20) Soybean (26)	**5**	**Corn** **Soybean** **Eucalyptus** **Rice** **White clove**
Traits	4	ns (2) IR (2) HT (5) IR/HT (3) Low lignin (1)	4[3]	IR (1) HT (7) IR/HT (4) or IRXHT (3) Delayed senescence (2)	2	HT (24) IR/HT (2) or IR X HT (11) or IR/HT X HT (3) or IR/HT X IR (4) or IR/HT X HT X IR (2)	**5**	• **IR** • **HT** • **IR/HT, IRXHT, IR/HTXHT, IR/HTXIR, IR/HTXHTXIR** • **Low ligning content** • **Delayed senescence**

Table A2.3. (cont'd).

Framework by period	**1995-2000 Resolution DGSA-CAAR**		**2000-2007 Decree No. 249/000 CERV**		**2008-present Decree No. 353/008 GNBIO/CGR/ERB/CAI**		**TOTAL 1995-2011**	
Genes	6	ns (2) *cry1Ab* (4) *cry9c* (1) *bar* (3) *pat* (1) *epsps* (4) *low lig* (1)	6[4]	*cry1Ab* (6) *cry1Fa2*(1) *pat* (4) *bar* (3) *epsps* (7) *ipt* (2)	9	*cry1Ab* (7) *cry1Fa2* (3) *cry2Ab2 (5)* *cry1A105* (5) *cry3Bb1* (4) *cry1Ac* (6) *Vip3Ab20* (2) *epsps* (34) *pat* (13)	**13**	***cry1Ab*** ***cry9c*** ***cry1Fa2*** ***cry2Ab2*** ***cry1A105*** ***cry3Bb1*** ***cry1Ac*** ***Vip3Ab20*** ***epsps*** ***pat*** ***bar*** ***low lig*** ***ipt***
Events	8	Corn (5) Soybean (1) Eucalyptus (2)	9[5]	Corn (5) Soybean (1) Rice (2) White clove (1)	13	Corn (9) Soybean (4)	**27**	**Corn (16)** **Soybean (6)** **Eucalyptus (2)** **Rice (2)** **White clove (1)**
Applications	13		17	Rescinded by use: (b): Soybean (1) (c): Corn (3), rice (1) (e): Corn (1), rice (1)	46	Under analysis by use: (b): Corn (2), Soybean (1) (d): Soybean (7) (e): Corn (2), Soybean (3)	**68**	
Permits issued	13		10		31[6]		**55**	

Table A2.3. (cont'd).

Framework by period	**1995-2000 Resolution DGSA-CAAR**		**2000-2007 Decree No. 249/000 CERV**		**2008-present Decree No. 353/008 GNBIO/CGR/ERB/CAI**		**TOTAL 1995-2011**	
Deregulations	1	RR (soybean)	2	Corn: MON810 BT11	5	Corn: GA21 GA21XBT11 TC1507 NK603 NK603XMON810	**8**	**Corn (7) Soybean (1)**

1. Seven of the original applications where rescinded leaving a total of 10 applications analyzed, distributed by use as follows: b) (6), c) (2), e) (2).
2. Seven of the original applications where rescinded leaving a total of 3 crops: corn (7), rice (1), white clove (2).
3. Seven of the original applications where rescinded leaving the following distribution by trait: IR (1), HT (3), IR/HT (2) or IR X HT (2), Delayed senescence (2).
4. Seven of the original applications where rescinded leaving a total of 5 genes analyzed: *cry1Ab* (5), *pat* (2), *bar* (1), *epsps* (4), *ipt* (2).
5. Seven of the original applications where rescinded leaving a total of 6 events analyzed: corn (4), rice (1), white clove (1).
6. The actual number corresponds to 32 resolutions issued by GNBio. The difference results from resolutions 32A and 32B that harmonize old events in corn (GNBio No. 32A for MON810 and GNBio No. 32B for BT11).

ns – non-specified in the record available at the Biosafety Office – MGAP- Uruguay.
IR – Insect resistance
HT – Herbicide resistance
IR/HT – individual event with IR and HT
IRXHT – stacked plant with two events incorporated by conventional crossing

Categories of uses: a) Contained experiment
b) Field trial for research
c) Field trial for National Register of Cultivars
d) Seed multiplication
e) Deregulation (commercial planting, food and feed)

(1) The period 2007-2008 is not included since Decree No. 037/007 suspended the treatment of any new applications.

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CHAPTER III: COMPARISON OF THE URUGUAYAN REGULATORY SYSTEM FOR GENETICALLY ENGINEERED CROPS WITH OTHER NATIONAL AND REGIONAL SYSTEMS AND POTENTIAL FOR HARMONIZATION

INTRODUCTION

The resulting biosafety regulatory system that each country has in place reflects the complex interaction between the political and economic situation, culture, environment and scientific and regulatory capacity of each individual country. A comparative analysis of the Uruguayan biosafety regulatory system with other frameworks is presented in this chapter. The Uruguayan approach is compared with those of Argentina, Brazil and Paraguay, which together form the Southern Common Market (MERCOSUR for its acronym in Spanish). The objective is to identify from divergences new strategies for improvement of the Uruguayan system, and also, to identify from commonalities potential approaches for harmonization toward a regional system taking advantage of the already existing regional trade agreement among the countries under analysis.

The information provided in this chapter is based on published documents and scientific literature. In addition, I present information and opinions based on my experience as the Technical Secretariat for the Interministries Working Group that elaborated the currently effective Decree 353/008, and subsequently as the coordinator for the analysis of the risk assessment working at the Risk Assessment on Biosafety agency (ERB). By working to develop the regulatory system and subsequently at the regulatory agency I had the opportunity to participate in various discussions and consultations among regulators from different countries. The comparisons that follow include a description of possible plusses and minuses of the risk analysis policies, with particular attention to guidelines and procedures

that could be adopted regionally. The overview and comparison of approaches is focused on field trials and commercialization of genetically engineered crops.

GENERAL DESCRIPTION OF BIOSAFETY FRAMEWORKS

Biosafety is a dynamic field and therefore the regulatory frameworks are in the process of continuous revision and updating. This section provides a general description of the history and current situation of the biosafety frameworks that have been implemented in the Regional Trade Agreement MERCOSUR countries of Brazil, Argentina and Paraguay in comparison with the regulatory system of Uruguay (see chapter 2 of this thesis).

Description of the Argentinean biosafety framework

Argentina was one of the first countries worldwide to establish a biosafety framework with policies dating back to 1991 (Argentina, 1991, Nap *et al*. 2003). Argentina employs a mixed regulatory system based on existing regulations in agriculture for plant protection chemicals and new crop-specific regulations created to determine the conditions for GE-crop development and release into the environment (Argentina, 1992, 2003a, 2003b, 2003c) and for food safety assessment (Argentina, 2002). Regulations applied for the National Advisory Commission on Agricultural Biotechnology (CONABIA for its acronym in Spanish) and the National Health Service and Food Quality (SENASA) for risk assessment of GE organisms are listed in Table 3.1 Additionally Argentina has in "parliament treatment" several proposed bills related to different aspects of biotechnology including one specifically on genetically modified organisms for agricultural, agroindustry and food industry production (Personal Communication, Godoy P., General Director of the Biotechnology Directorate of SAGYP, 2011).

Table 3.1. List of Argentinean specific regulations for GE organisms. (The date indicated in Resolutions corresponds to the date that the decision comes into force, and corresponds to the date of publication in the Official Gazette).

Norm	Purpose	Advisory Body to which it applies
SAGyPA Resolution No. 124/91 of 10/24/91	Creation of the National Advisory Commission on Agricultural Biotechnology (CONABIA)	CONABIA
SAGyPA Resolution No. 328/97 of 05/28/97	Modifies composition of CONABIA	
SAGyPA Resolution No. 244/04 of 02/27/04	Modifies composition of CONABIA and creates the Biotechnology Office	
SAGyPA Resolution No. 398/08 of 10/29/08 (modifies No. 328/97 and 244/04)	Modifies composition of CONABIA	
SAGyPA Resolution No. 656/92 of 07/30/92	Application form to analyze GE microorganisms and GE plants	
SAGyPA Resolution No. 39/03 of 07/17/03 (modifies No 656/92, 511/98 of 08/13/98 and derogates 289/97 of 05/14/97and 131/98 of 10/29/98)	Modifies application form to analyze environmental security of GE plants	
SAGyPA Resolution No. 644/03 of 12/18/03 (modifies Seed Law No. 20247 of 04/16/73)	Regulation of GM corn seed production with events still regulated in Argentina	
SAGyPA Resolution No. 212/06 of 05/10/06 (modifies No. 644/03)	Modifies regulation of GM corn seed production with events still regulated in Argentina	
SAGyPA Resolution No. 57/03 of 07/24/03 (modifies No 39/03 and Biological/ecological u organic production Law No.25127 of 09/13/99)	Application form for experiments with GE animals	

Table 3.1. (cont'd).

SAGyPA Resolution No. 396/08 of 11/05/08 (modifies No. 212/06 and 39/03).	Modifies administrative process for application submission.	
SAGyPA Resolution No. 400/10 of 08/24/10	Regulation of GM soybean seed production with events still regulated in Argentina	
SENASA Resolution No. 1265/99 of 11/09/99	Creation of the Technical Advisory Committee on the Use of Genetically Modified Organisms (CTAUOGM)	SENASA / CTAUOGM
SENASA Resolution No. 412/02 of 05/17/02 (modifies No. 289/97 of 05/14/97 and 511/98 of 08/13/98)	Application form to analyze food safety of GE products used as food/feed	
Source: Ministry of Economy and Production, Centre for Documentation and Identification http://www.infoleg.gov.ar/		

The National Advisory Commission on Agricultural Biotechnology (CONABIA) conducts environmental risk assessment, and the Directorate of Food Safety and Quality within SENASA conducts food/feed safety assessment. There is also the Directorate of Agrifood Markets (DAM), which in the case of applications for commercial use, evaluates the impact that the GE organisms could have on international trade of agriculture commodities.

At the beginning, the Argentinean biosafety framework depended on the Secretariat of Agriculture, Livestock, Fisheries and Food Supply (SAGyPA). SAGyPA was in the scope of the Ministry of Economy and the authority to approve or not the use of GE organisms was the Secretariat of SAGyPA. In 2009 the Ministry of Agriculture, Livestock and Fisheries (MAGYP for its acronym in Spanish) was created (Argentina, 2009a, b) and SAGyPA became one of its secretariats, named the Secretariat of Agriculture, Livestock and Fisheries (SAGYP). The biosafety regulatory system continues to depend on SAGYP, but SAGYP is now in the scope of the recently created Ministry of Agriculture. The authority to approve or

not, the use of GE organisms is the Secretariat of SAGYP. Figure 3.1 provides a schematic representation of the structure of the Argentinean regulatory system.

Since 1991 the National Advisory Commission on Agricultural Biotechnology (CONABIA), was the commission with the responsibility to carry out the environmental risk assessment and to design the biosafety conditions and risk management measures for the environmental release of GE organisms (Argentina, 1991, 2011). CONABIA had administrative support from the Technical Coordination of CONABIA, which later became the Biosafety Office (Argentina, 2004). Due to the increase in the number of applications requesting authorization for different uses of GE organisms, the Biosafety Office became the Biotechnology Directorate (Argentina, 2008), which has responsibility together with CONABIA for the follow-up and risk assessment of GE organisms.

The Biotechnology Directorate also participates in the development of the biotechnology policy. The Directorate has five members, the director, a scientific consultant, one responsible for the biosafety area, one responsible for the regulatory area and a technical coordinator. Members of the Biotechnology Directorate are also members of CONABIA. The General Director of the Biotechnology Directorate acts as the General Coordinator of CONABIA.

CONABIA is a multidisciplinary and inter-institutional advisory commission that provides technical support to the Secretariat of Agriculture. CONABIA is composed of representatives from the public and private sector of agriculture biotechnology. The composition of CONABIA, which was established originally in 1991 (Argentina, 1991), was modified several times (Argentina, 1993, 1997, 2004). It is currently composed of 25 members from 17 institutions according to SAGyPA's Resolution No. 398/008 (Argentina, 2008). A list of the institutions represented at CONABIA can be found in Table 3.3 at the end of this section. For each institution there is one regular representative and one alternate. In

those institutions with more than one area of knowledge, there is one representative from each area. The final recommendation to be forwarded to the Secretariat of Agriculture is decided by majority vote. In case of tie, the General Coordinator of CONABIA has a double vote.

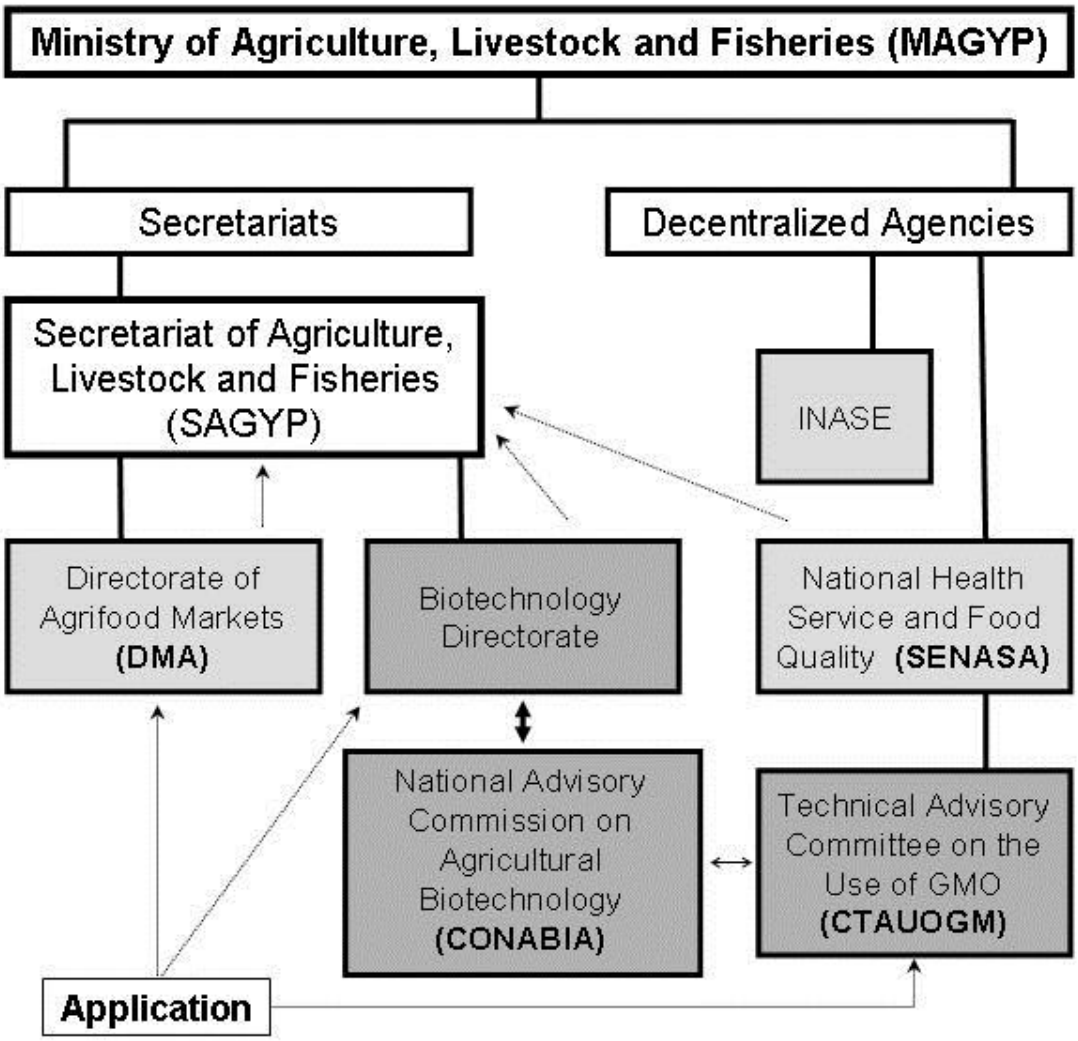

Figure 3.1. Scheme of the Argentinean Biosafety Framework design. The specific advisory bodies that compose the regulatory system and exclusively develop related tasks are indicated in dark shading. Divisions that collaborate with the regulatory system for particular tasks are indicated in light shading.

In the National Health Service and Food Quality Office (SENASA) works the Technical Advisory Committee on the Use of Genetically Modified Organisms (CTAUOGM for its acronym in Spanish). CTAUOGM was created in 1999 by Resolution of SENASA as indicated in Table 3.1 (Argentina, 1999). CTAUOGM is composed of representatives from the public and private sector from the area of health and agriculture. Table 3.3 lists the institutions represented at CTAUOGM. The National Directorate of Agrifood Safety and Quality of SENASA presides over CTAUOGM but the coordination is performed by the Agrifood Quality Directorate of SENASA.

The National Directorate of Agrifood Markets (DNM for its acronym in Spanish), which became the Directorate of Agrifood Markets (DMA) after the creation of the Ministry of Agriculture, assesses the economic appropriateness of commercialization of the GE product under consideration. DMA does not perform a socioeconomic analysis. Workers at DMA elaborate a business report with the analysis of markets and trade regarding the convenience of authorizing the event under consideration.

Independent non-binding recommendation reports from the Directorate of Agrifood Markets (DMA), the Biotechnology Directorate/CONABIA, and the National Health Service and Food Quality (SENASA) are forwarded to the Secretariat of SAGYP for his consideration and final decision.

Laboratory research activities with GE organisms are regulated by the Ministry of Science, Technology and Innovation (MINCYT for its acronym in Spanish.) In 2001 was created the Committee on Ethics in Science and Technology (CECTE for its acronym in Spanish) by MINCYT Resolution No. 004/2001 (Table 3.1).

Comparison between the Argentinean and Uruguayan regulatory systems

The operational design of the Argentinean regulatory system highlights the importance of the views of the trade office (DMA) in the decision-making and the lack of

public participation and socioeconomic considerations. Although the system is based on risk analysis methodology, its components: risk assessment, risk management and risk communication, are not well distinguished. The risk assessment phase is performed independently by the Biotechnology Directorate, based on CONABIA's analysis regarding environmental security, and SENASA's assessment of food/feed safety. The Biotechnology Directorate together with CONABIA, forwards a report to the Secretariat of SAGYP and likewise SENASA. Although CONABIA interchanges information during the analysis process with SENASA, the reports forwarded to the Secretariat of SAGYP are independent of each other.

Regarding the risk management phase, the Biotechnology Directorate performs the risk management, as it is responsible for the follow-up of released GE organisms and the control of compliance of imposed biosafety measures. In addition, the Directorate of Agrifood Markets (DMA) elaborates an independent report to the Secretariat of SAGYP with regard to issues usually considered in the risk management phase (markets and trade). The fact that the final decision is taken by the Secretariat of SAGYP based on non-binding recommendations from the Biotechnology Directorate/CONABIA, SENASA and DMA, suggests a separation between strictly biosafety issues and political, legal and economic issues.

A positive practice of the Argentinean regulatory system is the proceeding established in Regulation 39/2003 for the release into the environment of GE crops that considers a first phase of evaluation for field trials and a second phase for commercial use. The field trials performed under the so called "first phase" of the regulation Nº 39/2003 are mainly trials to test the efficacy of the events for the developer to decide which cultivar to move forward to commercialization, and so will require application for the "second phase". This procedure could be implemented in the Uruguayan framework as a way not only to include local

efficacy tests, but also for the assessment of any particular biosafety risk hypothesis that needs to be tested locally. The first and second phase approach is also discussed in the last section of this chapter as a procedure to be implemented regionally.

Comparing the structure and operation of the Uruguayan biosafety framework (chapter 2 of this thesis) with the Argentinean system, it seems that the Uruguayan Risk Assessment on Biosafety (ERB) committee performs similar tasks as the Argentinian Biotechnology Directorate with regard to coordination of the risk assessment phase and follow-up of released GE organisms. At the same time, the Institutional Coordination Committee (CAI) and Ad Hoc Groups from Uruguay, perform similar tasks as CONABIA does in Argentina. However, the Argentinean Biotechnology Directorate and CONABIA are responsible for environmental issues while the Uruguayan ERB committee, the Institutional Coordination Committee (CAI) and Ad Hoc Groups are also responsible for food and feed safety. In Argentina food/feed safety is performed independently by CTAUOGM at SENASA. The applicant submits the appropriate form at each office separately.

On the other hand, the Argentinian Biotechnology Directorate has a larger component of risk management than ERB. ERB participates in risk management by recommending biosafety measures, but the final recommendation to the Ministers is forwarded from the Uruguayan Risk Management Commission (CGR), which performs exclusively risk management tasks. In this sense, the Argentinian Biotechnology Directorate has similar responsibilities as CGR, while CGR commission performs part of the Biotechnology Directorate and the Directorate of Agrifood Markets (DMA)'s tasks. The Uruguayan CGR commission analyzes economic issues regarding markets and trades as the Argentinean DMA does, and performs the follow-up of released GE organisms regarding environmental security as does the Argentinean Biotechnology Directorate. The CGR commission also includes the

analysis of socio-economic considerations and carries out the risk communication phase, both of these aspects are not included in the Argentinean biosafety framework.

In summary, comparing the operation of both systems, the Uruguayan framework has the environmental risk assessment process integrated with the food/feed safety analysis and the risk management phase integrated at CGR. In the Argentinean system the signature of the Secretariat of SAGYP is required for approval while in Uruguay the signature of six Ministers is required.

Description of the Brazilian biosafety framework

Brazil has developed new norms specific for the regulation of genetically engineered organisms. The regulation is based on the Biosafety Law No. 11.105 of 03/24/05, which is implemented through the ordinances of Decree No. 5.591 of 11/22/05. Additionally there are several provisions issued by the National Biosafety Council (CNBS) and the National Biosafety Technical Commission (CTNBio) that regulate commercial release as well as research and development of genetically modified organisms. Specific regulations on GE organisms are listed in Table 3.2 The participating advisory bodies and commissions are shown in Figure 3.2.

The National Biosafety Council (CNBS for its acronym in Spanish) is linked to the Presidency of the Republic. CNBS is composed of 11 Ministers of State: the Minister of State Chief of the Civil House Office of the Presidency of the Republic (chairperson of CNBS), the Ministers of State of Science and Technology, Agrarian Development, Agriculture and Supply, Justice, Health, Environment, Development, Industry and Foreign Trade, Foreign Affairs, Defense and Special Secretary for Aquaculture and Fisheries of the Presidency of the Republic (Brazil, 2005).

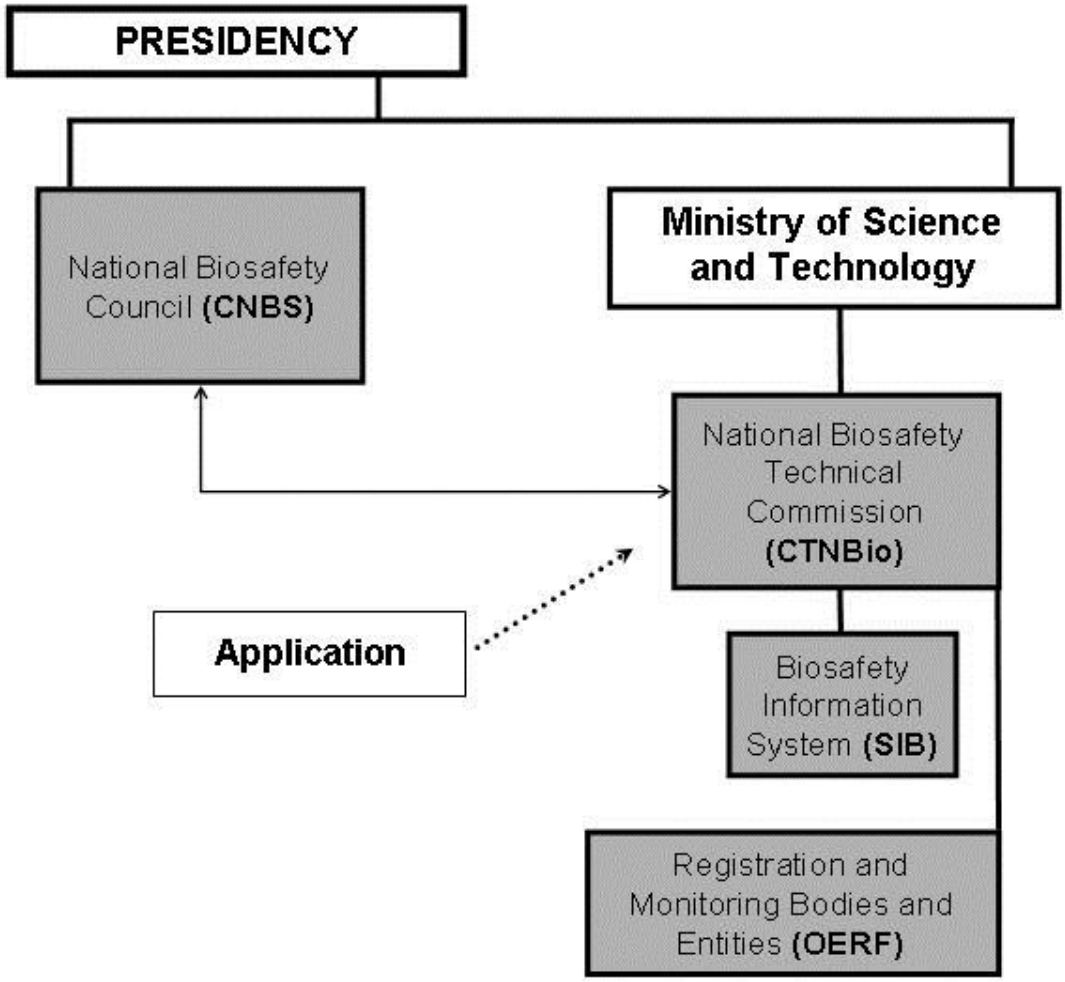

Figure 3.2. Scheme of the Brazilian Biosafety Framework design. The specific advisory bodies that compose the regulatory system and exclusively develop related tasks are indicated in shading.

CNBS defines the responsibilities of the entities involved in the regulatory system as the highest authority for formulating and implementing the national policy on biosafety. With regard to the risk analysis process itself, CNBS performs the analysis of socio-economic aspects and others of national interest if requested by the National Biosafety Technical Commission (CTNBio for its acronym in Spanish). CNBS also decides on appeals for commercial use of GE organisms and derivatives when the file has received a favourable opinion from CTNBio (Brazil, 2005 Article 52).

Table 3.2. List of Brazilian specific regulations for GE organisms.

Norm	Purpose	Advisory body to which it applies
Biosafety Law No. 11.105 of 03/24/05	Creation of the national biosafety framework for genetically engineered organisms.	CNB, CTNBio
Decree No. 5.591 of 11/22/05	For the implementation of the biosafety law, creation of advisory bodies of the regulatory system.	CNB, CTNBio
Decree No. 5950 of 10/31/06	Establishes limits for planting GEO in areas bordering protected areas.	CNB, CTNBio
Decree No. 6925 of 08/06/09	Designates Competent National Authorities and National Focal Points for the application of Article 19 of the Cartagena Protocol.	CNB, CTNBio
Provisional Measures from the President No. 327 of 10/31/06	Prohibition of planting GE plants in protected areas established in Law No. 9985 of 07/18/00.	CNB, CTNBio
CNBS Guideline No. 1 of 07/31/08	Gives capacity to CTNBio to authorize commercial releases of GE organisms.	CTNBio
CNBS Guideline No. 2 of 07/31/08	Approves monitoring plan for GE organisms and its derivatives.	CTNBio
Minister of Science and Technology Ordinance No. 146 of 03/06/06	Approves CTNBio Internal Regulation.	CTNBio
Minister of Science and Technology Ordinance No.373 of 06/07/11	Modifies CTNBio Internal Regulation.	CTNBio
CNBS Resolution No. 1 of 01/29/08	Approves CNBS Internal Regulation.	CNBS
CTNBio Normative Resolution No. 1 of 06/20/06	It disposes of installation and functioning of the Biosafety Internal Commissions (CIBios) and of criteria and procedure for request, issue, review, extension, suspension and cancelling of the Biosafety Quality Certificate (CQB).	CIBIos
CTNBio Normative Resolution No. 2 of 11/27/06	It disposes of risks classification of Genetically Modified Organisms (GMO) and biosafety levels to be applied to activities and projects with GMO and its derivatives in contention.	CTNBio
CTNBio Normative Resolution No. 3 of 08/16/07	It disposes of monitoring norms of genetically modified corn in commercial use.	CTNBio

Table 3.2. (cont'd).

CTNBio Normative Resolution No. 4 of 08/16/07	It disposes of minimal distance between the commercial cultivation of genetically modified and non-genetically modified corn, aiming at the coexistence between the production systems.	CTNBio
CTNBio Normative Resolution No. 5 of 03/12/08	Gives provisions on rules for commercial release of Genetically Modified Organisms and their derivatives.	CTNBio
CTNBio Normative Resolution No. 6 of 11/06/08	Provides on rules for the planned release to the environment of Genetically Modified Organisms (GMO) of plant origin and their derivatives.	CTNBio
CTNBio Normative Resolution No. 7 of 04/27/09	Provides on rules for planned release into the environment of Risk Class I Genetically Modified Microorganisms (GMM) and Genetically Modified Animals (GMAn) and their derivatives.	CTNBio
CTNBio Normative Resolution No. 8 of 07/03/09	Provides on simplified rules for Planned Release into the environment of Risk Class I Genetically Modified Organisms (GMO) and their derivatives.	CTNBio
CTNBio Normative Instruction No. 2 of 09/10/96	Provides rules for the import of GE plants with research purposes.	CTNBio
CTNBio Normative Instruction No. 4 of 12/19/96	Provides rules for transportation of GE organisms.	CTNBio
CTNBio Normative Instruction No. 8 of 07/09/97	Provides rules for genetic manipulation and cloning of human beings.	CTNBio
CTNBio Normative Instruction No. 9 of 10/10/97	Provides rules for genetic intervention on human beings.	CTNBio
CTNBio Normative Instruction No. 13 of 06/01/98	Provides rules for the import of GE animals with research purposes.	CTNBio
CTNBio Normative Instruction No. 17 of 11/17/98	Provides rules for the import, trade, transport, storage, human/animal consumption, environmental release and disposal of products derived from GE organisms.	CTNBio
CTNBio Normative Instruction No. 18 of 12/15/98	Establishes conditions for the environmental release of GE soybean with herbicide tolerance (glyphosate) including a monitoring plan.	CTNBio
CTNBio Normative Instruction No. 19 of 04/19/00	Establishes the procedure for public hearing by CTNBio	CTNBio

Table 3.2. (cont'd).

Communication CTNBio No. 1 of 08/09/06	Establishes conditions of isolation for authorization concession of planned release in the environment of genetically modified corn.	CTNBio
Communication CTNBio No. 2 of 07/12/07	Establishes conditions of isolation for authorization of planned release in the environment of genetically modified eucalyptus.	CTNBio
Communication CTNBio No. 3 of 11/28/07	Authorizes stacked events with herbicide tolerance and insect resistance derived from *Bacillus thuringiensis* obtained by conventional sexual crossing, to follow the simplified norm in force, in case there has already been analysis and approval of research or planned release with each of the events independently.	CTNBio
Communication CTNBio No. 4 of 06/24/08	Establishes conditions of isolation to enable authorization of planned release to the environment of genetically modified cotton plant.	CTNBio
Communication CTNBio No. 5 of 06/24/08	Authorizes Internal Biosafety Commissions (CIBio) to authorize activities of import, export and transportation of class 1risk GMO derivatives for the sole use in research under a regime of restraint.	CIBio
Communication CTNBio No. 6 of 03/18/10	Establishes conditions of isolation and disposal to grant planned authorisation for release into the environment of GE soybean.	CTNBio
Communication CTNBio No. 7 of 10/21/10	Establishes conditions of isolation and disposal to grant planned authorisation for release into the environment of GE sugar cane.	CTNBio
Source: National Biosafety Technical Commission (CTNBio), http://www.ctnbio.gov.br/index.php/content/view/55.html		

The National Biosafety Technical Commission (CTNBio) is part of the Ministry of Science and Technology. CTNBio is a consulting and deliberative, multidisciplinary collegiate body that provides technical and advisory support to the Federal Government in the formulation, updating and implementation of the National Biosafety Framework for GE organisms and their derivatives (Brazil, 2005). CTNBio is composed of twenty-seven (27)

members, Brazilian citizens specialists of recognized technical and scientific knowledge from different sectors (Table A3.1 in Appendix). From the 27 specialists, twelve are from academia, nine from the Federal Government, and six represent the civil society. Of the twelve specialists from academia, three are specialists on human health, three in animal health, three in plants and three in environmental issues. Of the nine specialists from the Federal Government there is one representative from each of the Ministries of: Science and Technology, Agriculture and Supply, Health, Environment, Agrarian Development, Development, Industry and Foreign Trade, Defense, Foreign Relations and of the Office of the Special Secretary for Aquaculture and Fisheries of the Presidency of the Republic. Of the six specialists that represent the civil society, there is one specialist from each of the following areas: consumer rights, health, environment, biotechnology, family agriculture and occupational health. The appointment of the above mentioned specialists is made by the respective Minister of State (Brazil, 2005).

CTNBio acts by developing Technical Reports on a case-by-case basis on GE organisms and its derivatives including security measures and restrictions if required by its use. If the technical opinion has majority vote, the event is authorized. In case of appeals to CTNBio's commercial approvals, the National Biosafety Council (CNBS) makes the final decision (Brazil, 2005 Article 52).

The Brazilian regulatory system also includes Registration and Monitoring Bodies and Entities (OERF for its acronym in Spanish), formed from the respective registration and monitoring bodies of the Ministries of Health, Agriculture and Supply, Environment and the Office of the Special Secretary of Aquiculture and Fisheries of the Presidency of the Republic. OERF's members, within their respective jurisdictions, are responsible for the compliance of CNBS deliberations and CTNBio's technical decisions set up by Decree 5591/005 (Brazil, 2005).

For laboratory research activities with GE organisms, CTNBio issues a Biosafety Quality Certificate (CQB). It is also the requirement for any institution committed to education, scientific research, technological development and industrial production that uses GE organisms, to set up an Internal Biosafety Commission (CIBio). CIBIO assigns a principal technician in charge of each specific project to ensure that the research is conducted in compliance with biosafety standards and measures established by CTNBio (Brazil, 2005, 2006).

The Biosafety Information System (SIB for its acronym in Spanish) is responsible for managing the information resulting from the risk analyses, including authorization, registration, monitoring and follow-up of activities involving GE organisms and their derivatives (Brazil, 2005). The SIB is under the Office of the CTNBio Executive Secretary.

Comparison between the Brazilian and Uruguayan regulatory systems

The Brazilian regulatory system highlights the legal support developed for the biosafety framework. Brazil has a National Biosafety Law and a vast number of regulations for the Law's implementation as observed in Table 3.2 In turn, the Brazilian regulatory system has had the ability to modify rules in order to provide greater efficiency to the system as the number of applications has increased.

Members of the National Biosafety Technical Commission (CTNBio) have changed so that its members are now required to be recognized Brazilian citizens with doctoral degree and an active professional career in biosafety, biotechnology, biology, human and animal health or the environment. The Brazilian high scientific and professional capacity as well as the strong political support for biotechnology, allowed the development of two transgenic crops generated in Brazil, soybean with herbicide tolerance (Brazil, 2009) and bean with virus resistance (Brazil, 2011).

A noteworthy feature of the Brazilian regulatory system is the broad participation of stakeholders in the discussions along the decision-taking process. Changes in legislation to make the system more efficient did not diminish public participation in the decision-taking process. The Brazilian regulatory system performs public hearing carried out by CTNBio, which is one of the public consultation mechanisms with the highest public participation.

Comparing the structure and operation of the Uruguayan regulatory system (chapter 2 of this thesis) with the Brazilian framework it seems that the Uruguayan advisory bodies: Risk Assessment on Biosafety (ERB), Institutional Coordination Committee (CAI) and Ad Hoc Groups corresponds with the Brazilian National Biosafety Technical Committee (CTNBio) for the risk assessment phase. CTNBio like ERB/CAI/Ad Hoc Groups, performs environmental security and food/feed safety analysis. However, the Uruguayan risk assessment phase forwards the technical opinion to the risk management phase, while the Brazilian CTNBio has the independence to approve applications either for research purposes under contained conditions or deregulation for commercial release.

In Uruguay the Risk Management Commission (CGR) leads the risk analysis process and is responsible for the risk assumed. Brazilian CTNBio seems to lead the risk analysis and authorizations are based mainly on technical decisions. CTNBio may request socio-economic analysis to the National Biosafety Council (CNBS), which corresponds to the Uruguayan National Biosafety Cabinet (GNBio). The Uruguayan GNBio is composed of six Ministers and the Brazilian CNBS is composed of eleven Ministers. Both are responsible for the formulation and implementation of the national policy on biosafety. The Brazilian CNBS may also perform socio-economic analysis if requested by CTNBio and gives the final decision if a favourable CTNBio's decision was appealed.

In Uruguay GNBio does not perform socio-economic analysis but issues resolutions for authorizations. In Uruguay socio-economic analysis is always considered by CGR for

authorizations of deregulation. There is not an equivalent figure of the Uruguayan CGR in the Brazilian regulatory system. The Brazilian CTNBio coordinates with the Brazilian Registration and Monitoring Bodies and Entities (OERF) and the Biosafety Information System (SIB), risk management tasks performed in Uruguay by CGR with regard to follow-up and control of authorized events and risk communication mechanisms, respectively.

The Brazilian criterion to consider as favourable a CTNBio's technical opinion with majority vote, would give efficiency to the Uruguayan regulatory system and could be applied for field trials authorizations. The Uruguayan CGR could keep management tasks as performed now regarding control of compliance of biosafety conditions.

In summary, in comparing their operations, both systems integrate environmental risk assessment with food/feed safety analysis in a similar structure for the risk assessment phase In the Brazilian system an approval of the CTNBio technical opinion by majority vote is considered as a favourable decision, while in Uruguay the signature of six Ministers is required and risk analysis is led by the risk management phase.

Description of the Paraguayan biosafety framework

Paraguay, like Argentina, has a mixed regulatory system based on existing regulations for agriculture and has created GE specific norms. The existing regulations include the Law No. 294/93 of Environmental Impact Assessment, the Law No. 2459/04 of the creation of the National Service of Quality and Plant Health and Seeds (SENAVE), the Seeds Law No. 385/94 and the Law No. 2426/04 of the National Service of Animal Health (SENASA). With regard to specific GE regulations, the Paraguayan biosafety framework is based on the Decree No. 12,706/08 that modifies the Decree No. 18,481/97 that created the Biosafety Commission (COMBIO) (Table 3.3). Under the former Decree No. 18,481/97, COMBIO had the power to issue resolutions with regard to the authorization for the introduction, field trials

and commercial use of GE plants. The authorities that supported COMBIO resolutions, within their respective jurisdictions, were the Ministries of Agriculture and Livestock (MAG) and Health and Social Welfare.

The Paraguayan regulatory framework regulates GE plants (including trees) and livestock products. In 2003 with the ratification of the Cartagena Protocol, a biosafety bill was presented to Parliament that includes the whole range of GE organisms. Additionally two proposals for a Biosafety Law, from the agricultural and green sectors respectively, were submitted to Parliament (Personal Communication, 2010, Rojas, L. Coordinator of COMBIO). The parliament has not yet enacted the biosafety bills. Meanwhile, the Decree No. 18,481/97 was modified and expanded by the Decree No. 12,706/08. COMBIO is now named the Agricultural and Forestry Biosafety Commission and the Decree No. 12,706/08 stipulates that the Ministry of Agriculture is the authority that issues authorization for the different categories of uses based on COMBIO's advisory body opinion (Paraguay, 1997, 2008).

But also, from 1997 to the ratification of the Cartagena Protocol in 2003, there were specific GE resolutions issued from the Ministries of Agriculture and Health and rules set by COMBIO that are summarized in Table 3.3 Administrative changes were implemented such as the creation of the Secretary of Environment (SEAM), and changes in the Ministry of Agriculture with the transition to independent agencies of Plant Health Services (SENAVE) and Animal Health and Quality (SENACSA) and the Paraguayan Agricultural Technology Institute (IPTA). A new decree, Decree No. 6733 of 06/13/11 has been recently approved, that establishes the National Policy and Program of Agricultural Biotechnology and Forestry of Paraguay (Paraguay, 2011)

COMBIO is a consulting and deliberative, multidisciplinary body that provides technical and advisory support to the Ministers of Agriculture and Health. COMBIO issues

technical opinions with regard to the authorization for field trials and commercial use of GE plants. COMBIO is composed of thirteen members, ten representing government institutions and three from academia (Table 3.3). The government participants include eight representatives of the Ministry of Agriculture, including three from the Secretary of Environment, and one representative each from the Ministry of Health and the Ministry of Industry. The academic representatives are from the National University of Asunción (UNA) with one representative from the School of Agricultural Sciences, School of Veterinary Science and one from the School of Natural Sciences. The final recommendation to the Ministry of Agriculture in cases of commercial release is decided by majority vote, although decisions to date have been reached by consensus.

Table 3.3. List of Paraguayan specific regulations for GE organisms.

Norm	**Purpose**	**Advisory Body to which it applies**
Decree No. 18481/97 of 09/18/97	Creation of the Biosafety Commission (COMBIO)	COMBIO
Decree No. 12706/08 of 08/13/08	Modifies Biosafety Commission (COMBIO) (created by Decree No. 18481/97) for Agricultural and Forestry Biosafety Commission.	
MAG Resolution No. 376/09 of 06/30/09	Establishes the procedure to be followed by field trial applications.	
MAG Resolution No.2128/10 of 10/29/10	Modifies the procedures for field trial applications not requiring the license of environmental impact assessment.	
MAG Resolution No. 158	Declares of Ministerial interest field trials for research in biotechnology and biosafety.	
Decree 6070/05	Creates the Biotechnology Coordination at SENAVE	SENAVE
SENAVE Resolution No. 555/10	In item 282 is charged a fee for the risk analysis process.	

The Decree No. 12,706/08 separates the risk analysis of plants and trees from livestock products. Thus, COMBIO has two secretariats, the Biotechnology Coordination, created by the Decree No. 6070/05, of SENAVE that performs the risk analysis of plants, while SENACSA performs risk analysis of livestock products. In turn, there is a sub-commission subordinated to COMBIO specialized in risk analysis. COMBIO also can request technical cooperation in national and international institutions with knowledge in biosafety. For commercial release, in addition to the environmental risk analysis performed by COMBIO, a license of environmental impact assessment must also be issued by the Secretary of Environment in accordance with the framework of the Law No. 294/93.

The requirement of an environmental license was already in place for conventional crops. The information reviewed is quite different for COMBIO and the Environmental Impact Assessment performed by the Secretary of Environment but it is coordinated between both authorities. COMBIO requests information specifically on environmental risk assessment based on risk hypothesis while the Environmental Impact Assessment requests the description of the project including information regarding the kind of activity whether forestry, agriculture, etc.; the investment and the technology that the project implies; raw materials and supplies; and waste volumes and treatments. It also requests a description of the size of the area and whether the area includes features such as natural sources of water, wetlands, or protected areas. It also requires a description of the vegetation and distance of the area to populated areas. An analysis of environmental impact, mitigation measures and a monitoring plan are also required according to SEAM Resolutions 368/08, 303/04 and 375/07. More details can be found in the Environmental Impact Assessment form called Basic Environmental Questionnaire (CAB for its acronym in Spanish).

Figure 3.3 outlines the biosafety regulatory system of Paraguay. The specific advisory bodies that compose the regulatory system and exclusively develop related tasks are indicated

in dark shading. Divisions that collaborate with the regulatory system in particular tasks are indicated in light shading.

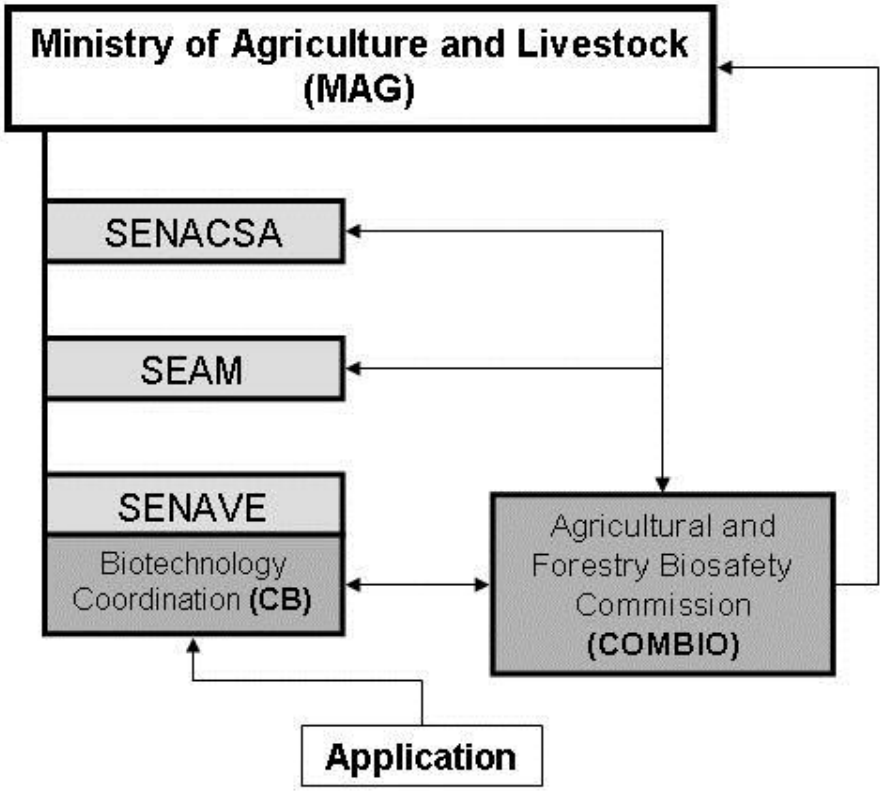

Figure 3.3. Scheme of the Paraguayan Biosafety Framework design. The specific advisory bodies that compose the regulatory system and exclusively develop related tasks are indicated in shading.

The Biotechnology Coordination (CB) at SENAVE receives the application form. CB in the case of plants and trees and SENACSA in the case of livestock products, forward to COMBIO a technical opinion as appropriate. Additionally, in the case of commercial releases SEAM forwards to COMBIO the environmental license. A final recommendation is forwarded by COMBIO to the Ministry of Agriculture.

Comparison between the Paraguayan and Uruguayan regulatory systems

The recently approved Decree No. 6733/11 establishing the National Policy and Program in Agricultural Biotechnology and Forestry demonstrates the beginning of political support in the area of agricultural biotechnology. The biosafety legislation in Paraguay covers GE plants and GE livestock products while does not yet include microorganisms and animals as in Uruguay. The Paraguayan biosafety framework is changing procedures to make the system more efficient. For example, the fact that field trials do not require environmental license accelerates the process.

Regarding the operation of the system, the Paraguayan COMBIO performs similar tasks as the Uruguayan ERB committee and CGR commission. The Paraguayan COMBIO commission performs coordination tasks for the risk assessment phase and participates in the follow-up of authorized events as ERB does. But also it recommends biosafety policy and forwards to the Ministry of Agriculture a recommendation whether to approve or not an event. In Uruguay, the recommendation is performed by the Risk Management Commission (CGR). In this sense, the Paraguayan system resembles the Argentinean biosafety framework in which there is not a clear separation between risk assessment and risk management. The Paraguayan regulatory system is also similar to the Argentinean one in that the signature of the Ministry of Agriculture in Paraguay and the signature of the Secretariat of Agriculture in Argentina is required for approval, while in Uruguay the signature of six Ministers is required.

COMMON AND DISTINCT ELEMENTS FOUND ACROSS THE MERCOSUR COUNTRIES

The comparative analysis provided in the following section is based on the description of the respective regulatory systems derived from relevant government documents presented in the previous section, and on opportunities for personal interaction with regulators from each of the countries reviewed while I was working at the Uruguayan Biosafety Office. The aspects compared include: legislative basis, regulatory triggers, methodology used for the decision making process, structure and operation of the risk analysis process, composition of the regulatory bodies, approaches to risk assessment, risk management and risk communication, the requirement of post-market monitoring, and the average number of applications submitted and events deregulated.

Table 3.4 indicates whether each of these aspects is similar or different among the regulatory frameworks examined. The objective of identifying common and distinct elements across the regulatory frameworks was to highlight practices that could be applied by the Uruguayan's regulatory system to improve its efficiency as well as to discuss aspects of the risk analysis organization that could be approached regionally.

With regard to common elements found across the four countries examined, the basic criteria for decision making regarding the use of GE organisms are shared among the countries of MERCOSUR. This baseline of concepts includes the recognition that biotechnology techniques *per se* are not inherently risky and so it is not the process itself (biotechnology techniques) what has to be regulated but *the product* of biotechnology (ICSU, 2005). However, in the four countries analyzed, what triggers the regulation is *the process* of genetic engineering. Products with the same traits but derived from conventional plant breeding, selection, or accelerated mutagenesis are not required to go through the regulatory systems.

Table 3.4. Common and different elements analyzed among the biosafety regulatory system of MERCOSUR countries.

Criteria of comparison	**Argentina**	**Brazil**	**Paraguay**	**Uruguay**
Regulatory triggers	Process based			
Methodology decision making process	Risk analysis			
Risk assessment approach	Case by case Step by step Comparative approach using the concepts of familiarity and substantial equivalence Risk factors for environmental and food safety evaluation High influence on the final decision of the risk assessment result			
Legislative basis	Existing and GE specific new regulation			
	Biosafety bill in parliament GE organisms used in agriculture Cartagena Protocol (CP) signed	Biosafety law GE organisms CP ratified	Biosafety bill in parliament GE plants and livestock products CP ratified	Biosafety bill in preparation GE plants. CP ratified
Structure and operating of the risk analysis process	See Table A3.1	See Table A3.1	See Table A3.1	See Table A3.1

Table 3.4. (cont'd).

Criteria of comparison	Argentina	Brazil	Paraguay	Uruguay
Risk management	Partially independent from risk assessment. Risk assessors also perform risk manager's tasks Weight-of-evidence approach Post-market monitoring not required	Partially independent from risk assessment. Risk assessors also perform risk manager's tasks Precautionary principle and Weight-of-evidence approach Post-market monitoring required	Partially independent from risk assessment. Risk assessors also perform risk manager's tasks Precautionary principle and Weight-of-evidence approach. Post-market monitoring not required.	Independent from risk assessment. Specific commission for risk management Weight-of-evidence approach. Precautionary principle under discussion. Post-market monitoring currently not required.
Risk communication	No procedures established.	Procedures established. Request for public comment and public hearing.	Not currently defined	Procedures established. Public notification and request for public comment.

Another basic criterion is the use of the risk analysis methodology applying the concepts of '*case by case'* and '*step by step*' (FAO, 2007) as well as the use of the comparative approach with the traditional counterpart using the concepts of '*familiarity*' and '*substantial equivalence*' in the risk assessment (OECD, 1993). The risk factors considered for the environmental and food safety evaluation are the same for each of the countries reviewed. Consequently the information required in the application forms for the molecular characterization, food safety as well as the biology of the plant and the environment where the GE organism would be released, is basically the same among the countries examined (FAO, 2007).

Generally within the countries examined, multidisciplinary and inter-institutional commissions or committees give scientific and technical support and advice to the public administration in the process of taking the final decision. In all cases, the authority with the final decision has to take into consideration the technical opinion of the biosafety commission regarding environmental and health risk assessment. These technical and scientific commissions have a high degree of diversity in terms of the specialists that participate. In Brazil the requirement to incorporate PhD-level accredited technicians or researchers involved in GE disciplines, made a difference in terms of the commission efficiency, leading to decrease in the average time for a commercial authorization from four to five years to one to two years (Biosafety Law, Article 11, Brazil-CTNBio, 2005).

Regarding the legislative basis of the biosafety frameworks, in the four countries analyzed, GE products must comply, and therefore the frameworks are supported in existing regulations related to plant and animal protection, environmental legislation and seed registration. In all cases, GE plants are under the norms applied in agriculture for conventional crop counterparts. But also, in all four countries, technology specific regulations

were developed for the creation of advisory bodies and to establish conditions for release of GE products.

Distinct elements were found across the regulatory frameworks with regard to the legislative basis. Other differences were found in the structure and operation of the risk analysis process, with respect to risk management approaches, risk communication procedures and post-market monitoring requirements. A summary of the legislative basis and the structure of the frameworks, regarding the main legislation, GE organisms regulated, the authorities responsible for decision-making, and the number of members that compose the different advisory bodies is presented in Table 3.5. Legislative differences are found in the legal status, whether the frameworks are based on a resolution, decree or law level. Brazil is the only country with a Biosafety Law in place (Brazil, 2005). Paraguay and Argentina have presented to the Parliament a Biosafety Bill (Personal Communication, Rojas, L. Coordinator of COMBIO, Paraguay, 2010; Personal Communication, Godoy P., General Director of the Biotechnology Directorate of SAGYP, Argentina, 2010) and Uruguay is working on this since the new regulatory system is being implemented (Uruguay, 2008).

As for GE organisms that are regulated by the different regulatory frameworks, there is a gradual increase in coverage among the countries; currently Uruguay covers the fewest GE organisms and Brazil the most. The current regulatory system in Uruguay covers GE plants while Paraguay covers GE plants and livestock products. In Argentina the regulatory system covers all GE organisms, i.e. plants, animals, microorganisms, and vaccines, but only for use in agriculture, and in Brazil the regulatory system incorporates all GE organisms for use in agriculture and medicine. Both Uruguay and Paraguay are working to increase the scope of regulation. Uruguay has included all GE organisms in the biosafety bill currently under development and all GE organisms are included in the bill that Paraguay has forward to the Parliament.

Table 3.5. Comparative description of the regulatory systems of the MERCOSUR countries: Argentina (ARG), Brazil (BRA), Paraguay (PAR) and Uruguay (URU).

Country	**Framework**	**Particularities of Risk analysis**	**GE organisms regulated**	**Resolution issued by:**		**Risk analysis done by:**	
				Authority	**Firms**	**Advisory body**	**Members**
ARG	SAGyPA Resolutions No 656/92, 39/03, 57/03, 644/03. SENASA Resolution No.412/02	Independent administrative route for environmental and food safety.	Plants Animals Microorganisms Vaccines	Secretariat of Agriculture, Livestock and Fisheries (SAGYP)	1	CONABIA (25 members)	Government Academia Private
						DNM (---)	Government
						CTAUOGM (12 members)	Government
BRA	Law No. 11105/05 Decree No. 5591/05	Resolutions not issued by a Minister or political entity.	Plants Animals Microorganisms Vaccines	CNB	Majority vote	CTNBIO (27 members)	Government Academia Civil Society
				CTNBio	Absolute majority vote and favorable vote of at least two thirds of members for deliberations on commercial approvals.		

Table 3.5. (cont'd).

Country	Framework	Particularities of Risk analysis	GE organisms regulated	Resolution issued by:		Risk analysis done by:	
				Authority	Firms	Advisory body	Members
PAR	Decree No. 12706/08	Environmental impact evaluation	Plants Livestock products	Ministry of Agriculture and Livestock (MAG)	1	COMBIO (9 members)	Government Academia Civil Society
URU	Decree No. 353/008	Independent commissions for risk assessment and risk management.	Plants	GNBio composed of the Ministers of: MGAP, MEF, MVOTMA, MSP, MIEM and MRREE.	6	CGR (6 members)	Government
						ERB (1member)	Government
						CAI (9 members)	Government Academia Non-state public entities
						Ad Hoc Groups (35 members)	Government Academia Non-state public entities

With regard to the Cartagena Protocol, Brazil and Paraguay ratified it in 2003 and 2004 respectively, while Uruguay recently ratified it (2011) and is in the three-month period of the process for its entry into force (BCH website, 2013; Uruguay, 2011). Argentina has instead only signed the Cartagena Protocol and so far there has not been political support for its ratification (Argentina, 2000). The Protocol has obligations to fulfil when ratifying that may help to harmonize the risk analysis procedures (Secretariat of the Convention on Biological Diversity, 2000).

With respect to the institutional organization of the regulatory systems, in each of these countries there are commissions or committees that address the assessment of risk to human, animal and plant health, and the environment, but their structure and operation vary from country to country. The Uruguayan framework seems to be the most complex in terms of different authorities involved in the decision making, and the number of signatures required for an authorization (Table 3.5). The complexity is observed in terms of coordination and time consumed in the requirement of the six GNBio's Ministers signatures for the authorization of any of the categories of uses. In Argentina, if there are no objections from CONABIA, CTAUOGM (SENASA) and DMA, the Secretariat of Agriculture (SAGYP) makes the final decision whether to authorize the GMO or not (Figure 3.1). Only one signature is also required in Paraguay, from the Ministry of Agriculture and Livestock (MAG) who receives the opinion from COMBIO, SENAVE, SENACSA and SEAM as appropriate (Figure 3.3). In the case of Brazil, the National Biosafety Technical Commission (CTNBio) has the authority to decide regarding applications for research and commercial release if there is a majority of votes.

Possibilities for Uruguay to positively impact the process efficiency would be to reduce the number of authorities from GNBio required to sign the authorizations or to enable the Risk Management Commission (CGR) to authorize contained field trials for research and

cultivar registration purposes. GNBio has recently authorized CGR to renew future permits for counter season seed production and research field trials (Uruguay, GNBio Resolution No. 46, 2012).

There is variation in the application review process among the different countries. In Argentina the environmental and food/feed safety evaluations follow different administrative routes. The applicant submits an application form at the office of the Biotechnology Directorate with environmental information, at the office of SENASA with food and feed safety information and at the office of Directorate of Agrifood Markets with economic information (Figure 3.1). Another particularity of the Argentinean framework is that delegates from industry (private sector), participate in the technical advisory commission (a delegate cannot participate when an application is from the company he represents). Brazil includes specialists representing the civil society as a distinct element compared to Argentina, Paraguay and Uruguay. Brazil is the only country where the resolutions are issued by a technical advisory body (CTNBio) and not by Ministers or political figures.

There is also lack of uniformity with respect to requirements for environmental impact evaluation. Environmental legislation in Paraguay requires environmental impact evaluation in addition to the environmental risk assessment performed by the advisory body COMBIO. The environmental impact evaluation is performed in parallel by the Secretary of Environment (SEAM). In Brazil, CTNBio also could request an environmental license, if it considers the commercial use of a GE plant as a potential factor that degrades the environment, but it is not mandatory as in Paraguay.

With regard to risk management, one difference indicated in Table 3.4 is the independence of the risk management phase. In Uruguay there is a clear separation in ERB/CAI vs GNBio/CGR performing the risk assessment phase and the risk management phase, respectively. Except in Uruguayan where there is a specific commission for risk

management with political delegates for advising ministers, in the other countries the risk management is only partially independent from risk assessment. The Secretary of Agriculture in Argentina, the Minister of Agriculture in Paraguay and the National Biosafety Council (CNBS) of Brazil can be considered exclusively "risk managers". However "risk assessors" also perform risk manager's tasks; thus they not only recommend approval of applications based on environmental and health safety, but also determine risk management measures, monitor compliance of biosafety conditions, and are responsible for the permits track.

Another difference indicated in Table 3.4 regarding risk management is the consideration of the precautionary principle or a weight-of-evidence approach in the decision making process. Argentina does not apply the precautionary principle in its regulatory system and uses the International Standards for Phytosanitary Measures (ISPM) No. 11 as criteria for risk analysis (FAO, 2009a). On the other hand, Brazil has incorporated the precautionary principle in its Biosafety Law to guide the decision making process regarding environmental risk (Brazil, 2005). Paraguay has incorporated the precautionary principle in the Biosafety Bill presented to the Parliament. In Uruguay it is being discussed whether it would be necessary to include the precautionary principle in the biosafety law. The precautionary principle is already incorporated in the Uruguayan General Law of Environmental Protection No. 17283 (Uruguay, 2000) and in the Law that ratifies the Convention on Biological Diversity (CBD) No. 16408, (Uruguay, 1993). Besides, Uruguay recently ratified the Cartagena Protocol. Although the Cartagena Protocol establishes a precautionary *approach* that is more flexible than a *principle*, Uruguay enacted a specific law for the Cartagena Protocol (Uruguay, 2011). These regulations will need to be coordinated with the biosafety law.

As mentioned in the Literature Review of this thesis, the precautionary principle can delay a decision based on hypothetical threats of harm even without evidence of occurrence

or negligible probability of occurrence (MacKenzie, 2000). The precautionary principle orients the process of decision making, which is always subject to uncertainty (MacKenzie, 2000). It is worth mentioning that the Cartagena Protocol refers to the "precautionary principle" as a "precautionary *focus*" that is more flexible than a *principle*. Besides the Protocol includes the requirement of a risk assessment for the decision making, which limits the application of precaution without any underlying basis. Generally within the MERCOSUR countries a weight of evidence approach, rather than the precautionary principle, is applied for the decision making (FAO, 2007). Thus, in the regulatory systems that have included the precautionary principle, it is not influencing the dynamic of the risk analysis. Table 3.6 shows the high number of events deregulated by Brazil in a short period of time even with incorporation of the precautionary principle into its Biosafety Law.

Table 3.6. Average of applications approved per year in the period 1995-2013 by category of use and country.

Country	**Average of applications approved per year in the period 1995-2013 by category of use and country**			
	Field trial	**Total in the period**	**Commercial (*)**	**Total in the period**
Argentina	105 ± 49.1	1790	1.6 ± 1.8	28
Brazil	--- (**)	---	2.0 ± 3.0	36
Paraguay	0 ± 0.2	1	0.3 ± 0.9	6
Uruguay	1.1 ± 1.4	20	0.8 ± 1.7	14
(*) The "commercial" category includes also events approved as of March 2013 (**) Brazil data for field trials are not available.				

The monitoring and control of the compliance of the biosafety conditions and ordinances established for commercial use of GE organisms are administrated differently in each country. In Argentina it is by the National Institute of Seeds (INASE) and SENASA according to their respective responsibilities. In Brazil it is by existing agencies within the

public administration with those competences that form the Registration and Monitoring Bodies and Entities (OERF), and in Paraguay by the Biotechnology Coordination, a specific body created for this purpose within SENAVE. In Uruguay it is by existing bodies within the ministries of the GNBio according to the risk management commission (CGR) ordinances which to date include the General Directorate of Agricultural Services (DGSA) and the National Institute of Seeds (INASE). Post-market monitoring has been required in Brazil (Brazil, 1998, 2005, 2007, 2008) but recently was approved the possibility to ask for its exemption (Brazil, 2011). It is being discussed its implementation in Uruguay and it is not required in Argentina and Paraguay.

Regarding risk communication, Brazil and Uruguay have formal procedures established. Argentina has not at this time set procedures for risk communication.

Regarding the average number of applications submitted and events deregulated in the MERCOSUR countries, Tables 3.6 and 3.7 list the average number of applications submitted in the last 17 years and the events deregulated in each country, respectively, as a way to analyse the dynamic of each regulatory system. Figure 3.4 and 3.5 shows the accumulated number and total number per year of commercial approvals over the period 1995-2011. With regard to the number of applications approved per year for field trials, there is an asymmetry comparing Argentina with Paraguay and Uruguay. Although data from Brazil was not obtained, it is estimated that in Brazil the average number of approved applications for field trials per year is similar to or higher than in Argentina.

Brazil is the country with the highest average number of applications approved for commercial release per year (1.9), followed by Argentina (1.2) and then Uruguay (0.5) and Paraguay (0.1). The average number of applications approved for commercial release in Brazil has rapidly increased in the last three years as shown in Figures 3.4 and 3.5. Since 1997 Argentina has had a steady increase over time with an average between 0.8 and 1.2

events approved per year. Argentina led with regard to the number of deregulated events until 2007, but was then surpassed by Brazil for number of approved events for commercial release. Paraguay and Uruguay have maintained an average lower than 0.4 deregulated events per year, with an increasing trend in Uruguay in the last two years.

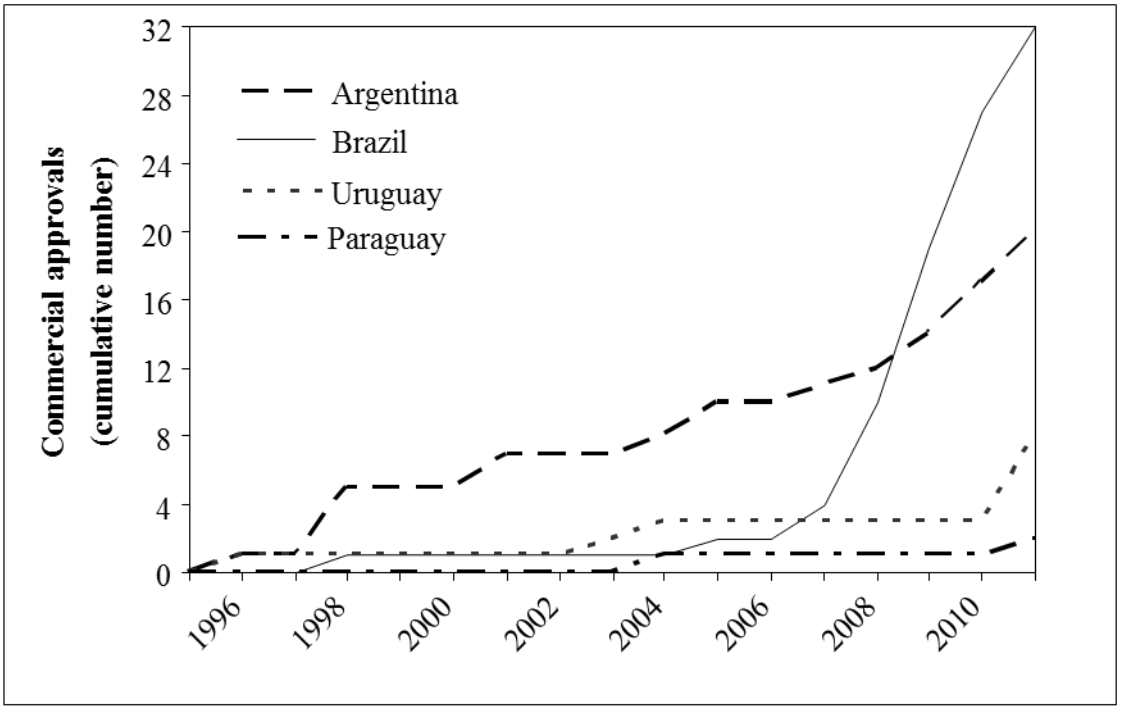

Figure 3.4. Accumulated number of commercial approvals over the period from 1995-2011 in MERCOSUR countries.

Both in Brazil and Uruguay the increase in the number of approvals coincides with adjustments in the regulatory system. In Brazil it corresponds with the promulgation of the Biosafety Law that re-structures CTNBio requiring to be comprised by experts with notable scientific and technical learning that hold a doctorate degree and are currently active professionals (Brasil, 2005). In the case of Uruguay the specific change that occurred around the relevant year is the promulgation of the Decree 353/008 after the moratorium period (Uruguay, 2008). As mentioned in first section of this chapter, Argentina has recently

adjusted specific aspects of its regulatory system in order to improve its efficiency. Protocols for counter season soybean seed production and field trials were adjusted. Also the way to present the information by the applicant and criteria for the analysis of stacked events were adjusted (Argentina, 2011).

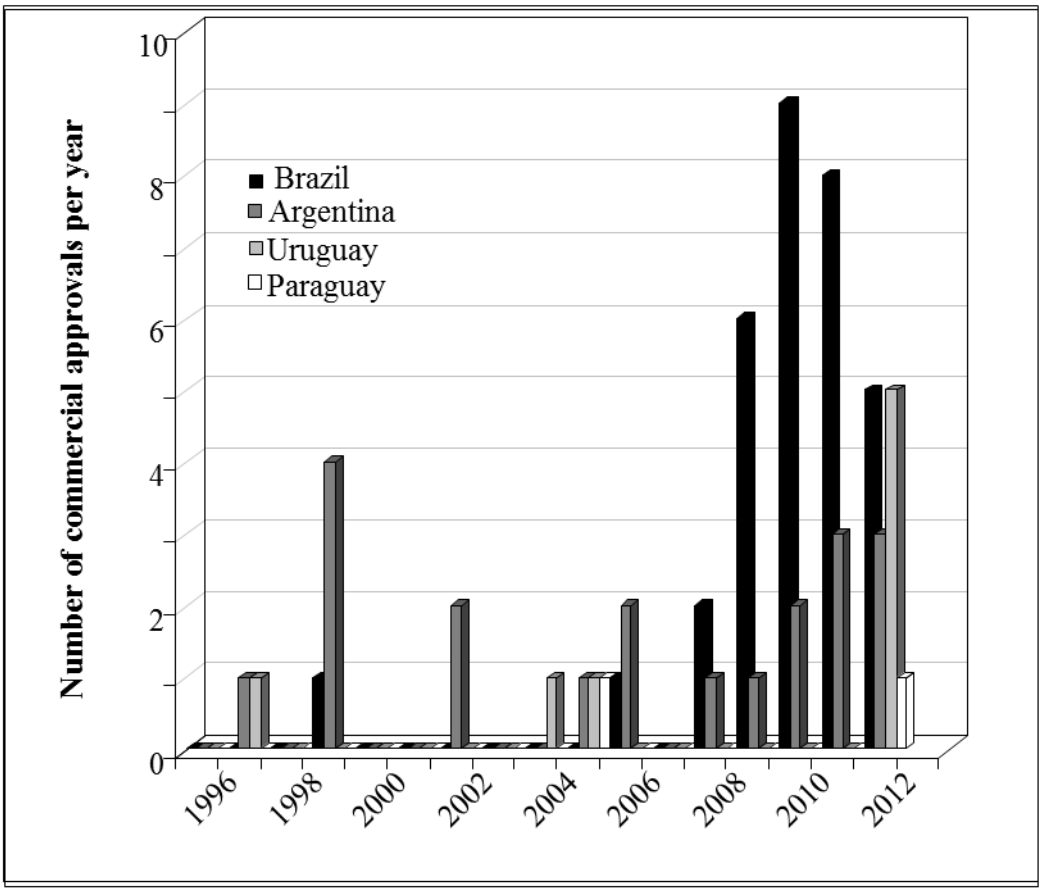

Figure 3.5. Total number of commercial approvals per year in MERCOSUR countries from 1995 to 2011.

In Figure 3.5 is observed the unevenness among years and countries with a concentration in the last five years. Brazil has the highest number of deregulated events with a total of 32 followed by Argentina with 20 events. In a second level is Uruguay with eight events approved for commercial release and then Paraguay with one event approved in soybean. Brazilian political and economic support for biotechnology allowed this increased in deregulated events and the development of the first national GE crop developed at the

Brazilian Enterprise for Agricultural Research (EMBRAPA for its acronym in Portuguese), a bean resistant to the golden mosaic virus approved in 2011 (Brazil, 2011).

Table 3.7. Events approved for commercial use (deregulated) by crop in the MERCOSUR countries.

Crop	**Event**	**Country**			
		Argentina	**Brazil**	**Paraguay**	**Uruguay**
Soybean	40-3-2	√	√	√	√
Soybean	MON89788XMON87701		√		
Soybean	A2704-12	√	√		
Soybean	A5547-127	√	√		
Soybean	BPS-CV127-9		√		
Total deregulated events in soybean		*3*	*5*	*1*	*1*
Corn	176	√			
Corn	T25	√	√		
Corn	MON810	√	√		√
Corn	BT11	√	√		√
Corn	NK603	√	√		√
Corn	GA21	√	√		√
Corn	TC1507	√	√		√
Corn	MIR162	√	√		
Corn	MON89034	√	√		
Corn	MON88017	√	√		
Corn	BT11XGA21	√	√		√
Corn	MON810XNK603	√	√		√
Corn	TC1507XNK603	√	√		
Corn	TC1507XMON810		√		
Corn	MON89034XNK603		√		
Corn	MON89034XMON88017	√			
Corn	BT11XMIR162XGA21		√		
Corn	MON89034XTC1507XNK603		√		
Corn	TC1507XMON810XNK603		√		
Total deregulated events in corn		*14*	*17*	*0*	*7*

Table 3.7. (cont'd).

Crop	Event	Country			
		Argentina	**Brazil**	**Paraguay**	**Uruguay**
Cotton	MON531	√	√	√	crop not planted
Cotton	MON1445	√	√		
Cotton	MON531XMON1445	√	√		
Cotton	LLCOTTON25		√		
Cotton	281		√		
Cotton	MON15985		√		
Cotton	GHB614		√		
Cotton	T304-40XGHB119		√		
Cotton	MON88913		√		
Total deregulated events in cotton		*3*	*9*	*1*	---
Bean	EMBRAPA5.1		√		
Total deregulated events in bean		*0*	1	*0*	*0*
TOTAL DEREGULATED EVENTS		**20**	**33**	**2**	**8**

In summary, the general criteria used for the application of the risk analysis methodology for genetically engineered crop technology as described for Uruguay in Chapter 2, are similar throughout MERCOSUR countries (FAO, 2007). Common elements are observed mainly in risk assessment criteria and information requested in the application form for environmental and food safety evaluation. The differences observed in risk assessment are regarding who does it, how it is organized, what principles are followed. Major differences among the regulatory systems are in structure and operation of aspects of risk management and risk communication. While these differences could mean barriers for the possibility of a regional system among MERCOSUR countries, the common elements found open an opportunity for harmonization of procedures. The greater complexity of the Uruguayan regulatory system with regard to its operation, results from the number of authorities required for authorizations. Possible solutions emerged when comparing the national systems of MERCOSUR countries.

REASONS FOR THE NEED OF HARMONIZATION AND ATTEMPTS THAT HAVE BEEN MADE

Although similar in some ways, the different institutional organizations and administrative procedures among the four countries analyzed, make it difficult to coordinate the approval of GE organisms for commercial use in the region (GAHBA, 2008, 2009). There are also different production, ecological, and socioeconomic conditions that cause growers to have different interests or needs for GE crops and traits. The market's size and dynamic also can motivate different interests by the developers of the technology, and public opinion about genetically modified organisms varies in the region. All this in part explains the asymmetric number of events approved for commercial use among these countries (Table 3.7).

However, the regulatory system is a complex, long and expensive process. Harmonization arises as a possible way to achieve a more efficient and effective framework in the use of economic, infrastructure and human resources, eliminating at the same time commercial problems of asynchronous authorizations (GHABA, 2009; OECD, 1995, 2012). When an event is authorized in one country, several issues arise as a consequence since there is an increased risk of *low-level* presence of non-authorized material (but approved in the exporter country), which could restrict or even interrupt trade between countries. It may reduce the offer of basic raw material for food or feed increasing the production costs. Importation or cross border transfer of unapproved products, either unintended or intentional, resulting in the illegal introduction of events as food, feed or seeds, may occur, especially close to border areas. For these reasons, it is among the objectives of the MERCOSUR's countries to harmonize to the best of the possibilities, the biosafety regulatory systems (MERCOSUR, 2003).

Harmonization, in the sense of "to accept mutually the authorizations among different countries" while seeming an ideal situation, requires a high level of political, commercial and

risk analysis criteria coordination among nations. The European Union is an example of harmonization at this level, an approach which has different layers of complexity. The regional framework that is in place in the European Union (EU), dates back to 1990 (Directive 90/220/EEC). The EU system was built with the elaboration of new regulations specific for genetically engineered organisms. The legislation is currently based on the GM Food and Feed Regulation 1829/2003/EC that replaces the former regulation on novel food No.258/97, on the Directive 2001/18/EC finished in 2002 that replaces Directive 90/220/EEC of 1990 and on a series of Guidelines elaborated by the European Food Safety Authority (EFSA) for risk assessment.

The European Commission (EC) that centralizes the operating system coordinating with each EU Member State regulatory system was created by Directive 90/220/EEC. Each EU Member State has its own regulatory system and therefore the Directive can have different mechanisms and criteria for its implementation. The legislation establishes that any EU Member State that receives an application for authorization of the use of a GE organism, must forward the results of the initial risk assessment to the European Commission (EC). From the EC the application is put under consideration to all EU Member States. If there are objections or open questions regarding the initial assessment performed by the national agency where the application was submitted, while the application is being reviewed by each EU Member State regulatory system, EC consults with the scientific panel of the European Food Safety Authority (EFSA) regarding food and feed safety and environmental risk as appropriate according to the proposed use. Applications for environmental release can be submitted, as explained above to a Member State national agency, or may be submitted directly to EFSA.

EFSA was created in 2002 as the central European authority for the scientific evaluation of food and feed safety (European Union Regulation 178/2002). EFSA is located

in Parma, Italy and operates separately from the European Commission. EFSA operates eight scientific panels composed of independent scientist from different EU Member States. One of these panels is the GMO panel with specialists on GE organisms and genetically modified food and feed. The EFSA's criteria for environmental risk assessment and food safety are the same as those applied by MERCOSUR's countries. Generally within the MERCOSUR´s countries EFSA's criteria are taken as a guide for risk assessment due to its scientific robustness and because Europe is the destination of exports of many GM products.

EFSA's scientific opinion is forwarded to the EC, which embraces other components in its opinion such as political, socio-economic and cultural factors, taking the final decision. If there are objections from any Member State, the application enters into a field of exclusively political action involving the Regulatory Committee (RC) and the Council of Ministers (CM). The EC forwards the application to the Regulatory Committee; it then moves back and forth between the Regulatory Committee and the Council of Ministers until the application is accepted, requiring 2/3 of votes, or it is rejected.

The complexity of the European regional system refers on the one side to the coordination among Member States and on the other to the differences in the implementation of the directive in each Member State (Ostrovsky *et al*., 2007). , Difficulties faced in the linkage between the scientific opinion of the European Food Safety Authority (EFSA) and the political opinion of the European Union executive body, i.e., the European Commission, have obstructed the system, resulting in only two authorized events for cultivation at present, insect resistance MON810 corn and EH92-527-1 potato with altered starch composition for starch production (non-food) (COMPASS web site, 2011). Under the EU regulation, the authorization licensed by the EC is valid in all Member States and can only be superimposed in a specific country if it demonstrates a scientific reason to restrict its use. Thus, oppositions to authorizations are disputed at the EC resulting in the deadlock of the system. As a way to

unlock the flow of the system, a proposal from the European Commission is currently being discussed in which countries that oppose an event would be able to ban it from cultivation on non-scientific reasons.

In the countries of the Regional Trade Agreement MERCOSUR, the European level of harmonization, although theoretically possible, is functionally impossible to achieve. Several efforts have been implemented to analyze harmonization possibilities in the region including: i) the creation of an *Ad Hoc* Working Group on Agricultural Biotechnology (GAHBA) within the Agriculture Group No. 8 of Common Market Group of MERCOSUR; ii) the creation of the Group No.5 on Public Policy in Biotechnology and Biosafety of the Southern Agricultural Council (CAS), and iii) the project titled *"Development of reference tools for the risk management of biosafety in the expanded MERCOSUR"* (FAO TCP/RLA/3109).

The GAHBA within the MERCOSUR had as terms of references: the evaluation and elaboration of alternatives to harmonize and coordinate the biosafety regulatory systems as well as norms related among the MERCOSUR's countries (GAHBA, 2009). From the diagnostic analysis performed and interchange of information, the general conclusion reached by the Group was that there were practical limitations that make harmonization hard to achieve at least in the short term (GAHBA, 2009). It was observed that it would be difficult to harmonize the frameworks of each country in terms of their structure and operation, given the different traditions in the regulation and national standards. Further complications arise from the different agricultural, ecological, socio-economic and political situations of each country. Companies that commercialize GE crops have different interest in each country's market, and farmers have different necessities and interests in GE products. In addition, public perception as well as general response to change is different among the countries examined (GAHBA, 2009).

The GAHBA Group recommended that proposals for harmonization and coordination should focus on actions aimed at the adoption of internationally recognized principles and guidelines, in particular those for risk assessment, such as the Cartagena Protocol, Codex Alimentarius and the International Convention for the Protection of Plant (item 1.3 of the International Standard for Phytosanitary Measures No. 11). The GAHBA Group proposed that each country apply its own criteria for the resolution of matters such as the structure of the decision-making process and implementation of specific issues (GAHBA, 2007).

The Group No.5 (GT5) on Public Policy in Biotechnology and Biosafety of the Southern Agricultural Council (CAS) was created in 2004 within the Coordination Network for Agricultural Policies (REDPA for its acronym in Spanish). CAS is composed of the Ministers of Agriculture of the MERCOSUR's countries plus Bolivia and Chile, what is called the "expanded MERCOSUR". The technical administrative secretariat is performed by the Inter-American Institute for Cooperation on Agriculture (IICA for its acronym in Spanish). The terms of references for GT5 were not as specific with regard to harmonization as were GAHBA's. The objective of GT5 is to advise CAS's Ministers on biotechnology public policy. The GT5 has elaborated and maintained updated a publication with the description of the Biosafety Regulatory Systems and status of commercial approvals in the countries of CAS, the 'expanded MERCOSUR', (CAS, 2010) as an information source for the analysis of harmonization possibilities. The basis for this publication was the information gathered by GAHBA (GAHBA, 2007, 2008) and a questionnaire sent to the regulatory offices asking regarding institutions involved in the regulatory system, requirements for commercial authorizations, approach for risk assessment of stacked events and if the country applies coexistence measures.

The aim of the FAO project was to discuss and reach regional agreed on minimum reference criteria for the management of biosafety. Several workshops and courses where

carried out during this project. Two regional workshops, in Paraguay and Brazil, were performed for the discussion and elaboration of a final document with agreed technical tools and criteria for risk assessment and risk management. This document also includes a sample of an application form (FAO, 2009b). The FAO final document, although useful to the countries of the expanded MERCOSUR, has no binding power, leaving to each country´s criterion its application and use. Uruguay used the FAO project's final document for the development of its own application form and discussion of criteria for risk assessment and risk management, since at the time of the FAO project, Uruguay was at the beginning of the process for the implementation of a new regulatory system. Other activities in the framework of the FAO project included a regional workshop on biosafety research held in Argentina and two courses, on introduction to risk assessment and risk communication held in Uruguay (FAO project TCP/RLA/3109).

The GAHBA Group and the FAO project ended in 2009. The GT5 Group remains active and its last meeting was in Paraguay last August (CAS, 2011). Although the GT5 does not have specific objectives on harmonization, it is important that there is a group at a regional level on biotechnology and biosafety. The fact that GT5 Group was created and is being maintained demonstrates political interest for the matter in the region.

SUGGESTIONS FOR FURTHER PROGRESS TOWARDS A REGIONAL SYSTEM

While the above mentioned facts are bottlenecks to move towards a regional system, other actions can be taken to reach harmonization, although not as extensive as for the EU regulatory system. Harmonization could be reached for specific procedures to minimize the consequences of the asymmetry. Three suggestions are proposed in this section with actions toward harmonization while maintaining an effective and competent risk analysis process for safe introduction of genetically engineered crops in each of the MERCOSUR's countries.

The first suggestion is the re-establishment of the *Ad Hoc* Group in Agricultural Biotechnology (GAHBA) that finished its tasks in 2009 (GAHBA, 2009). The second suggestion has to do with the implementation of a regional advisory body for food and feed safety assessment. The third suggestion is the creation of a coordinated system for field trials as the "first and second phase" approach applied in Argentina.

Regional Ad hoc Group on agricultural biosafety of GE organisms

The *Ad Hoc* Group on Agricultural Biotechnology (GAHBA) was created within Agriculture Group No. 8 of the Common Market Group of MERCOSUR. GAHBA was comprised of regulators from the regulatory agencies of Argentina, Brazil and Paraguay. The participants from Uruguay were from the Unit of International Affairs of the Ministry of Agriculture, which participated with development of the biosafety framework. The richness of GHABA was the qualifications of the individuals involved with regard to regulatory issues and on the substance of the topics they dealt with. The documents elaborated by GHABA helped to inform this analysis since they contain valuable information regarding the operation of the regulatory systems that is not published or written in the legislation. At the regional level, groups created from MERCOSUR have binding and mandatory force. Thus, the request to conform the GAHBA group again, would create a regional qualified working group for the discussion of the current agenda topics and elaboration of regional proposals, with the eventual possibility of implementation in the countries of MERCOSUR.

The GT5 within the Southern Agricultural Council (CAS) is also a regional working group for discussion. However, the elaboration of regional proposals for harmonization is not an objective of GT5, and GT5 does not have mandatory force. Agreements signed by Ministers at CAS have political and moral binding force but it does not mean that countries will necessarily change their systems. On the other hand, if GHABA forwards a proposal of

harmonization that is accepted by the Common Market Group of MERCOSUR, MERCOSUR's countries have the obligation to incorporate the agreed upon harmonization into each country's legal system. This explains why GHABA acted with caution when making recommendations on harmonization, since decisions by MERCOSUR require a strong political will.

At the time that GAHBA was reviewing MERCOSUR's countries' regulatory systems for harmonization, they had already independently developed biosafety policies according to their particular national regulations, as well as agricultural, ecological, socioeconomic and political situations. Although there are similar criteria, procedures and operation are different, as described in previous sections, which led GHABA to conclude that harmonization was functionally impossible to achieve (GHABA, 2009). However, GAHBA recommended implementing a permanent mechanism of interchange of information, interaction and mutual cooperation among the expanded MERCOSUR regulatory agencies as a way to build confidence among authorities to move toward harmonization by joining efforts of economic and technical/scientific human resources (GAHBA, 2009).

From the current agenda of the regulatory agencies, the issue of *low level* presence and *adventitious presence* (that is related to coexistence) are urgent topics to approach regionally. *Low level* presence refers to the necessity to establish tolerance limits for presence of events that are not in the importing country but are approved in the exporting country. *Adventitious presence* refers to the incidental, small amounts of approved GE material in conventional commercial product (seed, feed, food) (SAA, 2010). There are currently no standards in the region either for *low level* or *adventitious presence.* In May, 2010 there was a specific Workshop on regional approaches to *low level* and *adventitious presence* organized by the Seed Association of the Americas (SAA) in which the main conclusions, shared by GAHBA (2009), were that zero tolerance is functionally impossible, and that it is necessary

to establish regional standards under international guidelines for risk assessment (SAA, 2010). In addition, the Working Group on Biotechnology of the Organization for Economic Cooperation and Development (OECD), on Harmonization of Regulatory Oversight in Biotechnology, is preparing a document with recommendations on how to proceed when a *low level* presence situation happens.

On the other hand, adventitious presence is related to coexistence. Brazil has a specific policy on coexistence that is under review (Brazil, 2007) and Uruguay is discussing its implementation as was explained in chapter 2 of this thesis. There is a general regional interest in coexistence manifested at the GT5 group of CAS (Personal Communication, Ing. Agr. Benech E., Uruguayan representative at GT5 and president of the Uruguayan risk management commission on biosafety (CGR), 2011).

These topics involve issues at a regional level justifying consideration by MERCOSUR. These topics are being discussed individually in each MERCOSUR country, but there is not yet a final decision, opening the opportunity for harmonization. Uruguay could promote the request to re-convene the GAHBA group based on the knowledge that this is an interest also for the other MERCOSUR countries (Personal Communication, GHABA's members from Argentina, Brazil and Paraguay (Godoy P., Cohelo M., Rojas L., respectively), 2010).

Uruguay took a first step toward creating a formal agreement between regulatory systems, in this case with Chile. A formal agreement was established between the Uruguayan and Chilean regulatory systems for interchange of information and mutual cooperation that is already functioning (CGR, 2010). In the framework of this agreement there has been interchange of information regarding biosafety protocols for winter seed production. A visit of Uruguayan regulators to Chile was held in September 2011 in which winter seed production facilities were observed with regard to biosafety conditions; regulatory procedures

also were discussed (CGR, 2011). Although the agreement with Chile is not about harmonization, it is a first step toward harmonization feasibility. Mutual collaboration and interchange of experience allow regulators to know each other regarding capacities, processes and robustness of resolutions issued by each country. This agreement is being extended to the other countries of the expanded MERCOSUR. The Ministry of Agriculture of Uruguay has recently signed a memorandum of understanding with the Ministry of Agriculture of Argentina, regarding sharing of information, capacity building and research in biosafety and biotechnology (Uruguay, 2011).

Regional advisory body for food and feed safety assessment

International standards and trade require that decisions regarding food and feed safety be based on solid scientific information. The criteria and information required by all MERCOSUR's countries for food and feed safety are the same, and follow the internationally recognized guideline *Codex Alimentarius* (GAHBA, 2007, FAO, 2009b). This requirement has led authorities to be aware of each country's limitations in capacities and infrastructure for risk assessment as well as the costs implied. From one side it is the necessity to enhance human resources and capacities which could be regionally coordinated. Furthermore, it is also the technical conviction that a regional approach is the right way to comply with the information required with the necessary quality and efficiency.

Based on the above reasons it is proposed that a regional scientific and technical committee or commission be created that could act as a regional advisory body regarding food and feed safety of GE organisms. The structure and operation of this advisory body could be discussed within the GAHBA group proposed in the previous item. A similar idea was supported at the Workshop on research in *biosafety* organized in the framework of the FAO project TCP/RLA/3109 (FAO, 2010) whose participants included regulators, scientists

from academia and research institutes and representatives from the governments of MERCOSUR countries and Bolivia and Chile (was is called as the "expanded MERCOSUR).

Authorization for food and feed could be issued regionally independently from authorization for planting, i.e. when an event is authorized in one country for all uses, it is authorized in the other countries for food and feed, although restricted for cultivation. This is a possible scenario since there are different interests in events among MERCOSUR's countries as explained above. This kind of split approval, recommended also by GAHBA (2009), would allow an event to be authorized regionally for food and feed, but each country to decide whether to approve it or not for planting. To be able to approve a GE plant for food and feed but not for planting, would also eliminate the commercial troubles generated by the asymmetric number of events that are approved for commercial use among MERCOSUR's countries (Table 3.7).

The European Food Safety Authority (EFSA) and the bi-national government agency Food Standards Australia New Zealand (FSANZ) list requirements for GM food and controls labeling that could provide examples from which MERCOSUR could develop a multi-country approach for harmonization. A regional agency for health risk assessment could be created to enable events to obtain wide authorization in MERCOSUR for animal feed and human consumption. This proposal has the challenges of defining a common application form, at least regarding food and feed safety evolution, and strong political support from the national health authorities to the suggested advisory body that would perform the safety evaluations.

Regional coordinated system for contained field trials

Authorizations for commercial use take into account, apart from biosafety issues, several other factors related to each country's particular agricultural, ecological,

socioeconomic and political situation, making it impossible, at least in the short term, to achieve harmonization with regard to commercial approvals. The proposal described in this item refers to the possibility of coordinating at regional level contained field trials as a way to synchronize future deregulations. The advantage of coordination for field trials lies in the ability to make the risk assessment process more efficient and effective to save time, work and money for regulatory systems, and to better coordinate product development for the region by helping to avoid asynchronous approvals.

Although there are differences among regulatory systems in their structures and operation, generally all of them adopt the same criteria for environmental risk assessment (FAO, 2009b). Field trials for efficacy tests and environmental risk assessment could be performed in the same time frame by MERCOSUR countries and information generated could be shared. Consensus documents regarding plant biology and molecular characterization could be used for risk assessment. Sharing of information could be formalized regarding protocols of field trials for efficacy tests and for environmental risk assessment. Biosafety protocols could be adjusted for each country as needed due to different environmental conditions.

Based on the common elements used for environmental risk assessment, a regional system for environmental risk assessment similar to the approach used by Argentina with "first" and "second" phases of evaluation could be adopted. The "first phase" could be coordinated regionally, while an eventual "second phase" of analysis for deregulation would be performed individually by each country. A common system could be established for contained field trials as a "first phase" of evaluation that includes efficacy tests as well as parameters to test risk hypothesis that may arise in an eventual commercial release.

The field trials performed under the so called "first phase" of the Argentinean regulation No.39/2003 are mainly trials to test the efficacy of the events. The efficacy of an

event is evaluated by looking at the agronomic performance, its commercial success and whether varieties maintain market interest (Mihaliak, 2002). Trails are also performed to provide information about factors that may influence environmental safety such as: vigor, flowering time, root strength, weediness potential, and possible effects on non-target organisms. Since agronomic and environmental safety parameters are highly influenced by environment it is important to perform trials in each receiving environment. Thus, the "first phase" would have two parts, one would be the original trial with a common protocol for efficacy tests and additional biosafety parameters included as appropriate. The second part of the first phase would be a review of information from the original trial to determine if there are unanswered or environment specific questions needed for specific countries if the event would move forward to the "second phase".

A regional technical commission could coordinate the field trials of the "first phase" and process the data. It could be possible to develop procedures and methodologies to assess the environmental risk meeting national and international obligations, including the Cartagena Protocol. Once an application is submitted to a national agency, it is forward to a regional commission and to the rest of the MERCOSUR's countries. The regional commission would set a time frame for each country to issue the permit for field trials and to define whether additional data is needed from local field trials to eventually authorize the commercial use of the GE plant. The structure and operation of the regulatory system of each country would remain the same, assuming they met regionally agreed upon standards.

The limitation of this proposal would be if an event were developed with a specific trait for one country's particular situation. In this case, field trials performed in countries where there is no interest for an eventual commercial release, could be used to generate risk safety information to approach the inconvenience of low level presence.

This approach, although incorporating a regional commission for the coordination of the first phase evaluation, offers efficiency to the regulatory system, predictability to the applicant and helps to increase the synchrony of commercial approvals among MERCOSUR's countries. Events that pass to a "second phase" of risk analysis could be analyzed independently since each country will shape the final decision to its environment, economic, politics, social and legal situation.

APPENDIX

APPENDIX

Table A3.1. List of institutions represented on the advisory bodies by country. The color code refers to the institution origin: government (□), academia (□), civil society (□) and private sector (□)

INSTITUTIONS BY COUNTRY	
ARGENTINA	
Biotechnology Directorate	
1	General Director
2	Technical Coordinator
3	Scientific Consultant on biosafety
4	Scientific Consultant on regulatory issues
CONABIA	
1	National Institute of Agricultural Technology (INTA) of MAGYP, specialist in plants.
2	National Institute of Agricultural Technology (INTA) of MAGYP, specialist in animals and/or microorganisms.
3	National Council Scientific and Technical Research Ministry of Science, Technology and Innovation, specialist in plants.
4	National Council Scientific and Technical Research Ministry of Science, Technology and Innovation, specialist in animals and/or microorganisms.
5	National Institute of Seeds (INASE), one representative from the Coordination of Special Projects on Biotechnology.
6	National Institute of Seeds (INASE) of MAGYP, one representative from the Laboratory of Molecular Markers.
7	National Health Service and Food Quality (SENASA). Specialists in plants.
8	National Health Service and Food Quality (SENASA), specialist in microorganisms.
9	National Health Service and Food Quality (SENASA), specialist in animals.
10	Secretariat of environment and sustainable development.
11	Secretary of Policies, Regulations and Health Relationships of the Ministry of Health.
12	National Institute for Fisheries Research and Development (INIDEP).
13	Biotechnology Office of SAGyPA (now corresponds to the Biotechnology Directorate of SAGYP), the General Director.
14	Biotechnology Office of SAGyPA (now corresponds to the Biotechnology Directorate of SAGYP), the Technical Coordinator.
15	University of Buenos Aires, College of Agriculture.

Table A3.1. (cont'd).

16	University of Buenos Aires, College of Natural Sciences.
17	National University of La Plata, College of Agriculture.
18	National University of La Plata, College of Science.
19	National University of Rosario. Specialist in areas related to biotechnology.
20	National University of Comahue. Specialist in areas related to biotechnology.
21	Argentine Association of Ecology (ASAE).
22	Argentine Forum of Biotechnology (FAB).
23	Biotechnology Committee of the Argentine Seed Association (ASA).
24	Chamber of Agricultural Health and Fertilizers (CASAFE).
25	Argentine Chamber of Industry of Veterinary (CAPROVE).
CTAUOGM of SENASA	
1	National Health Service and Food Quality (SENASA)
2	Ministry of Health
3	National Council of Scientific and Technical Research (CONICET)
4	Food Area of the Secretariat of Agriculture, Livestock and Fisheries (SAGYP)
5	National Advisory Commission on Agricultural Biotechnology (CONABIA)
6	Academia
7	Food traders
8	Groups of Growers
9	Consumers Organizations.
10	Biotechnology Committee of the Argentine Seed Association (ASA).
11	Argentine Forum of Biotechnology (FAB).
12	Chamber of Food Products (COPAL)
DMA	
---	Not specified, members of the office.

Table A3.1. (cont'd).

BRAZIL	
CNBS	
1	Minister of State Chief of the Civil House Office of the Presidency of the Republic (chairperson of CNBS),
2	Minister of State of Science and Technology
3	Minister of Agrarian Development,
4	Minister of Agriculture and Supply.
5	Minister of Justice.
6	Minister of Health.
7	Minister of Environment.
8	Minister of Development Industry and Foreign Trade.
9	Minister of Foreign Affairs.
10	Minister of Defense.
11	Special Secretary for Aquaculture and Fisheries of the Presidency of the Republic
CTNBio	
1	Ministry of Science and Technology.
2	Ministry of Agriculture and Supply.
3	Ministry of Health.
4	Ministry of Environment.
5	Ministry of Agrarian Development.
6	Ministry of Development, Industry and Foreing Trade.
7	Ministry of Defense.
8	Ministry of Foreign Relations.
9	Office of the Special Secretary for Aquiculture and Fisheries of the Presidency of the Republic
10	Specialist on human health.
11	Specialist on human health.
12	Specialist on human health.
13	Specialists on animal health.
14	Specialists on animal health.
15	Specialists on animal health.

Table A3.1. (cont'd).

16	Specialists on plants.
17	Specialists on plants.
18	Specialists on plants.
19	Specialists on environmental issues.
20	Specialists on environmental issues.
21	Specialists on environmental issues.
22	Specialist on consumer rights.
23	Specialist on health.
24	Specialist on environment.
25	Specialist on biotechnology.
26	Specialist on family agriculture.
27	Specialist on occupational health.
PARAGUAY	
COMBIO	
1	Ministry of Agriculture and Livestock, National Service of Quality and Plant Health and Seeds (SENAVE)
2	Ministry of Agriculture and Livestock, National Service of Animal Health (SENACSA)
3	Ministry of Agriculture and Livestock, Secretary of Environment (SEAM), General Directorate of Environmental and Natural Resources Quality Control (DGCCARN)
4	Ministry of Agriculture and Livestock, Secretary of Environment (SEAM), General Directorate of Biodiversity (DGB).
5	Ministry of Agriculture and Livestock, Secretary of Environment (SEAM), Directorate of Fisheries (DPE).
6	Ministry of Agriculture and Livestock, Paraguayan Institute of Agricultural Technology (IPTA)
7	Ministry of Agriculture and Livestock, General Directorate of Planning (DGP),
8	Ministry of Agriculture and Livestock, (SSEG)
9	Ministry of Health and Social Welfare, National Institute of Food and Nutrition (INAN).
10	Ministry of Industry and Commerce (MIC)
11	National University of Asunción (UNA), School of Agricultural Sciences (FCA).
12	National University of Asunción (UNA), School of Veterinary Science (FCV).
13	National University of Asunción (UNA), School of Natural Sciences (FACEN).

Table A3.1. (cont'd).

URUGUAY	
CGR	
1	Ministry of Livestock, Agriculture and Fisheries (MGAP)
2	Ministry of Economy and Finances (MEF)
3	Ministry of Housing, Land and Environment (MVOTMA)
4	Ministry of Public Health (MSP)
5	Ministry of Industry, Energy and Mining (MIEM)
6	Ministry of Foreign Relationships (MRREE)
ERB	
1	Specialist in environmental risk assessment
2	Specialist in food/feed safety (to be incorporated)
CAI	
1	Ministry of Housing, Land and Environment. Specialist in environment (MVOTMA).
2	Ministry of Public Health (MSP).
3	Ministry of Livestock, Agriculture and Fisheries (MGAP)
4	National Institute of Agriculture Research (INIA)
5	National Institute of Seeds (INASE)
6	Technological Laboratory of Uruguay (LATU)
7	Institute Pasteur of Montevideo (IP)
8	Biological Research Institute Clemente Estable (IIBCE)
9	University of the Republic (UDELAR)

Table A3.1. (cont'd).

Ad Hoc Groups			
1	Molecular Characterization and Identification (GAHCIM)	1	National Institute of Agriculture Research (INIA), specialist on biotechnology
2		2	National Institute of Agriculture Research (INIA), specialist on molecular biology
3		3	National Institute of Agriculture Research (INIA), specialist on molecular biology
4		4	National Institute of Seeds (INASE), specialist on molecular identification.
5		5	Technological Laboratory of Uruguay (LATU), specialist on molecular identification.
6		6	Technological Laboratory of Uruguay (LATU), specialist on molecular identification.
7		7	Ministry of Livestock, Agriculture and Fisheries (MGAP), specialist on molecular identification.
8		8	Ministry of Livestock, Agriculture and Fisheries (MGAP), specialist on molecular identification.
9		9	Ministry of Livestock, Agriculture and Fisheries (MGAP), specialist on molecular identification.
10		10	University of the Republic (UDELAR), School of Agronomy, specialist on biotechnology.
11		11	University of the Republic (UDELAR), School of Science, specialist on molecular biology.
12		12	University of the Republic (UDELAR), School of Science, specialist on molecular biology.
13	Gene flow and coexistence (GAHFG)	1	Ministry of Housing, Land and Environment (MVOTMA), specialist on biodiversity
14		2	Ministry of Housing, Land and Environment (MVOTMA), specialist on biodiversity
15		3	Ministry of Housing, Land and Environment (MVOTMA), specialist on biodiversity
16		4	Ministry of Livestock, Agriculture and Fisheries (MGAP), Planning and Budget Office (OPYPA).
17		5	National Institute of Agriculture Research (INIA), specialist on crop biology.
18		6	National Institute of Agriculture Research (INIA), specialist on crop biology and physiology.
19		7	National Institute of Seeds (INASE), specialist on certified seed production.
20		8	University of the Republic (UDELAR), School of Agronomy, specialist on botany.
21		9	University of the Republic (UDELAR), School of Agronomy, specialist on crop biology.
22	Non-target organisms (GAHONOB)	1	Ministry of Housing, Land and Environment. Specialist in environment (MVOTMA), specialist on biodiversity
23		2	National Institute of Agriculture Research (INIA), specialist on entomology.
24		3	National Institute of Agriculture Research (INIA), specialist on entomology.
25		4	National Institute of Agriculture Research (INIA), specialist on soil microorganisms.
26		5	University of the Republic (UDELAR), School of Agronomy, specialist on entomology.

Table A3.1. (cont'd).

27	Food/feed safety (GAHSHA)	1	Ministry of Public Health (MSP), specialist on human nutrition.
28		2	Ministry of Public Health (MSP), specialist on human nutrition.
29		3	Ministry of Public Health (MSP), specialist on human nutrition.
30		4	Institute Pasteur of Montevideo (IP), specialist on molecular biology.
31		5	Institute Pasteur of Montevideo (IP), specialist on genetically engineered animals.
32		6	National Institute of Agriculture Research (INIA), specialist on animal health.
33		7	Ministry of Housing, Land and Environment (MVOTMA), specialist on animal health and welfare.
34		8	University of the Republic (UDELAR), School of Medicine, specialist on human nutrition.
35		9	University of the Republic (UDELAR), School of Agronomy, specialist on animal health.

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CHAPTER IV: APPLICATION OF THE PROBLEM FORMULATION APPROACH WHEN ASSESSING ENVIRONMENTAL RISKS OF CROPS ENGINEERED WITH COMPLEX TRAITS - A CASE STUDY OF DEHYDRATION STRESS TOLERANCE IN CUCUMBER

INTRODUCTION

Crops engineered with complex traits such as dehydration stress tolerance, may utilize genes for which the protein product is indirectly responsible for the desired phenotype. For example, genes being used for the new traits may encode transcription factors that regulate expression of other genes; they may code for signalling factors that initiate response to perceived changes in the cellular environment; they may produce metabolic pathway enzymes that result in the production of new cellular compounds, among others.

These sorts of genes have the potential to initiate a cascade of cellular changes and thus may produce unanticipated effects on plant metabolism, physiology, and/or development with biosafety implications. Adding to this complexity, the interaction between genotype and the highly variable environmental conditions might affect the expression of the intended phenotypes. As a result, the application review for environmental biosafety may be more complex as we move from the first wave of genetically engineered crops, such as insect- or herbicide tolerant crops for which the gene product directly confers the trait of interest, to crops with more complex traits. It should be noted, however, that while important to evaluate, the possibility of pleiotropic effects is not a hazard in itself. Rather, the same ultimate harms must be evaluated, for example increased weediness or harmful effects on non-target organisms, whether those harms arise due to primary effects of the gene, or pleiotropic or unintended effects. While pleiotrophy means that there is a greater potential for unintended effects to occur, unintended effects that do not result in a significant harm are

not a regulatory concern. The regulatory system needs to adapt the risk assessment process according to the dynamic of the technology, focusing on data that aids the decision-making process. The objective of this chapter is to develop a conceptual framework for regulators when analyzing environmental risk assessments of transgenic crops with complex traits. A case study is performed for release of cucumber expressing the *Arabidopsis thaliana* dehydration stress tolerance transcription factor gene, *CBF*, into the Uruguayan environment.

It is critical to ensure an effective risk assessment process by appropriately outlining the Problem Formulation to define the right hazard and risk scenarios where harm could happen. The roadmap begins with a list of possible harms that could occur as consequence of cultivation of a specific GE crop. The list is refined by establishing an exposure pathway for each harm with its corresponding testable risk hypotheses according to Raybould (2010). The first stage to determine acceptance or rejection of these hypotheses is based on existing data and baseline information. Those harms for which risk hypotheses can be sufficiently corroborated by analyzing existing baseline information are eliminated from consideration. For those harms for which it is necessary to obtain additional information, an analysis plan is discussed. Biosafety parameters are discussed from a regulatory point of view in order to contribute to the decision-making process of these soon-to-be-commercialized second generation complex traits.

PROBLEM CONTEXT

The study case refers to cucumber plants that are genetically engineered for dehydration-related environmental stress resistance using the *CBF1* or *CBF3* genes from *Arabidopsis thaliana*. *CBF* genes encode members of the C-repeat binding factor (CBF) [also termed DREB (dehydration-responsive element binding factor)] transcription factor family, which induce expression of a set of abiotic stress-related genes (Jaglo-Ottosen *et al*., 1998;

Kasuga *et al*., 1999). Overexpression of *CBF/DREB* genes has led to enhanced freezing, drought, and salt stress resistance (Jaglo-Ottosen, *et al*., 1998; Liu *et al*., 1998; Kasuga *et al*., 1999; Gilmour *et al*., 2000). In this case study, Uruguay is considered as the receiving environment where the GE cucumber plants would be released if a favorable regulatory decision is issued. Figure 4.1 shows a schematic of the complex interplay of factors influencing environmental safety considerations with respect to the case study.

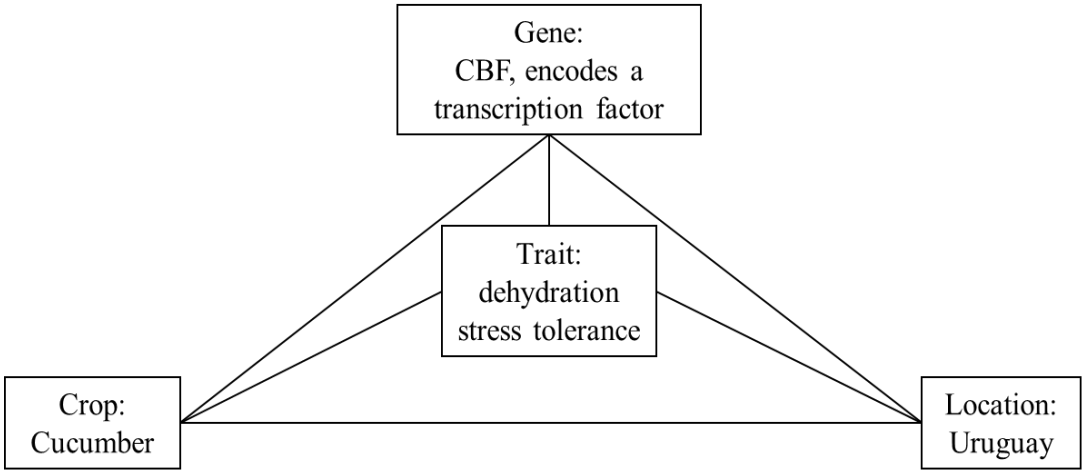

Figure 4.1. Scheme of interconnection between factors that determine possible environmental concerns: the crop, gene, trait and location of the study case (Schematic adapted from Grumet *et al*., 2011).

According to the risk assessment methodology, the first step of risk assessment is the identification of possible harms that the GE plant could cause to the environment. A list of potential harms classified according to agronomic and ecological effects, compiled from Ellstrand (2001), Dale *et al*. (2002), Conner *et al*. (2003), Snow *et al*. (2005), Chapman and Burke (2006) and Craig *et al*. (2008), is presented in Table 4.1. The exercise to be performed in Problem Formulation is to determine, for a specific combination of GE plant/gene/trait/receiving environment (Figure 4.1), which potential harms could be considered as real concerns and to postulate risk hypotheses to be tested relevant for decision-making.

Table 4.1 Possible environmental harms that may require further consideration.

<table>
<tr><td colspan="3">Possible environmental harms that may require further consideration</td></tr>
<tr><td rowspan="3">Environmental biosafety issues</td><td rowspan="2">Agronomic</td><td>Weediness:
1)- GE crop itself becomes a weed in agricultural systems (phenotypic effect of the gene causes volunteer plants with increased persistence).
2)- Generation of more aggressive weedy relatives (harder to kill, population expansion) due to gene flow from pollen.</td></tr>
<tr><td>Negative effects on organisms (non-target species and food webs):
3)- Toxic effect of the gene product on non-target organisms.
4)- Harmful effect on organisms due to changes in agricultural practices that may change the agroecological system.
5)- Development of resistance to products that confer herbicide or insect protection.</td></tr>
<tr><td>Negative effects on soil functions:
6)- Toxic effect of the gene product on soil microorganisms.
7)- Negative impact on soil physical properties and soil microorganisms due to changes in agricultural practices.</td></tr>
<tr><td>Ecological</td><td>Negative effects on natural resources (soil, water, wildlife and flora) and its biodiversity:
8)- GE crop itself becomes an invasive species because it could be more persistent.
9)- Gene flow from pollen to wild relatives causing one of the following (implies hybridization and introgression success of the transgene):
a) Increase abundance of wild relatives /become invasive if transgene confers fitness advantage (population size);
b) Decrease abundance of rare wild relatives/become extinct if it confer fitness disadvantage (outbreeding depression) (loss of biodiversity) (population size);
10)- Harmful effect on non-target species (insects, natural enemies, soil microorganisms):
11)- Soil degradation due to a change in agricultural practices.
12)- Destruction of refuge areas due to agricultural expansion over natural environments.</td></tr>
<tr><td>Commercial issues</td><td>Coexistence (adventitious presence)</td><td>Negative effects on trade:
13)- Negative impact on trade due to gene flow from pollen to conventional or organic crop.
14)- Negative impact on trade due to seed mixing (seed dispersal).</td></tr>
</table>

The first step is to establish the *problem context* by: defining *protection goals* and the *environmental scope* and analyzing *baseline information* regarding the GE crop, trait, gene and receiving environment (Figure 4.1). These steps are discussed below according to the Uruguayan biosafety framework.

Protection goals

Protection goals are defined based on the country's law, statutes, regulations or guidance (US EPA, 1998; Raybould, 2006; Raybould and Quemada, 2010; Wolt, *et al*., 2010; Sanvido *et al*., 2012). Considering the study case to be released in Uruguay, protection goals derive from the laws listed in Table 4.2 regarding the environment. Protection goals derived from legislation include conservation and sustainable use of the biological diversity and natural resources (air, soil, water, wildlife and flora), preservation of ecological protected areas, and avoidance of invasive alien species.

Table 4.2. Uruguayan laws related to environmental protection.

Year	Law No.	Comments
1935	9463	Creation of the Ministry of Livestock, Agriculture and Fisheries.
1990	16112	Creation of the Ministry of Housing, Land and Environment
1993	16408	Ratification of the Convention on Biological Diversity
1994	16466	Declaration of national interest in environmental protection, definition of environmental impact and prescription of environmental impact studies for certain endeavors.
2000	17283	General Law of Environmental Protection passed. Establishes specific considerations for living modified organisms.
2006	17942	Approval of FAO Treaty on genetic resources for food and agriculture
2008	18381	Law of land and sustainable development passed.
2011	18792	Ratification of the Cartagena Protocol
		Adapted from Uruguay, 2007

Environmental scope

One of the criteria for *environmental scope* is whether environmental impacts of the event itself will be analyzed, or whether impact of the associated technology will be considered. In Uruguay the scope has been defined by the Risk Management Commission (CGR) with the support of the National Biosafety Council (GNBio) that together perform the risk management phase, to focus mainly on the event. The impact of the associated technology is considered in a coordinated way between the regulatory system with the respective government bodies with competence in the matter: the Division of Natural Renewable Resources (RENARE) that has responsibility for soil conservation (considered as a non-renewable natural resource) and Good Agricultural Practices (GAP) plans; the General Directorate of Agricultural Services (DGSA) of the Ministry of Agriculture (MGAP); and the National Directorate of Environment of the Ministry of Environment (MVOTMA). *(*See Chapter 2 of this thesis for an example of integration of these divisions with the regulatory system for commercial release authorizations).

Another criterion to be defined as part of the environmental scope is whether the event is going to be analyzed in the context of the agroecosystem or more broadly in the wild ecosystem. The focus in Uruguay has been defined to be the agroecosystem, but introducing, when justified, parameters of the natural ecosystem (CGR, 2010). The small size of the country determines that agriculture is carried out in close proximity to native areas and environmental protection areas. In the case of abiotic stress tolerance traits, such as the study case, it could be argued that it would be justified to not only include the agricultural environment where the GE plant will be released, but also a wider environment including semi-natural and natural environments where exposure to the new gene/trait could happen either by gene flow or accidental release.

In the case of Uruguay another criterion that frames the risk assessment is the political definition established in Decree 353/008 to set coexistence between different production systems (organic, conventional and transgenic). (See chapter 2 of this thesis for the progress made on coexistence legislation). Coexistence rules and crop management measures are set to regulate the planting of GE crops in order to avoid the unintended presence of GE material in products obtained from other production systems for which trade markets does not allow GE organisms. As mentioned in the previous section, coexistence does not refer to biosafety issues; it refers to commercial issues to prevent potential economic losses. Scientific analysis is required to assess its feasibility and to establish crop management measures to regulate the coexistence.

Baseline information

The next step of problem formulation is to analyze the case-specific baseline information. Although all potential harms listed in Table 4.1 fall within the defined protection goals and environmental scope criteria, analysis of the *baseline information* regarding the receiving environment and plant biology, indicates that not all potential harms are relevant for the study case. In the following section is provided the evidence why concerns can be eliminated from consideration and why others are required to be examined further. For each potential harm listed in Table 4.1, the *exposure pathway* including specific steps that would need to occur for the harm to happen, and the corresponding risk hypothesis, are described according to Raybould (2010) (Table 1.10 from chapter 1 of this thesis). The risk hypothesis postulated for each step of the pathway is formulated in a way that indicates that the cultivation of GE cucumber crop will be harmless. If the hypothesis survives a rigorous search trying to falsify it and the evidence actually corroborates it, the testing will have high confidence showing that cultivation of GE cucumber is harmless (Raybould, 2010). Some of

these hypotheses can be sufficiently corroborated by analyzing existing baseline information and therefore the particular harm can be rejected early in Problem Formulation due to the low probability of occurrence. For those risk hypotheses that it is necessary to find out additional information an *analysis plan* is discussed.

PROBLEM DEFINITION

Agronomic harms related to weediness

Agronomic harm: The GE crop itself becomes a weed in agricultural systems (harm 1).

The factor that determines this concern is the phenotypic effect of the gene product that may cause volunteer plants with increased persistence. The protection goal (conservation of the environment) is defined more concretely as the *environmental value* to avoid weediness in agricultural fields. In other words, the *environmental value* to be protected is the fact that the current cucumber crop (non-GE) is not weedy in agricultural fields. To evaluate the risk of GE cucumber to become a weed, it is necessary to consider its crop biology and reproductive characteristics, the phenotype that resulted from introduction of the *CBF* gene, as well as conditions of the receiving environment and stress encountered (Ellstrand, 2001).

In this case, the *exposure pathway* or *risk scenario* that determines a real possibility for the harm to occur would be if GE cucumber crop with dehydration stress tolerance is deregulated and planted under conditions where lack of adequate moisture or salinity in the soil or low temperature, is the main environmental factor limiting plant growth. Harm 1 is discussed considering water stress condition as outlined in Table 4.3.

Dehydration stress tolerance is a trait expected to confer a fitness advantage to the GE crop if the characteristic ‘dehydration stress’ currently limits crop growth (Hancock, 2003; Chapman and Burke, 2006). In other words, in order for harm 1 to occur it is necessary that

water stress conditions in the receiving environment are a major factor limiting plant growth. Thus by conferring dehydration stress tolerance, the *CBF* gene, could release plant growth from an environmental limitation, making GE cucumber plants able to grow and reproduce in an environment in which they were previously not able to grow. GE cucumber plants may be more competitive, increasing persistence and survival in the planting and nearby natural areas, resulting in volunteer plants in subsequent crops, increasing weediness or invasiveness. The broad risk hypothesis to test is that *"transgene will not confer a selective advantage to GE crop resulting in increased weediness or invasiveness"*.

Table 4.3. Harm 1: Exposure pathway and risk hypotheses to characterize harm 1 of Table 4.1 regarding weediness in agricultural systems of the GE crop.

Exposure pathway	**Hypothesis**
Cultivation of GE cucumber plants with dehydration stress tolerance.	
↓	GE crop is not planted under lack of soil moisture
GE cucumber crop is planted under lack of soil moisture.	
↓	GE crop growth is not only limited by water stress conditions.
GE cucumber crop growth is only limited by water stress conditions.	
↓	GE crop does not leave viable seeds in the field after harvest.
GE cucumber crop leaves viable seeds in the field after harvest.	
↓	GE seeds do not survive winter conditions.
GE cucumber seeds survive winter conditions.	
↓	Volunteer plants do not complete the life cycle and do not persist more than one year.
Volunteer GE cucumber plants complete their life cycle and persist more than one year sufficient to increase its weed potential in agricultural systems (Harm).	

The first risk hypothesis of the exposure pathway to test is: *'GE crop is not planted under lack of soil moisture'*. The evidence regarding characteristics of the receiving environment indicates that in Uruguay cucumber crop is planted under field conditions with irrigation and in greenhouses where water is also provided with no limitation (Uruguay, 2010, 2012a,b). However, a few growers at the south of the country occasionally can grow cucumber under field conditions without irrigation and may have years of reduced moisture (Personal Communication Ing. Agr. Luis Aldabe, Department of Crop Production, School of Agronomy-UDELAR, 2012). The trait dehydration stress tolerance analyzed in the study case may be useful for these growers that could plant cucumber under field conditions with water stress or for those that apply irrigation and may avoid the cost of the irrigation system. Thus, it is necessary to continue to risk hypothesis of step 2 of the exposure pathway.

The risk hypothesis of step 2 is '*GE crop growth is not only limited by water stress conditions'*. The information needed to test this hypothesis is whether the crop management practices typically address other possible growth limitations such as insect and disease damage, physiological disorders, nutrient limitation, weed pressure, pollination requirements, etc. Information regarding cultivation practices can be obtained from agronomists who work and advise growers regarding the cultivation of cucumber. If the hypothesis is falsified by corroborating that water stress condition is the main limitation, this means that the *CBF* gene could release GE plants from that environmental limitation compared to non-GE plants and the analysis requires advancing to step 3.

Evidence for crop management factors such as insect and disease control, fertilization and weed control, indicates that these cultivation practices are not limiting cucumber production in Uruguay (Aldabe, 2000). The main limitations to obtain a high yield of cucumber production in Uruguay are lack or excess of water and low temperatures at the beginning and end of the growing season (Aldabe, 2000). Cucumber can be grown on a

variety of soils, but it has to be fertile and well-drained (Gildemacher and Jansen, 1993). Irrigation is required if the soil moisture is not maintained by appropriate rainfall, and it cannot tolerate waterlogging (Purseglove, 1968, Tindall, 1983). Specifically, insufficient irrigation results in low fruit quality due to poor fruit set that negatively affects seed formation resulting in fruit defects as well as early maturation due to small fruit size (Aldabe, 2000). Additionally, lack of water increases the damage by powdery mildew (*Erysiphe cichoracearum* and/or *Spharotheca macularis*) (Aldabe, 2000), which normally appears in old senescent leaves. Therefore, information about cultural practices indicates that cucumber crop growth in the receiving environment is mainly limited by water stress conditions. Thus, the risk hypothesis 2 can be considered as false, necessitating progressing to step 3 of the exposure pathway.

The risk hypothesis for step 3 is: *'GE crop does not leave viable seeds in the field after harvest'*. Baseline information regarding the cucumber crop indicates that fruits that remain in the field after harvest, are degraded in a few weeks and seeds released (Personal Communication, Ing. Agr. Luis Aldabe, Department of Crop Production, School of Agronomy-UDELAR, 2012). This evidence corroborates that cucumber crop may leave viable seeds in the field after harvest falsifying hypothesis 3 of the exposure pathway and the analysis advances to step 4.

The risk hypothesis to test step 4 is: *'GE seeds do not survive winter conditions'*. Cucumber seeds have no dormancy mechanism and require protective conditions to survive the winter (Purseglove, 1968; Bates *et al*., 1990). Cucumber plants are chilling sensitive and need a warm climate with temperatures between 20°C and 30° C with frost-free conditions for the entire growth period from sowing to harvest. The optimum day-night range of temperature for growth is 30°C during the day and 18-21°C at night. Therefore, in cool temperate countries it can be grown outside only during the summer months or year-round in

greenhouses (Gildemacher and Jansen, 1993; Tindall, 1983). The average temperature according to data from the Agroclimatic Information System (GRAS) of INIA at http://www.inia.org.uy/gras/) is below 15°C (Table 2 in Appendix). Local agronomists that work with the cucumber crop in Uruguay agree based on personal observation, that although fruits remain in the field after harvest, by the time seeds are mature and there is humidity in the soil to germinate, the low temperature does not allow germination. The excess water in the soil during winter for about five to six months degrades the seeds further eliminating the possibility of seed over-wintering (Personal Communication, Ing. Agr. Luis Aldabe, Department of Crop Production, School of Agronomy-UDELAR, 2012). This evidence would block the exposure pathway on step 4 and harm 1 can be eliminated without further consideration.

Agronomic harm: Gene flow from pollen to weedy relatives causing more aggressive weedy relatives (harder to kill, population expansion) (harm 2)

The potential to cause more aggressive weedy relatives requires the occurrence of gene flow from GE cucumber plants to compatible relatives. To evaluate the risk of gene flow from GE pollen to weedy relatives it is necessary to first consider the baseline information of the receiving environment regarding the origin and geographic distribution of cucumber. Table 4.4 represents the pathways with the sequence of steps that are necessary to happen in order for the cultivation of GE cucumber plants to cause agronomic harm 2.

The first condition is the presence of compatible, interfertile weedy relatives in the receiving environment and so the first risk hypothesis of the exposure pathway is: *'No presence of weedy relatives in the receiving environment'*. The evidence to look for to test this hypothesis is the presence of compatible relatives in Uruguay.

The analysis of the gene pool of cucumber (*C. sativus* L. var. s*ativus)* and its related species indicate that *C. sativus* L. var. s*ativus* only produces fertile hybrids when crossed with species from the subgenus *Cucumis* with the same basic chromosome number (x=7) such as *C.sativus* L. (var. *hardwickii*) (Deakin *et al*., 1971; Puchalski and Robinson, 1990; Gildemacher and Jansen, 1993; Bates and Robinson, 1995).

Table 4.4. Harm 2: Exposure pathway and risk hypotheses to characterize harm 2 of Table 4.1 regarding generation of more aggressive weedy relatives.

Exposure pathway	**Hypothesis**
Cultivation of GE cucumber plants with dehydration stress tolerance under lack of soil humidity	
↓	No presence of weedy relatives in the receiving environment
Presence of weedy relatives in Uruguay	
↓	No pollen flow to weedy relatives
Pollen flow to weedy relatives	
↓	No hybridization between weedy relatives and GE cucumber plants
Hybridization between the GE cucumber plants and weedy relatives	
↓	No hybrid survival and reproduction
Hybrid survival and reproduction	
↓	No introgression of transgene into weedy relatives
Introgression of CBF genes into weedy relatives	
↓	Transgene does not confer a selective advantage to hybrids
CBF genes confer dehydration stress tolerance to weedy hybrids	
↓	Abundance of weedy hybrids is not only limited by lack of soil humidity
More aggressive weedy relatives (harder to kill, population expansion (Harm)	

The other species in the subgenus *Cucumis*, *C. hystrix* Chakr, is diploid 2n=24 and the only successful interspecific hybridization between *C. sativus* L. and *C. hystrix* Chakr has been obtained with the laboratory intervention of embryo rescue (Chen *et al*., 1997; Chen *et al*., 2002). Backcrosses of the reciprocal F_1 hybrids to either parent and self-crossing indicated that the hybrids were male and female sterile (Chen *et al*., 1997). Attempts to obtain hybrid plants between subgenera of *Cucumis* have not been successful. No hybrids have ever been obtained in crosses between cucumber and any species of the subgenus *Melo*, even when aided by embryo culture and/or other techniques (Bates and Robinson, 1995).

Cucumber (*C.sativus* var. *sativus* L) is native to India or southern Asia, probably originating in the northwest of India (Bates *et al*., 1990). Cucumber (*C. sativus* L. var. s*ativus)* can be found native in temperate regions of Asia in the Arabian Peninsula (Oman and Yemen), in Burma and China (Yunnan Province); in tropical areas of Asia, Sri Lanka, Indo-China (Myanmar and Thailand) and in the Indian subcontinent. Its closest related species, the wild *C. sativus* L. var. *hardwickii*, is not considered a weed and occurs only in its center of origin (Gildemacher and Jansen, 1993) in temperate regions of Asia: China (Guangxi, Guizhou, Yunnan), and in tropical regions of Asia: India (Nepal) (USDA-GRIN online database).

In summary, according to the information provided above, there are no weedy relatives and outcrossing to wild relatives would only be a consideration if the wild relative *C. hardwickii (Royle) Alef.* coexists with GE cucumber plants (*C.sativus* var. *sativus* L.) in the receiving environment. Considering Uruguay as the receiving environment, Uruguay is located in the southeastern part of South America not being part of the center of origin of cucumber (*Cucumis sativus* L.). In Uruguay there are no weedy relatives, wild relatives or landraces of *Cucumis sativus* L. (Brussa, 2007; Lombardo, 1984; Zuloaga and Morrone, 1999). The existing baseline information regarding cucumber origin and geographic

distribution is sufficient to corroborate the risk hypotheses that there are no weedy or wild relatives in Uruguay. Thus the exposure pathway of harm 2 is blocked in step 1 and can be eliminated from consideration.

Agronomic harms related to a negative effect on organisms

Agronomic harm: The gene product is toxic to non-target organisms (harm 3)

The factor that determines this concern is the occurrence of a direct or indirect toxicity effect of the gene product on non-target organisms. 'Organisms' in this context are globally considered as non-target, since the trait introduced was not intended to have any toxic properties i.e., does not have intended pesticide effect. A gene product could be toxic to organisms due to molecular features and/or its mode of action. In the study case the gene product (i.e., the CBF protein) is not the trait *per se* but the way to obtain the desired trait (dehydration stress tolerance). Thus, not only a *direct* mechanism but a possible *indirect* mechanism of the *plant attribute* (*CBF* genes) could change gene expression through pleiotropic effects (unexpected secondary effects) ultimately resulting on an altered plant metabolism with toxic effects on non-target organisms (Raybould *et al*., 2010). Therefore these hypotheses require the analysis of molecular characteristics of the CBF protein as a possible toxin *per se*, and the analysis of the proteins and other metabolic changes expressed as result of its action. Table 4.5 represents the pathway with the sequence of steps that are necessary to happen in order for the cultivation of GE cucumber plants to cause the agronomic harm 3.

The first condition is that the gene product or metabolic changes caused by the gene product are toxic to non-target organisms. The risk hypothesis to test is *'the gene product or metabolic changes are not toxic to non-target organisms'*. First the gene product will be

analyzed and then possible metabolic changes. The first evidence to look for is whether the CBF protein is toxic to non-target organisms.

Characteristics of the CBF protein that may help to evaluate its intrinsic hazard include its physio-chemical and biochemical properties; biological function; mode of action and source (Carlini and Grossi-de-Sa, 2001; Delaney *et al*., 2008). Physio-chemical and biochemical properties refer to protein size, isoelectric point, stability to pH, temperature, and chemical or biochemical agents. Structural characteristics refer to post-translational modifications, amino acid sequence and secondary and tertiary structure which influence mode of action at the molecular level and facilitate evaluation of the potential risk to non-target organisms. These kinds of analyses are typically performed as part of the toxicity review for food safety (FAO, 2007; Codex Alimentarius, 2003).

Table 4.5. Harm 3: Exposure pathway and risk hypotheses to characterize harm 3 of Table 4.1 regarding toxicity of the gene product on non-target organisms.

Exposure pathway	**Hypothesis**
Cultivation of GE cucumber plants with dehydration stress tolerance.	
↓	The gene product or metabolic changes resulting from the gene product are not toxic to non-target organisms.
The gene product or metabolic changes are toxic to non-target organisms (insects, soil microorganisms or plants)	
↓	Severity of toxicity of the gene product or metabolic changes does not modify non-target organism populations
Toxicity of gene product negatively affects abundance of non-target organisms (Harm)	

For a protein to be a toxin it must be capable of causing disease due to contact with or absorption by the organism's tissues by interacting with biological macromolecules such as

enzymes or cellular receptors. A protein toxin could be related to a protein with enzymatic activity, enzyme inhibition or binding certain nutrients causing anti-nutritive effects or acting as carrier molecules, hormones or toxins that will bind to specific cell receptors and trigger a reaction sequence ending in different possible toxic effects (Craig *et al*., 2008).

Plant toxic proteins that are thought to be involved in defense mechanisms include lectins, ribosome-inactivating proteins (RIPs), inhibitors of proteolytic enzymes, glycohydrolases, arcelins, chitinases, canatoxin and modified forms of storage proteins (Carlini and Grossi, 2002 and references within it). *CBF* genes do not encode any of the mentioned proteins. *CBF* genes encode members of the C-repeat binding factor (CBF) or DREB (dehydration-responsive element binding factor) transcription factor family. The *CBF* family is a small family of three transcriptional activators, *CBF1* (Stockinger *et al*., 1997), *CBF2* and *CBF3* (Gilmour *et al*., 1998; Medina *et al*., 1999), also identified as *DREB1B, DREB1C* and *DREB1A*, respectively (Liu *et al*., 1998). The CBF proteins, and transcription factors in general, have not been identified as toxins for plants, microorganisms or insects.

It is also possible that the insertion of the *CBF* gene and its promoter cause altered expression of a native gene(s) that produces a toxin. Another possibility is that the insertion disrupts a known plant gene or generates new open reading frames that could produce unintended new proteins. These possibilities are evaluated by standard molecular characterization of the transformation event that examines sequence integrity of the introduced DNA as well as the region surrounding the insertion site to ensure that such changes have not occurred.

The existing baseline information regarding CBF protein molecular characteristics and mode of action is sufficient to corroborate the risk hypotheses that gene product is not toxic to non-target organisms. Thus, the exposure pathway of harm 3 would be blocked in step 1 and would be eliminated from consideration.

The second consideration is whether expression of CBF causes other transcriptional or metabolic changes resulting in production of toxins by the plant. Transcription factors do not generate different or new proteins but regulate time and amount of gene expression (Pabo and Sauer, 1992; Riechmann *et al*., 2000; Hussain *et al*., 2011). Because *CBF* is a transcription factor it may influence gene expression in such a way that it causes production of a toxin not usually made under the circumstances.

The history of use (concept of familiarity) of cucumbers indicates that cucumber plants may naturally produce toxins such as cucurbitacins (a secondary plant metabolite, oxygenated tetracyclic triterpenoids) (Chambliss and Jones, 1966; Horie *et al*., 2007; Zehnder *et al*., 1997). Thus, as part of normal food safety assays would be necessary to test for the production of cucurbitacins. When the *CBF* genes are constitutively overexpressed in other species several changes have been observed, many of which are associated with-responses to abiotic stress (Jaglo-Ottosen, *et al*., 1998; Liu *et al*., 1998; Kasuga *et al*., 1999; Gilmour *et al*., 2000; Fowler and Thomashow, 2002; Chan *et al*., 2011); such as elevated levels of proline, total sugars, catalase, and hydrogen peroxide (Hsieh *et al*., 2002a,b). In addition, expression of abiotic stress related genes also is frequently associated with biotic stress related genes, and so may influence expression of defense molecules (e.g., Little *et al*., 2009; Atkinson and Urwin, 2012) and CBF overexpression has resulted in enhanced expression of genes associated with response to biotic stimulus (Chan *et al*., 2011).

Overexpression of CBF has also shown growth retardation (dwarfing) in the absence of stress and delayed reproductive development (Liu *et al*., 1998; Kasuga *et al*., 1999; Gilmour *et al*., 2000; Achard *et al*., 2008). However, this seems to be related to a decrease in the biosynthesis of the phytohormone gibberellin (GA) that targets DELLAs, a family of nuclear growth-repressing proteins, for degradation (Achard *et al*., 2008). The resulting growth restraint and late flowering should not be understood as a toxic effect due to the

action of the proteins whose expression is regulated by CBF, but as a way to promote survival under abiotic stress conditions (Achard *et al*., 2008).

Due to, the difficulty of predicting plant response to abiotic stresses and the possibility of cross talk among stresses, an analysis plan is recommended to evaluate possible unexpected secondary effects of CBF gene products in the releasing environment that would result in toxic products that adversely affect non-target organisms. The analysis plan would include comparative compositional analyses, performed as part of food safety, to determine substantial equivalence between the GE crop and its conventional counterpart except for the gene product introduced. In this study case, compositional data is relevant also for environmental risk assessment since small molecules with potential toxic effects on non-target organisms could be detected (Nickson, 2008). If there is a significant compositional difference when comparing fruit samples, the analysis plan could extend compositional analysis to leaves and roots in order to obtain more information before moving to field trials. The analysis plan would include field trials if it is necessary to assess specific negative impact on beneficial insects and/or soil microorganisms.

Agronomic harm: Negative effect on organisms due to changes in agricultural practices (harm 4)

To evaluate whether possible changes in cultivation practices resulting from use of the genetically engineered crop could lead to a harmful effect on non-target organisms, it is necessary to analyze the baseline information regarding cultivation practices of cucumber plants in Uruguay. Harm 4 generally has been considered with respect to herbicide-tolerant GE crops that may increase the use of a specific chemical and thereby affect particular beneficial organisms. While chemical use is not a factor for CBF crops, if efficacy tests indicate that GE cucumber plants have drought tolerance to the point of not requiring

irrigation, GE cucumber plants grown under field conditions may not be irrigated. If frost tolerance is corroborated, the farmer would have the option of planting earlier in the spring or harvest later in the fall coinciding with low temperature conditions and frosts. Table 4.6 represents the exposure pathway with the sequence of steps and the corresponding risk hypothesis that are necessary in order for cultivation of GE cucumber plants to cause harm 4.

Table 4.6. Exposure pathway and risk hypotheses to characterize harm 4 of Table 4.1 regarding the GE crop to change cultivation practices that negatively affect the agroecosystem.

Exposure pathway	Hypothesis
Cultivation of GE cucumber plants with dehydration stress tolerance.	
	CBF gene does not confer dehydration stress tolerance to GE crop in the receiving environment.
CBF gene confers dehydration stress tolerance to GE cucumber plants under Uruguayan environment.	
↓	There are no differences in agricultural practices between the GE crop and conventional crop.
There are differences in agricultural practices between the GE cucumber crop and conventional cucumber.	
	The change in agricultural practices does not negatively affect non-target organisms
The change in agricultural practices negatively affects non-target organisms (Harm).	

The first condition in order for harm 4 to occur is that *CBF* gene confers dehydration stress tolerance to GE cucumber plants under Uruguayan environmental conditions. The first risk hypothesis of the exposure pathway to test is: '*CBF gene does not confer dehydration stress tolerance to GE crop in the receiving environment'*. To test this hypothesis it is necessary to evaluate the efficacy of the trait in the receiving environment. If it is

corroborated that *CBF* gene under Uruguayan environmental conditions confers dehydration stress tolerance, the analysis advances to step 2.

The second risk hypothesis of the exposure pathway to test is: *'There are no differences in agriculture practices between the GE crop and conventional crop'*. The first step here would be to determine the changes in agronomic practices that are likely according to the performance of the CBF crop in the field. If CBF protects against some water stress but does not eliminate need for irrigation completely, or would still be within range of irrigation seen due to seasonal variation, can be considered as not causing changes in agricultural practices and the exposure pathway be stopped at this step. If the levels of stress tolerance conferred are sufficiently strong to cause significant changes in agriculture practices, then the analysis is advanced to step 3.

The risk hypothesis to test in step 3 is: *'The change in agriculture practices does not negatively affect non-target organisms'*. Changes such as modified irrigation regimes or planting times are associated with agriculture in general, any crop that is planted and requires irrigation or not, would have possible impacts on the agroecosystem. This is evaluated by the ministry of environment when evaluates the environmental impact of agriculture in a particular area but is not under the terms of references of risk analysis specific for the biosafety of a GE crop. The question at hand is whether the changes that would occur as a result of planting CBF cucumber is outside the range of normally observed agricultural impacts. If not, the exposure pathway of harm 4 is blocked in step 3 and can be eliminated from consideration. If novel agronomic changes result, for example, the cold tolerance results in planting at a time when no other crops would be planted in the area, rather than simply causing cucumbers to be planted earlier instead of an alternate crop that would have been planted at that time, then a more detailed analysis of possible harms would be relevant.

Agronomic harm: Development of resistance to products that confer herbicide or insect protection (harm 5)

This risk is not relevant to CBF as it is not an insecticide or herbicide. There is also no anticipated increase in use of a specific chemical. Table 4.7 represents the corresponding exposure pathway with the sequence of steps and risk hypotheses that are necessary to happen in order for the cultivation of GE cucumber plants to cause the agronomic harm 5.

Table 4.7. Exposure pathway and risk hypotheses to characterize agronomic harm 5 of Table 4.1 regarding resistance development to gene products that confer herbicide or insect protection.

Exposure pathway	Hypothesis
Cultivation of GE cucumber plants with dehydration stress tolerance.	
↓	No increased use of a specific chemical product and/or adequate management practices to avoid resistance development.
Increased use of a specific chemical product or inadequate resistance management measures making weeds/insects/disease to evolve resistance to gene product or chemicals being more difficult to manage (Harm)	

In order for harm 5 to occur there has to be a change in the crop management practices regarding the use of chemical products. GE crops that constitutively express a protein toxic to a specific insect may lead to insect resistance development to the particular toxin. Similarly GE crops with herbicide tolerance increase the use of that specific herbicide and may lead to weed resistance development to the particular herbicide. Resistance development eliminates the GE crop technology and makes pest control management more difficult. The evidence to look for to test the risk hypothesis is whether there will be an adequate management to avoid resistance development or will be an increased use of a

specific chemical product. In the case study the trait introduced is dehydration stress tolerance rather than insect or viral resistance or herbicide tolerance. There is not expected to be a change in agricultural practices regarding the use of chemical products. The exposure pathway for harm 5 is blocked in the first step and therefore eliminated from consideration.

Agronomic harms related to a negative effect on soil functions

Agronomic harm: The gene product is toxic to soil microorganisms (harm 6)

The factor that determines this concern is the occurrence of a direct or indirect toxicity effect of the gene product on organisms as was discussed for harm 3 (see page 269). This harm shares the same exposure pathway and risk hypotheses as for harm 3 (Table 4.5). Evidence-provided is enough to corroborate that the *CBF* gene product *per se* does not have a *direct* toxic effect on non-target organisms. However, as for harm 3, comparative compositional analyses are relevant for environmental risk assessment to determine whether there is a change in plant composition that can alter the ecological interactions of cucumber plants with the biotic community.

Agronomic harm: Negative impact on soil physical properties and soil microorganisms due to changes in agricultural practices (harm 7)

The factor that determines this concern is a possible change in cultivation practices, specifically regarding tillage, that could lead to a harmful change in the soil ecosystem. This harm shares the same exposure pathway and risk hypotheses as for harm 4 (see pages 273-274, Table 4.6) but for the soil ecosystem (Table 4.8).

Table 4.8. Exposure pathway and risk hypotheses to characterize harm 7 of Table 4.1 regarding the GE crop to change cultivation practices that negatively impact on soil physical properties and soil microorganisms.

Exposure pathway	Hypothesis
Cultivation of GE cucumber plants with dehydration stress tolerance.	
	There are no differences in tillage practices between the GE crop and conventional crop.
There are differences in tillage practices between the GE crop and conventional crop.	
↓	The change in tillage practice does not negatively affects the soil ecosystem.
The change in tillage practices negatively affects the soil ecosystem (Harm).	

For this hypothesis testing it is necessary to analyze the technology and agricultural practices applied for cucumber crops in Uruguay. Harm 7 has generally been considered when examining the effect of no-tillage practices associated with herbicide tolerant GE soybean. The traits conferred by CBF are not anticipated to influence tillage practices for cucumber. The exposure pathway for harm 7 is blocked in the first step and therefore eliminated from consideration.

Ecological harms related to negative effects on natural resources

Ecological harm: The GE crop itself becomes an invasive species in natural habitats (harm 8)

The factor that determines this ecological concern is the phenotypic effect of the gene product that may cause volunteer plants with increased persistence, as was discussed for the agronomic harm 1 (GE crop itself becomes a weed, see page 262). The *environmental value* is to avoid invasiveness of alien species in natural habitats since cucumber is not currently an invasive alien species in natural habitats of Uruguay. Similar to harm 1, to evaluate the risk of GE cucumber to become invasive it is necessary to consider its crop biology and reproductive characteristics, the phenotypic effect of the *CBF* gene introduced, as well as conditions of the receiving environment and stress encountered (Ellstrand, 2001). In the case of the ecological harm 8, it is expected the same phenotypic effect of the gene as in harm 1, that the trait 'dehydration stress' be able to change GE crop fitness under water stress conditions becoming more persistent, expanding the range where GE cucumber plants could grow and eventually invade in natural habitats.

The steps and hypotheses to test harm 8 are similar to those analyzed for harm 1. Evidences from conventional cucumber plants indicate that seeds have no ability to survive low temperatures and waterlogged winter conditions. The discussion of Harm 1 stopped in step 4 since cucumber is not a weedy species and GE seeds would not survive winter conditions requiring for survival warm temperatures, frost-free period as well as well irrigated and drained soils (Bates *et al*., 1990; Gildemacher and Jansen, 1993). The exposure pathway of harm 8 also stops at step 4 and can be eliminated from consideration without further testing.

Table 4.9. Exposure pathway and risk hypotheses to characterize harm 8 of Table 4.1 regarding the GE crop itself becoming an invasive species in natural habitats.

Exposure pathway	Hypothesis
Cultivation of GE cucumber plants with dehydration stress tolerance.	
	Natural habitats have no lack of soil moisture.
Natural habitats have lack of soil moisture.	
↓	GE plants growth in natural habitats is not only limited by lack of soil moisture.
GE cucumber plants growth in natural habitats is only limited by water stress conditions.	
↓	GE crop does not leave viable seeds after harvest in the crop field that is close to natural habitats.
GE cucumber plants leave viable seeds in the crop field that is close to natural habitats.	
↓	GE seeds do not survive outside of cultivation winter conditions in natural habitats.
GE cucumber seeds survive outside of cultivation winter conditions in natural habitats.	
↓	Volunteer GE plants do not complete the life cycle outside of cultivation and do not persist more than one year in natural habitats.
Volunteer GE cucumber plants complete the life cycle outside of cultivation and persist more than one year in natural habitats increasing its invasive potential (Harm).	

Ecological harm: Gene flow from pollen to wild relatives causing one of the following: wild relatives increase/become invasive or decrease abundance/extinct (harm 9)

The factor that determines this concern is the occurrence of gene flow from GE cucumber plants to compatible relatives such as in harm 2 (gene flow from GE pollen to weedy relatives, see page 266). Similarly, to evaluate the risk of gene flow of GE pollen to

wild relatives is necessary to consider the baseline information of the receiving environment regarding the origin and geographic distribution of cucumber.

In order for the ecological harm 9 to occur, there must be compatible, interfertile wild relatives in the vicinity of the GE cucumber crop (Tables 4.4 and 4.10). In the section corresponding to harm 2 (page 266) are the reasons in detail providing the relevant known and background information regarding the presence/absence of such relatives that allows blocking the exposure pathway on step 1, eliminating this harm from consideration.

Table 4.10. Exposure pathway and risk hypotheses to characterize harm 9b of Table 4.1 regarding gene flow form pollen to wild relatives becoming invasive.

Exposure pathway	**Hypothesis**
Cultivation of GE cucumber plants with dehydration stress tolerance under lack of soil humidity	
↓	No presence of wild relatives in the receiving environment
Presence of wild relatives in Uruguay	
↓	No pollen flow to wild relatives
Pollen flow to wild relatives	
↓	No hybridization between wild relatives and GE cucumber plants
Hybridization between the GE cucumber plants and wild relatives	
↓	No hybrid survival and reproduction
Hybrid survival and reproduction	
↓	No introgression of transgene into wild relatives
Introgression of CBF genes into wild relatives	
↓	Transgene does not confer a selective advantage to hybrids
CBF genes confer dehydration stress tolerance to hybrids	
↓	Abundance of hybrids is not only limited by lack of soil humidity
Increase abundance of wild relatives/ become invasive (Harm)	

Ecological harm: The gene product is toxic to non-target species (insects and soil microorganisms) (harm 10)

The factor that determines this concern is the possible toxic effect of the gene product on non-target species, specifically in reference to the native environment, rather than the agroecosystem. As discussed in harms 1 and 8 (pages 262 and 279 respectively), the crop itself is not weedy and not likely to come in contact with the native environment. Additionally the trait will not move into the native environment due to gene flow to wild relatives as it was discussed in harms 2 and 9 relative to gene flow (pages 266 and 280 respectively). Thus, this harm can be eliminated from consideration without developing an exposure pathway and risk hypotheses.

Ecological harm: Destruction of the natural resource soil due to a change in cultural practices (harm 11)

Like in harm 10, as cucumber plants are not likely to come in contact with the native environment and the trait will not move into the natural habitats due to gene flow to wild relatives, then this harm can be eliminated from consideration without characterizing the exposure pathway.

Ecological harm: Destruction of refuge areas due to agricultural expansion over natural environments (harm 12)

The factor that determines this concern is an expansion of cucumber planting over natural environments, specifically to native habitats currently not cultivated. To evaluate the risk of agricultural expansion is necessary to consider whether the CBF trait would result in planting cucumber in land that is currently not used for agriculture (e.g., dry or saline soils).

Table 4.11 represents the exposure pathway with the sequence of steps that are necessary to happen in order for the cultivation of GE cucumber plants to cause the agronomic harm 12.

The risk hypothesis to test is *'the crop is not planted in large extensions or new regions over natural environments'*. The baseline information indicates that in Uruguay cucumber is not among the main horticultural crops. There are no large cucumber producers in Uruguay. It is planted in small areas under a crop rotation pattern traditional of the horticultural system and in greenhouses in the north of Uruguay (Uruguay, 2000, 2010).

Table 4.11. Exposure pathway and risk hypotheses to characterize ecological harm 12 of Table 4.1 regarding destruction of refuge areas due to agricultural expansion over natural environments.

Exposure pathway	**Hypothesis**
Cultivation of GE cucumber plants with dehydration stress tolerance.	
↓	The crop is not planted in large extensions or new regions over natural environments.
GE cucumber crop expanded over natural environments destroying habitats considered natural protected areas (Harm).	

Growers that produce tomato and pepper grow cucumber as a minor crop to complement the production together with melon, beans and eggplant (Personal Communication, Ing. Agr. Luis Aldabe, Department of Crop Production, School of Agronomy-UDELAR, 2012). According to the last General Agricultural Census (2000) there are 199 growers in the south of Uruguay with a total of 50 ha planted under field conditions, and 71 growers in the north of Uruguay with a total of 3 ha planted in greenhouses (Table 4.12). A new General Agricultural Census has recently finished but the data is not yet available (Uruguay, 2011). A recent partial horticultural survey indicates an increase in the area planted in greenhouses in the north of the country to 4 ha with a total of 32 growers

(Uruguay, 2010). This survey did not consider the area planted in the south of the country under field conditions due to the small area. Agronomists specialized in horticulture agree with a shift of the area planted of cucumber from the field to the north of the country in greenhouses that allow a better control of its management (Personal communication, Ing. Agr. Luis Aldabe, Department of Crop Production, School of Agronomy-UDELAR, 2012).

While dehydration stress tolerance will be interesting for growers, it is not expected to result in an expansion of planting to the point of eliminating natural areas or to cause cucumber to be planted in new regions where it would not have been planted before. Drought tolerance would be useful for growers since makes the crop less dependent on irrigation but would not expand the crop to natural areas with water stress. Cold tolerance also would be useful by extending the growing period since low temperature and early frost of fall limits the growing season. Additionally in the north of Uruguay where cucumber is produced in greenhouses, salt tolerance would be useful to make cucumber plants less sensitive to irrigation water with high content of salt (Personal Communication, Ing. Agr. Carlos Barros and Luis Aldabe, Department of Crop Production, School of Agronomy-UDELAR, 2012). Under greenhouse conditions the main source of water for irrigation is from water wells with generally high content of dissolved salts (Zamalvide, 2000). This kind of water determines a high conductivity in soils under greenhouse conditions since the rainfall does not reach the soil to leach the excess of salt. This effect is greatly intensified by the high fertilization generally applied with the irrigation in greenhouse production systems. However, under field conditions the Uruguayan soils have no salinity problems. Soils are exposed to leaching of salts by rainfall resulting in a low conductivity. Thus, the dehydration stress tolerance may be valuable for growers both in the south of Uruguay under field conditions (to avoid irrigation and expand growing season), and in the north under greenhouse conditions (to tolerate

salinity), but will not result in significant new production areas not used previously for this crop.

On the other hand, a possible expansion of the crop due to an increased consumer interest will depend on the market. There is a low demand in the Uruguayan market for cucumber fruit since its consumption is low, mainly by immigrants and foreign people, not being a vegetable of the Uruguayan diet (Personal Communication, Ing. Agr. Luis Aldabe, Department of Crop Production, School of Agronomy-UDELAR, 2012). Additionally the short postharvest life of its fruit makes it not well suited for export.

In summary, an expansion of the crop or its production in new areas is not expected. Harm 12 regarding destruction of native areas due to planting expansion, can be ruled out without further testing after corroborating the risk hypotheses *'the crop is not planted in large extensions or new regions over natural environments'*, blocking step one of its exposure pathway.

Table 4.12. Number of growers and area planted of cucumber crop in Uruguay.

Cucumber crop	**Number of growers**	**Area planted (ha)**	
		Total	**Average per grower**
Under field conditions (south of Uruguay)	199	50	0.25
In greenhouse (north of Uruguay)	71	3	0.04
Source: MGAP, General Agricultural Census, 2000			

Commercial harms related to negative effects on trade

Commercial harm: Negative economic impacts to conventional or organic crops due to gene flow (harm 13)

This harm is related to the commercial situation of the cucumber crop in Uruguay. The factor that determines the harm is the occurrence of adventitious presence of the transgene in conventional and/or organic crops due to gene flow from GE cucumber pollen. The protection goal related to this harm is the political definition of Uruguayan authorities to allow coexistence among different production systems.

The concept of 'gene flow' in coexistence is different from the concept used in environmental biosafety, for example in harms 2 and 9. In environmental biosafety 'gene flow' is not harmful in itself, what is harmful is the phenotype that the transgene confers, for example fitness enhancement that may lead in increase weediness (harm 2) or invasiveness (harm 9) of the receiving plant. In coexistence, the presence of 'gene flow', *per se*, is defined as the harm and not the effect of the gene. The biosafety consideration of gene flow on the environment is evaluated first, before considering coexistence. Coexistence is analyzed once the effect of the gene was considered harmless for the environment, otherwise the event would not be authorized and there is no need to analyze coexistence.

Table 4.13 represents the pathway with the sequence of steps necessary to happen in order for the cultivation of GE cucumber plants to cause agronomic harm 13. The general risk hypothesis will be *"transgene will not result in adventitious presence in conventional and/or organic crops growing according to risk management measures for coexistence"*.

Table 4.13. Exposure pathway and risk hypotheses to characterize harm 13 of Table 4.1 regarding adventitious presence of the transgene in conventional and/or organic crops due to gene flow.

Exposure pathway	Hypothesis
Cultivation of GE cucumber plants with dehydration stress tolerance.	
↓	No presence of conventional or organic crops in the receiving environment.
Presence of conventional or organic crops in the receiving environment near enough one another to make possible pollen exchange.	
↓	No gene flow from GE pollen to conventional or organic crops.
Gene flow from GE cucumber pollen to conventional or organic crops (Harm).	

The first step of the exposure pathway for harm 13 to occur is the intentional growing of GE cucumber plants in fields where conventional or organic production systems are present in the nearby area. As mentioned above, the cucumber crop in Uruguay is planted under field conditions in the horticultural area of states at the south of Uruguay (Uruguay, 2000). The horticultural region is characterized by small fields one next to each other making highly probable the presence of conventional and/or organic growers in the proximity of the GE cucumber fields. Thus, the first risk hypothesis, *'no presence of conventional or organic crops in the receiving environment'*, is falsified and the analysis goes to step two of the exposure pathway.

The second risk hypothesis to test is: *'there is no gene flow from GE pollen to conventional or organic crops'*. Since the mating system of cucumber is by cross-pollination with honeybees as the main pollinating agent, which are able to transfer pollen up to three kilometers (Dadant and Dadant, 1976; Root, 2005), if authorized, the GE cucumber crop would likely be planted by horticultural growers that are concentrated in a small area next to

each other and step 2 of the risk scenario described above is probable (Hokanson *et al*., 1997a, b).

Another factor to consider is environmental conditions. Collison (1973) described conditions influencing pollen viability and bee activity in cucumber crops. Environmental conditions directly influence pollination since bee activity, pollen viability and longevity as well as flowering time and stigma receptivity are correlated each other and depend on thresholds of temperature, light intensity, humidity and wind. Flowers (males and females) open in the morning and normally close in the afternoon (evening) on the day of anthesis. Bees foraging activity starts at the same time that anthesis occurs, triggered by the anther dehiscence in staminate flowers and nectar secretion in pistillate flowers. Pollen is viable and the stigmas are receptive once anther dehiscence occurs. Pollen longevity varies with the variety and the environmental conditions, but generally decreases during the day and is greatly reduced in day-old staminate flowers. Although it could be the case that the following morning the petals of one day-old flowers are withered, the pollen may still be viable, the pistillate flowers may contain some nectar and the stigma can be receptive. Environmental conditions with low humidity and wind cause pollen to lose viability sooner. Stigma receptivity also depends on environmental conditions but it remains receptive longer than pollen viability. The conditions above described required for optimal flower formation, anthesis, bee activity and pollination are met under the Uruguayan receiving environment (Aldabe, 2000).

The other condition that must occur for GE pollen to effectively pollinate non-GE plants is phenological overlap in flowering time. This would be probable since horticultural growers from the same area sow crops according to climate conditions and market demand. Thus, the second risk hypothesis is falsified corroborating the possibility for gene flow from GE cucumber pollen to conventional or organic crops. Additional experimentation for

hypothesis testing of this harm (Table 4.13) is not required. However, once a harm is characterized as being possible to occur, the next step of risk assessment methodology is the *'consideration of possible adequate strategies for the management of the risk, which could reduce the probability of occurrence and/or its consequences and to meet contingencies'*. Therefore an analysis plan with experiments to find risk management measures to avoid adventitious presence by cross pollination would be required according to Uruguayan regulation of coexistence (Uruguay, 200)

An issue that has recently raised concern in the case of crops that are pollinated by honeybees, is the prohibition by the European Union market of the importation of honey that contains pollen from transgenic crops if the transgenic events are not authorized in the European Union for all categories of food and feed (Uruguay, 2012-2013). This new market situation has already damaged the honey production in Uruguay due to the presence of pollen from GE corn containing the event MON810 as well as other countries for which main honey market is Europe. Similar to the risk management approaches for corn that is wind pollinated, discussions are underway regarding the location of beehives. It is proposed that beehives from which honey is going to be exported be at least ten kilometers from any GE crop. However in the study case, cucumber requires honeybees for its normal pollination and therefore it will be necessary to locate beehives in the crop and make sure the honey is not exported to Europe.

Commercial harm: Negative economic impacts to conventional or organic crops due to seed mixing (harm 14)

In this case the factor that determines the harm is the occurrence of adventitious presence of the transgene in conventional and/or organic crops due to seed mixing. Table 4.14 represents the pathway with the sequence of steps that are necessary to happen in order for the cultivation of GE cucumber plants to cause the agronomic harm 14.

Table 4.14. Exposure pathway and risk hypotheses to characterize harm 14 of Table 4.1 regarding adventitious presence of the transgene in conventional and/or organic crops due to seed mixing.

Exposure pathway	Hypothesis
Seed production of GE cucumber plants with dehydration stress tolerance.	
↓	No seed production of conventional or organic cucumber in the receiving environment.
Seed production of conventional or organic crops in the receiving environment.	
↓	No seed mixing of GE seed in conventional or organic cucumber seed production.
Seed mixing from GE cucumber seed to conventional or organic cucumber seed production (Harm).	

The exposure pathway described in Table 4.14 is blocked in step 1 since there is no cucumber seed production in Uruguay at the commercial level [(Personal Communication, Sanguinetti G., Seed certification manager of the National Seeds Institute (INASE), 2012]. Thus, harm 14 is eliminated from consideration of the study case.

SUMMARY OF PROBLEM FORMULATION

The roadmap began with a list of possible environmental harms that could occur as consequence of cultivation of GE crops. The list was refined by analyzing the exposure pathway and corresponding risk hypotheses using existing data and baseline information. Those harms for which risk hypotheses were sufficiently corroborated were eliminated from consideration. Table 4.15 shows the original table (Table 4.1) with the harms that require further analysis highlighted in bold black and the harms eliminated in normal black.

In summary, harms that require further considerations in the study case include concerns related to a negative impact on non-target organisms such as beneficial insects and/or soil microorganisms of the agroecosystem that may result from secondary effects of expression of CBF leading to increased production of natural toxic products in cucumber (harms 3 and 6 respectively). This consideration can be evaluated by testing for substantial equivalence and levels of known cucumber toxins. The harm caused by the hazard associated with the commercial situation of potential adventitious presence due to gene flow from GE cucumber pollen to conventional and/or organic crops (harm 13), is also relevant for the study case and requires further considerations. According to the Uruguayan legislation it would be necessary the implementation of management measures to prevent adventitious presence.

If harm requires further consideration an analysis plan is elaborated describing the experimental design, assessment endpoints and methodology to measure them. Table 4.16 summarizes the process of Problem Context and Problem Definition with respect to the harms defined as relevant for the study case.

In conclusion, the list of potential environmental harms to be considered, which is based on environmental protection goals and scope, would be the same either for genes with direct effects, such as insect- herbicide tolerant crops, or for genes conferring more complex

traits such as the study case of an abiotic stress tolerant crop. A difference with respect to analysis of traits that may confer a selective advantage in the receiving environment and/or the use of genes whose products regulate the expression of other genes, is the necessity to consider potential for a broader range of possible phenotypes that could cause environmental impact.

Table 4.15. Possible environmental harms that may require further consideration.

<table>
<tr><th colspan="3">Possible environmental harms that may require further consideration</th></tr>
<tr><td rowspan="3">Environmental biosafety issues</td><td rowspan="3">Agronomic</td><td>Weediness:
1)- GE crop itself becomes a weed in agricultural systems (phenotypic effect of the gene causes volunteer plants with increased persistence).
2)- Generation of more aggressive weedy relatives (harder to kill, population expansion) due to gene flow from pollen.</td></tr>
<tr><td>Negative effects on organisms (non-target species and food webs):
3)- Toxic effect of the gene product on non-target organisms.
4)- Harmful effect on organisms due to changes in agricultural practices that may change the agroecological system.
5)- Development of resistance to products that confer herbicide or insect protection.</td></tr>
<tr><td>Negative effects on soil functions:
6)- Toxic effect of the gene product on soil microorganisms.
7)- Negative impact on soil physical properties and soil microorganisms due to changes in agricultural practices.</td></tr>
<tr><td>Ecological</td><td>Negative effects on natural resources (soil, water, wildlife and flora) and its biodiversity: 8)- GE crop itself becomes an invasive species because it could be more persistent.
9)- Gene flow from pollen to wild relatives causing one of the following (implies hybridization and introgression success of the transgene):
a) Increase abundance of wild relatives /become invasive if transgene confers fitness advantage (population size);
b) Decrease abundance of rare wild relatives/become extinct if it confer fitness disadvantage (outbreeding depression) (loss of biodiversity) (population size);
10)- Harmful effect on non-target species (insects, natural enemies, soil microorganisms)
11)- Soil degradation due to a change in agricultural practices.
12)- Destruction of refuge areas due to agricultural expansion over natural environments.</td></tr>
<tr><td>Commercial issues</td><td>Coexistence (adventitious presence)</td><td>Negative effects on trade:
13)- Negative impact on trade due to gene flow from pollen to conventional or organic crop.
14)- Negative impact on trade due to seed mixing (seed dispersal).</td></tr>
</table>

Table 4.16.Summary of Problem Formulation performed in the context of a hypothetical environmental risk assessment process of GE cucumber plants engineered for dehydration stress tolerance using a transcription factor.

	Protection Goeal: Conservation of the agricultural environment (agroecosystem)	**Protaction Goal: Commercial situation: Coexistance between different production systems**
Plant attribute (hazard)	*CBF* genes confer dehydration stress tolerance. .	Cucumber is a cross-pollinated species.
Mechanism of the plant attribute to cause harm	*CBF* genes could negatively impact on non-target organisms, insects (Harm 3) and/or soil microorganisms (Harm 6) by causing substantially increased production of toxic compounds normally produced by cucumber.	No specific mechanism due to the transgene. Gene flow from GE cucumber pollen to conventional or organic cucumber plants (Harm 13).
Environmental value	Balanced agro-ecosystem regarding organisms population	Absence of adventitious presence in conventional and/or organic cucumber crops.
Assessment endpoint	Abundance of non-target organisms.	Cross-pollination between GE cucumber plants and conventional or organic cucumber plants.
Assessment methodology	1) General plant phenotypic characterization under water stress and non-stress conditions. 2) Compositional analysis from field produced samples. If necessary according to results from 1 and 2, confirmatory studies with non-target organisms to confirm risk hypothesis of lack of toxicity.	Identification of *CBF* genes at the molecular level (PCR analysis) in conventional or organic cucumber plants.
Exposure pathway	Commercial release of GE cucumber plants.	Commercial release of GE cucumber plants growing adjacent/nearby to conventional or organic cucumber.
Risk hypothesis	*CBF* genes will not negatively impact the abundance of non-target organisms.	GE cucumber plants will not determine adventitious presence of *CBF* genes in conventional and/or organic cucumber crops growing according to risk management measures for coexistence.

APPENDIX

APPENDIX

Table A4.1. Minimum temperatures (°C) from March 15th to April 30th in the south and north regions of Uruguay in the period 2006 to 2010.

Region	Year	Average minimum temperature from 03/15 to 04/30 (°C)
South	2006	12.0
South	2007	13.8
South	2008	11.8
South	2009	10.0
South	2010	11.3
Average South		**11.8**
North	2006	13.3
North	2007	16.8
North	2008	12.3
North	2009	13.8
North	2010	10.4
Average North		**13.3**
Source of data: Agroclimatic Information System (GRAS) of the National Agricultural Research Institute (INIA), http://www.inia.org.uy/gras/		

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