E. THOMAS, PH.D. NICKSON

Ecological Technology Center, Monsanto Company,
800 N. Lindbergh Blvd., St. Louis, Missouri, USA, 63141

ENVIRONMENTAL RELEASE OF LIVING MODIFIED ORGANISMS: CURRENT APPROACHES AND CASE STUDIES

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Agricultural biotechnology is being rapidly adopted as evidenced by the acreage of genetically modified (GM) crops planted and tonnes of product (grain and fiber) harvested. Concurrent with this technological progress, is a growing concern that the world's biological diversity is coming under increasing threat from human activities. As such, ecological risk assessment approaches are being developed for GM crop plants as international agreements regulating the transboundary movements of these products are being implemented. This paper reviews the ecological risk assessment approach that has been used to date to approve GM crops to date. The process has been case-by-case, using a comparative, science-based approach balancing the potential risks and benefits of the new technology versus those present with the currently accepted practices. The approach used to evaluate and approve these products is consistent with the conditions and requirements outlined in the Cartagena Protocol.

Introduction. Agricultural biotechnology is being rapidly adopted as evidenced by the acreage of genetically modified crops planted and tonnes of product (grain and fiber) harvested [1]. Adoption first occurred in Argentina, Canada, China and the United States; but other large agricultural producing countries including Australia, South Africa and India are now growing these products on significant acreage. Regulatory approval of each product was obtained through a formal, scientifically based process prior to the production of the crops in these countries. To date, most commercial experience has been with approved crops containing herbicide tolerant and insect protected traits [1]. As such, a few biotechnology-derived products predominate in terms of acreage of production and their availability to global markets.

Concurrent with this technological progress, is a growing concern that the world's biological diversity is coming under increasing threat from human activities. In response to this concern, representatives from many nations negotiated the Convention on Biological Diversity. The Convention recognizes the use of crop products derived through modern biotechnology as living modified organisms (LMOs) in agriculture as a technology with potential benefits and risks. It further recommends that these products should be developed carefully, with appropriate safeguards to ensure that implementation proceeds without unnecessary risk to biodiversity and human health. Accordingly, the parties to the Convention developed the Cartagena or Biosafety Protocol (CP) [2] to provide oversight regarding transboundary movement and assessment of the risk of LMOs used in agricultural production and commodity trade.

The products currently being marketed have undergone a rigorous environmental risk assessment in the countries where they have been approved. Countries that have approved LMOs for intentional (uncontained) release have done so after developing environmental risk assessment guidelines for their use in agriculture. Scientifically robust risk assessments provide a basis approving LMOs for intentional release. Some examples are discussed in more detail below.

General Principles of Ecological Risk Assessment for LMOs

Ecological risk assessment for LMOs should be based on the general principles of ecological risk assessment (*era*). These principles have been described in several sources [3, 4]. According to the

Phenotypic and Compositional Analyses Complete on HT Corn

Phenotypic CharacteristicsPhenotypic Characteristics	Compositional Characteristics
Early stand count	Protein
Days to 50 % pollen shed	Total fat
Days to 50 % silking	Ash
Ear height (in)	Fiber (various)
Plant height (in)	Carbohydrates
Stay green (1—9 scale)	Moisture
# ears dropped	Individual minerals
% moisture in grain	Amino acids
Yest weight (lbs/bu)	Fatty acids
Yield (bu/acre)	Secondary metabolites

literature, a valid *era* should be science-based, utilize a systematic approach that is inclusive of all available information and iterative based on new information. In addition, the fundamental elements of risk (hazard + exposure/frequency) should be explicitly described in order to accurately characterize and analyze the overall risk. *Era* approaches to genetically modified plants have been described [5—9]. Every model recommends that assessments of LMOs be case-by-case, giving detailed consideration to the biology of the crop, the nature of the trait and the environment into which the LMO will be released. For countries signatory to the Convention on Biological Diversity, *era* must be in accordance with principles outlined in Annex III of the CP [1]. Annex III of the CP states that *era* should acknowledge expert advice as well as guidelines developed by «relevant international organizations». The CP also notes that risks should be viewed in a comparative context (i.e., relative to the non-modified counterpart) focusing on the likely receiving environment. Finally, the CP recommends a precautionary approach stating that: «*Lack of scientific knowledge or scientific consensus should not necessarily be interpreted as indicating a particular level of risk, an absence of risk, or acceptable risk*». Importantly, all the era models described above have been developed with the intention to apply them for intentional release.

The method discussed below to assess the environmental risk associated with LMOs is a tiered approach focusing on the LMO relative to the recipient plant, the introduced trait, including the donor organism, and the likely environment for release based on the proposed use. A LMO is characterized in detail for its biological and ecological attributes using detailed phenotypic and compositional evaluations. In addition, the trait is evaluated for its potential to confer a selective advantage (i.e., alter the weediness/invasiveness of the plant) to the LMO or adversely impact nontarget organisms (nto's). Since the nature of the trait will dictate the intended use of the LMO, its properties will define the likely environment where the LMO will be released. Based on the outcome of this phenotypic and compositional assessment, a regulatory official can determine if the properties of the LMO have been meaningfully modified in terms of its ecological properties compared to a conventional counterpart. Many LMOs are not meaningfully changed compared to the non-modified crop in their phenotypic and compositional characteristics. This outcome allows a regulator to conclude that the LMO is «familiar» [6, 10, 11] and thereby focus the *era* on the introduced trait and the likely receiving environment. As such, «familiarity» in not a risk endpoint; rather, it is a characterization that enables scientists to focus the risk assessment on meaningful differences.

Case Studies

Herbicide Tolerant Corn: Several products have been developed to give growers improved tools for weed management in corn production systems. As noted above, the steps in the era of herbicide tolerant (HT) corn have been to:

- assess familiarity through agronomic and compositional analysis,
- evaluate the trait for potential selective advantage or impacts to nto's,
- evaluate the LMO for the site of likely release into the environment.

The HT corn products evaluated to date have been examined in detail by phenotypic and compositional analyses. The phenotypic characters evaluated were based on the experience and information available for traditionally bred corn. Summarized in Table are the phenotypic characteristics that have been assessed in HT corn products.

Also listed in Table are the nutritional and antinutritional (secondary metabolite) compositional components that have been evaluated in HT corn. All analyses were conducted on plants that were

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grown in field trials at multiple locations in replicated plots. Furthermore, the results of every analysis were compared with information that is known for corn either from the literature or based on expert opinion.

In addition to the detailed characterization of the plant outlined in Table, HT corn would be examined for the potential selective advantage of the trait and its potential impact on nto's. In the case of Roundup Ready® corn (RR corn), the introduced trait is a variant of an endogenous protein already present in corn. Since the protein already present in corn, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), is a member of a family of proteins that confer no selective advantage to plants in general and have no toxicity toward nto's, it was concluded that the introduced EPSPS will not meaningfully change the ecological and environmental risk of this HT corn. As such, no further assessment has been necessary in the countries that have approved RR corn to date. Similarly, the HT trait only confers a selective advantage when the specific herbicide is being used. The HT corn that has been evaluated to date has been shown to have no increased potential to become a weed. Were HT corn accidentally released into the environment outside of the agricultural field, the risk of an environmental impact would be no greater than the risk present from conventional corn because the HT trait does not confer a selective advantage. In areas where sexually compatible relatives are present (e.g., Mexico for corn), a more detailed examination of the frequency and consequences of pollen-mediated gene flow would be needed.

Lastly, the likely environment of release of the HT corn was considered as part of the assessment process. Since the HT trait is an agronomic input trait (useful only in agricultural production systems), and because >95 % of corn in the US is treated with herbicide, it was concluded that the likely environment for release is identical to that of any other conventional corn. Consequently, the era focused on those areas where corn is currently produced and where it is known to establish and persist, which is only in cultivated fields. After the data were analyzed, the HT trait was shown to have no greater affect on the environment than that already experienced with non-modified corn where weed control is currently practiced. Furthermore, recent studies with RR corn have shown that the environment can benefit from the use of a more efficacious, environmentally superior herbicide [12].

The overall era conclusion for the HT corn products currently approved for use in corn production systems is: *no meaningful environmental risk exists compared to non-modified corn*. Recently, the Office of Science and Technology Policy (OSTP) of the United States conducted a review on the risk assessment approaches taken and decision-making processes for LMO products. One of these products was a herbicide tolerant soybean plant [13]. Despite the fact that the analysis focused on HT soybeans, this reference provides valuable insight into the regulation and approval process used in the United States for intentional release of HT crops including corn.

Insect Protected Corn: Insect protected (IP) corn is currently based on the insertion of well-characterized genes from a common soil bacterium, *Bacillus thuringiensis* (Bt). The first products targeted lepidopteran pests through expression of the Cry1Ab protein, which is know to be specific to this family of insects. Currently, growers in the US, Canada, South Africa, and some parts of Europe are witnessing the benefits of using insect protected corn in their production systems. These benefits include modest reduction in pesticide use and markedly reduced levels of mycotoxins in their harvested grain [14]. The era approach used to approve IP corn for intentional release involved collecting the same data used to approve HT corn (see above) as well as additional information on environmental fate and impacts to nontarget organisms.

Detailed characterization of IP corn revealed that it was unchanged in terms of its phenotypic and compositional characteristics compared to non-modified corn (i.e., familiar) with the exception of the IP trait conferred by the presence of a single Bt protein. As with HT corn, the environment for likely release of IP corn is the same as that in which non-modified corn is grown. In fact, IP corn is only used in areas where specific lepidopteran pests are known or likely to occur. As a result, the environment of intentional release is a subset of the entire corn production area. After familiarity was established for IP corn, the focus of the risk assessment centered on the IP trait and its potential to confer a selective advantage or adversely impact nto's.

Detailed evaluation of the compositional and phenotypic characteristics of the IP corn plant (Table) demonstrated that IP corn was not meaningfully changed in its weediness characteristics. No changes were observed for levels of key nutrients and anti-nutrients, germination, dormancy, growth habit, reproductive potential, seed dispersal and persistence. Because there are no wild relatives of corn in the countries where intentional release will occur, the frequency of pollen-mediated gene flow to a compatible species was zero. As such, it was concluded that IP corn posed no meaningful risk of increased weediness or transfer of a selective advantage to a wild relative in the regions where this product has been approved. The focus of the risk assessment then became the potential to adversely impact nto's.

A tiered approach was taken to assess impact to nto's based on guidelines established in the US for biological agents (US EPA Subdivision M Guidelines). Selected species representing diverse, ecologically important functions within agricultural systems were chosen for testing. Representative pollinators, predators, parasitoids, decomposers, aquatic organism and avian species were tested, many at very high doses, to evaluate potential toxicity:

Nontarget Organism Testing and No Effect Levels for Bt Protein

Adult honey bee	>20 ppm
Larval honey bee	>20 ppm
Lady bird beetle	>20 ppm
Green lacewing	>16.7 ppm
Parasitic wasp	>20 ppm
Earthworm	>200 mg/kg*
Collembola	50.6 mg/g*
Daphnia magna	>100 mg/L*
Bobwhite quail	>100,000 ppm*

* Tests were conducted with plant tissue, otherwise pure protein was tested.

The test substances were either pure protein or plant tissues incorporated into diets. In all tests conducted, no evidence of toxicity was detected (No Effect Levels) at the highest doses tested. For all species except the bobwhite quail, these doses were >10x the expected environmental concentrations. Based on US EPA testing guidelines, these tests indicate minimal risk to nto's.

A well-publicized, preliminary report from a university in the US alleged that monarch butterflies (*Danaus plexippus*) might be at risk from consumption of pollen produced by IP corn [15]. Since *era* is inclusive of all information and iterative, an extensive program including academicians, industry and government scientists was launched to resolve this issue. The conclusions of this investigation were ultimately published in a peer-reviewed journal [16], and the US EPA concluded that IP corn poses minimal risk to monarch butterflies. However, this is a good example of how era conclusions can be revisited based on new information.

Lastly, because the insecticidally active Bt protein is present in IP corn tissues at the end of the season, the environmental fate of the protein was studied as part of the era. The approach taken was to place either pure Bt protein or plant tissues from IP corn into soils and monitor the degradation of the protein. Results published by [17] showed that the Cry1Ab protein is rapidly degraded in soil. When this research was extended to high clay soils know to bind proteins, academic scientists reported that the Cry1Ab protein could persist for longer periods of time [18]. Based on these results, several additional studies have been launched showing that the Cry1Ab protein may bind tightly in high clay soils in the lab, but that there are no effects to a broad group of nto's [19]. As such, if the Cry1Ab protein is binding to soil in the field, the risk to nto's is still minimal.

The *era* conclusion for the IP corn products currently approved for use in production systems is: *no meaningful environmental risk compared to non-modified corn*. However, two conditions of registration were instituted by the US EPA, which are: manage the potential to develop resistance in target pests and monitor the Cry1Ab protein levels in soils within the fields where IP corn has been grown. These conclusions are based on a detailed characterization of the IP corn plant as well as an assessment of its potential impact to nto's. The conditions of registration were designed to maintain the product's efficacy and to collect additional information. Importantly, they were imposed to ensure that the efficacy and longevity of the product would be maximized, and not for any identified hazard. Recently, the Office of Science and Technology Policy (OSTP) of the United States conducted a review on the risk assessment approaches taken and decision-making processes for LMO products. One of these products was an IP corn plant [20]. This reference provides valuable insight into the regulation and approval process used in the United States for intentional release of IP corn.

Conclusion

In summary, the careful development of LMOs requires that a precautionary-based, case-by-case, scientifically sound, *era* approach be developed that is protective of local biodiversity and appropriate to the level of risk present. Detailed *era's* have been completed on the several LMO-based products that are currently being traded around the world. This paper describes the approaches taken to approve HT and IP corn products that are now being produced on millions of acres in several countries. Other countries should evaluate the quality and rigor of these *era's* in light of the CP, with an eye to protecting their local biodiversity, while also giving careful consideration to the important issues of agricultural economy and efficiency.

РЕЗЮМЕ. Сельскохозяйственная биотехнология быстро внедряется, о чем свидетельствует увеличение площадей возделываемых генетически модифицированных (ГМ) сельскохозяйственных растений и объемов собираемого урожая (зерно и волокно). Наряду с технологическим прогрессом возрастает беспокойство, что мировое биологическое разнообразие находится под возрастающей угрозой из-за хозяйственной деятельности человека. В связи с этим развиваются подходы к оценке экологического риска использования ГМ сельскохозяйственных культур, чтобы обеспечить выполнение международных соглашений, регулирующих трансграничное перемещение таких продуктов. В статье рассматривается использованный до настоящего времени для санкционирования ГМ культур подход к оценке экологического риска. Этот подход является сравнительным, научно обоснованным и уравновешивает потенциальные риски и преимущества новой технологии в сравнении с теми, которые уже используются в настоящее время. Подход к оценке и санкционированию этих продуктов согласуется с условиями и требованиями Картахенского Протокола.

РЕЗЮМЕ. Сільськогосподарська біотехнологія швидко втілюється, про що свідчить збільшення площі, оброблюваної генетично модифікованими (ГМ) сільськогосподарськими рослинами, і обсягів зібраного врожаю (зерно та волокно). Разом з технологічним прогресом зростає занепокоєність, що світова біологічна різноманітність знаходиться під зростаючою загрозою від діяльності людини. В зв'язку з цим розвиваються підходи до оцінки екологічного ризику використання ГМ сільськогосподарських культур, щоб забезпечити виконання міжнародних угод, які регулюють трансграничне переміщення таких продуктів. В даній статті розглядається використовуваний до цього часу для санкціонування ГМ культур підхід до оцінки екологічного ризику. Цей підхід є порівняльним, науково обгрунтованим і зрівноважує потенційні ризики і переваги нової технології в порівнянні з тими технологіями, які вже використовуються в наш час. Підхід до оцінки і санкціонування цих продуктів узгоджується з умовами та вимогами Картахенського Протоколу.

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