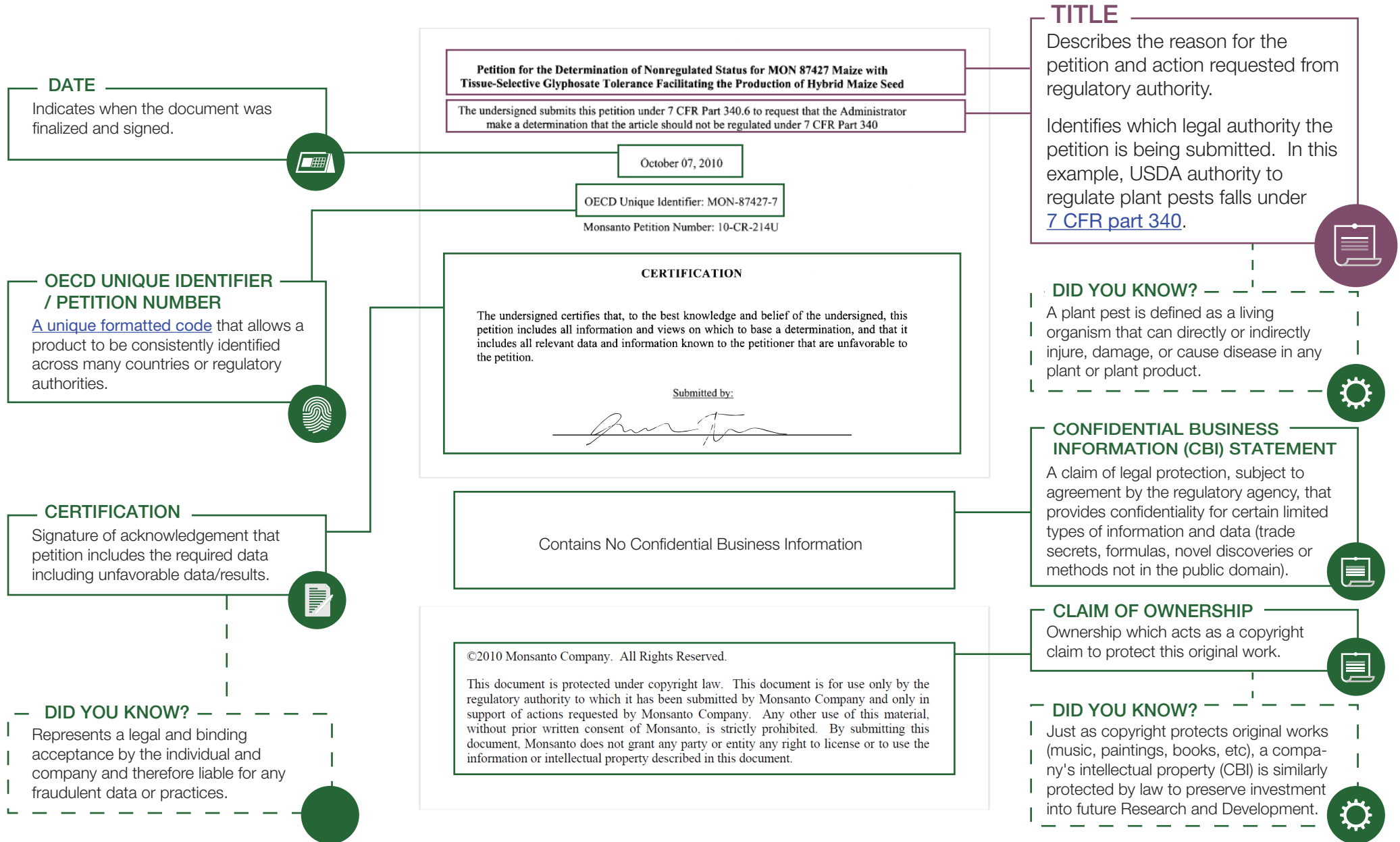


How to read a U.S. GM crop submission document

Submission documents can be quite long and not always easy to understand. To help you with reading and understanding, this guide will explain the most important parts and sections of a USDA document in detail and highlight the EPA Study Index.





EXECUTIVE SUMMARY

Brief description on what information, data and conclusions the entire petition will contain.



EXECUTIVE SUMMARY

Monsanto Company has developed biotechnology-derived MON 87427 maize with tissue-selective glyphosate tolerance to facilitate the production of viable hybrid maize seed. MON 87427 produces the same 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein that is produced in commercial Roundup Ready® crop products, via the incorporation of a *cp4 epsps* coding sequence.

The data and information presented in this petition demonstrate MON 87427 is agronomically, phenotypically, and compositionally comparable to conventional maize with the exception of the introduced trait. Moreover, the data presented demonstrate MON 87427 is unlikely to pose an increased plant pest risk, including weediness or adverse environmental impact, compared to conventional maize.

Based on the data and information presented in this petition, it is concluded that MON 87427 is not likely to be a plant pest. Therefore, Monsanto Company requests a determination from APHIS that MON 87427 and any progeny derived from crosses between MON 87427 and conventional maize or deregulated biotechnology-derived maize be granted nonregulated status under 7 CFR Part 340.

1. RATIONALE

Narrative to describe why this product was developed and the benefits and utility of the product. Also, describes the legal basis for the agency's action (the decision to/not to deregulate). Additionally, this section outlines the coordinated framework among US agencies, to ensure we are complying with all necessary regulations by other agencies that may be required for food (FDA) and environment (EPA).



I.B. Rationale for the Development of MON 87427 Maize with Tissue-Selective Glyphosate Tolerance

Monsanto Company is submitting this request to APHIS for a determination of nonregulated status in whole for the new biotechnology-derived maize product, MON 87427, any progeny derived from crosses between MON 87427 and conventional maize, and any progeny derived from crosses of MON 87427 with other biotechnology derived maize that has been granted nonregulated status under 7 CFR § 340.

Maize hybrids have been, and still are, developed and used based on the positive yield increases and plant vigor associated with heterosis, which is also known as hybrid vigor (Duvick, 2001). Inherent to the cultivation of hybrid plants, seed produced from hybrid plants is typically not used for replanting, due to the loss of hybrid vigor.

Under the Coordinated Framework for Regulation of Biotechnology, the responsibility for regulatory oversight of biotechnology-derived crops falls primarily on three federal agencies: EPA, FDA and USDA (USDA, 1986). Deregulation of MON 87427 by USDA constitutes only one component of the overall regulatory oversight and review of this product. As a practical matter, MON 87427 cannot be released and marketed until FDA and USDA have completed their reviews and assessments under their respective jurisdictions.

- DID YOU KNOW? -

Under the Plant Protection Act, USDA-APHIS has regulatory oversight over products of modern biotechnology that could pose a plant risk. Since 1986, the import, handling, interstate movement, and release into the environment of regulated organisms have been regulated.



Zea mays subspecies *mays* (L.), referred to as maize in this petition, is a versatile crop that provides food, feed, and fuel to the global economy. The biology of maize is well understood and documented. The Organization for Economic Co-operation and Development (OECD) Consensus Document on the Biology of *Zea mays* subsp. *mays* (Maize) (OECD, 2003) provides key information on:

- i. general description of maize biology, including taxonomy, morphology, and the use of maize as a crop plant
- ii. agronomic practices in maize cultivation
- iii. geographic centers of origin
- iv. reproductive biology
- v. cultivated maize as a volunteer weed
- vi. inter-species/genus introgression into relatives and interactions with other organisms
- vii. summary of the ecology of maize

MON 87427 was developed through *Agrobacterium*-mediated transformation of maize immature embryos from line LH198 × HiII utilizing PV-ZMAP1043. This section describes the plasmid vector, the donor gene, and the regulatory elements used in the development of MON 87427 as well as the deduced amino acid sequence of the CP4 EPSPS protein produced in MON 87427. In this section, transfer DNA (T-DNA) refers to DNA that is transferred to the plant during transformation. An expression cassette is comprised of sequences to be transcribed and the regulatory elements necessary for the expression of those sequences.

Characterization of the DNA insert in MON 87427 was conducted by Southern blot analyses, PCR and DNA sequencing. The results of this characterization demonstrated that MON 87427 contains a single copy of the *cp4 epsps* expression cassette, also referred to in this petition as transfer DNA (T-DNA), that is stably integrated at a single locus and is inherited according to Mendelian principles over multiple generations. These conclusions were based on several lines of evidence: 1) Southern blot analyses assayed the entire maize genome for the presence of DNA derived from PV-ZMAP1043, and demonstrated that only a single copy of the T-DNA was inserted at a single site and no plasmid vector backbone sequences were detected in MON 87427; 2) DNA sequencing analyses determined the exact sequence of the inserted DNA and allowed a comparison to the T-DNA sequence in the plasmid vector confirming that only the expected sequences were integrated; 3) Southern blot fingerprint analyses demonstrated the stability of the T-DNA present in MON 87427 over five generations; and 4) segregation analyses showed expected heritability and stability of the insert occurred across multiple generations. Taken together, the characterization of the genetic modification demonstrates that a single copy of the T-DNA was stably integrated at a single locus of the genome.

2. BIOLOGY OF THE PLANT

Evaluation of a plant pest potential relative to the conventional counterpart. This sets a foundation for the comparative assessments of what we already know of an acceptable plant today to ensure the GM plant is no different than what is currently grown.



3. DESCRIPTION OF THE GENETIC MODIFICATION

Description of how the genetic material (DNA) was introduced into the host plant. Answers the question, "What did we intend to do?"



4. CHARACTERIZATION OF THE GENETIC MODIFICATION

Detailed information on the introduced DNA and the stability (unchanged over time) of the introduced genetic material. Answers the question, "What did we actually do?"



- DID YOU KNOW? -

Stability testing is important to ensure when the trait is conventionally bred into other genetic backgrounds, it still delivers the same characteristic(s).



RAW DATA

Defined as the original data as they were taken during the study, i.e. before summarizing them for analysis. Included throughout the submissions in relevant sections discussing results.

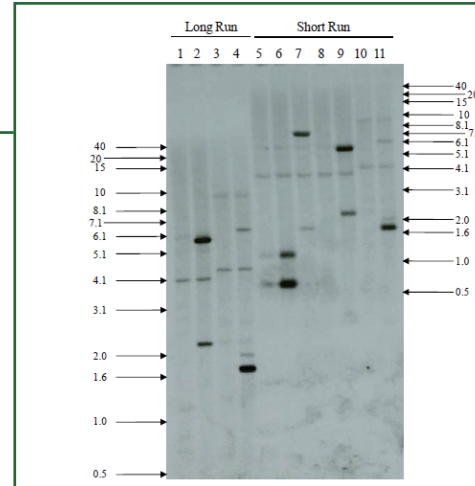


Figure IV-2. Southern Blot Analysis to Determine Insert and Copy Number of the T-DNA in MON 87427: Probe 1 and Probe 4

The blot was hybridized with two ^{32}P -labeled probes that spanned portions of the T-DNA sequence (Figure III-1, Probe 1 and Probe 4). Each lane contains ~10 μg of digested genomic DNA isolated from maize seed. Lane designations are as follows:

Lane	
1	Conventional control (<i>Nco</i> I)
2	MON 87427 (<i>Nco</i> I)
3	Conventional control (<i>Nsi</i> I)
4	MON 87427 (<i>Nsi</i> I)
5	Conventional control (<i>Nco</i> I) spiked with Probe 1 and Probe 4 [-0.1 genome equivalent]
6	Conventional control (<i>Nco</i> I) spiked with Probe 1 and Probe 4 [-1.0 genome equivalent]
7	Conventional control (<i>Nco</i> I) spiked with PV-ZMAP1043 (<i>Sph</i> I) [-1.0 genome equivalent]
8	Conventional control (<i>Nco</i> I)
9	MON 87427 (<i>Nco</i> I)
10	Conventional control (<i>Nsi</i> I)
11	MON 87427 (<i>Nsi</i> I)

Arrows denote the size of the DNA, in kilobase pairs, obtained from the 1 Kb DNA Extension Ladder (Invitrogen) on the ethidium bromide stained gel.

5. CHARACTERIZATION AND SAFETY ASSESSMENT OF THE PROTEINS PRODUCED

Analyses of the introduced protein(s) to evaluate the safety for human and animal consumption as well as the plant pest risk.

Some elements of the protein studies include:

- // history of safe consumption
- // physical and functional properties
- // levels in various plant tissues
- // similarity to known protein toxins/allergens
- // simulated mammalian digestibility
- // high dose toxicity evaluations in mammals



Characterization of the introduced protein in a biotechnology-derived crop is important to establishing food, feed, and environmental safety. As described in Section IV, MON 87427 contains a *cp4 epsps* expression cassette that, when transcribed and translated, results in the expression of the CP4 EPSPS protein.

This section summarizes: 1) the identity and function of the CP4 EPSPS protein produced in MON 87427; 2) demonstration of the equivalence of the plant-produced and *E. coli*-produced proteins; 3) the level of the CP4 EPSPS protein in MON 87427 plant tissues; 4) assessment of the potential allergenicity of the CP4 EPSPS protein produced in MON 87427; and 5) the food, feed, and environmental safety assessment of the CP4 EPSPS protein produced in MON 87427. The data support a conclusion that MON 87427 is safe for the environment and human or animal consumption based on several lines of evidence summarized below.

DID YOU KNOW?

DNA directs the production of a protein(s) which gives a plant a specific or particular characteristic (trait) - like herbicide tolerance, insect resistance.



Safety assessments of biotechnology-derived crops typically include comparisons of the composition of forage and grain of the GE crop to that of conventional counterparts (Codex Alimentarius, 2003). Compositional assessments were performed using the principles and analytes outlined in the OECD consensus document for maize composition (OECD, 2002).

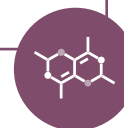
A recent review of compositional assessments conducted according to OECD guidelines that encompassed a total of seven GE crops, nine countries and 11 growing seasons concluded that incorporation of biotechnology-derived agronomic traits has had little impact on natural variation in crop composition; most compositional variation is attributable to growing region, agronomic practices, and genetic background (Harrigan, et al., 2010). Compositional quality therefore implies a very broad range of endogenous levels of individual constituents. Numerous scientific publications have further documented the extensive variability in the concentrations of crop nutrients, anti-nutrients, and secondary metabolites that reflects the influence of environmental and genetic factors as well as extensive conventional breeding efforts to improve nutrition, agronomics, and yield (Harrigan, et al., 2007; OECD, 2002; Reynolds, et al., 2005; Ridley, et al., 2004).

This section provides an evaluation of the phenotypic and agronomic characteristics and the environmental interactions of MON 87427 compared to the conventional control (LH198 × LH287). The data support a determination that MON 87427 is similar to conventional maize with the exception of the tissue-selective glyphosate tolerance and, therefore, is no more likely to pose a plant pest risk or to have a significant environmental impact than conventional maize. The conclusions are based on the results of the multiple evaluations reported herein.

Phenotypic, agronomic, and environmental interaction characteristics of MON 87427 were evaluated in a comparative manner to assess plant pest potential (OECD, 1993). These assessments included evaluation of five seed germination parameters, 14 plant growth and development characteristics, observations for plant responses to abiotic stress, plant-disease and plant-arthropod interactions, and two pollen characteristics. Results from the phenotypic, agronomic, and environmental interaction assessments indicate that MON 87427 does not possess weedy characteristics, increased susceptibility or tolerance to specific abiotic stresses, diseases, or arthropods, or characteristics that would confer a plant pest risk or a significant environmental impact compared to conventional maize.

6. COMPOSITIONAL ASSESSMENT OF THE TRAIT

Analyses of key nutrients and anti-nutrients (those that diminish the usefulness of nutrients/nutritive value) in the parts of the plant consumed by humans and animals compared to the levels of a conventional plant grown and harvested under similar conditions.



— DID YOU KNOW? —

These evaluations identify if there any unintended effects due to the introduction of DNA or the production of the introduced protein within the metabolism of the plant.



7. PHENOTYPIC, AGRONOMIC, AND ENVIRONMENTAL INTERACTIONS

Comparative assessment of the visual observation of growth and development of the plant. Additional analyses are conducted to evaluate how the plant is interacting with its physical surrounding and biological organisms through a combination of field and lab assessments.



TABLE

Tables efficiently present a summary of data in a numerical or statistical format versus text of the same content.

Table VII-4. Plant Growth and Development across 16 Locations during 2008

Phenotypic Characteristic (units)	Mean (S.E.)		Reference Range ¹	
	MON 87427	Control	Min	Max
Seedling vigor (1-9 scale)	2.7 (0.19)*	2.4 (0.17)	1.0	5.0
Early stand count ² (#/plot)	67.7 (1.69)	70.3 (2.01)	55.7	80.3
Days to 50% pollen shed	63.9 (1.15)	63.5 (1.19)	45.7	78.0
Days to 50% silking	62.9 (1.07)	62.7 (1.09)	46.7	75.0
Stay green ³ (1-9 scale)	5.9 (0.34)	5.6 (0.35)	2.0	9.0
Ear height (in)	42.0 (0.89)	41.9 (0.97)	26.3	56.1
Plant height ⁴ (in)	91.4 (1.50)	90.8 (1.50)	73.9	103.1
Dropped ears ⁵ (#/plot)	0.6 (0.22)	0.5 (0.17)	0.0	2.7
Stalk lodged plants ⁵ (#/plot)	7.7 (2.58)	5.5 (1.77)	0.0	71.3
Root lodged plants (#/plot)	7.5 (2.49)	5.3 (2.08)	0.0	25.9
Final stand count ⁶ (#/plot)	60.8 (0.89)	60.4 (0.88)	54.7	65.8
Grain moisture ⁶ (%)	19.6 (0.55)	20.3 (0.62)	16.0	27.4
Test Weight ⁶ (lbs/bu)	55.3 (0.33)	55.2 (0.38)	51.6	58.6
Yield ⁶ (bu/a)	156.9 (5.47)	165.4 (6.71)	94.6	193.8

Note: the experimental design was a randomized complete block design with three replications at each site. S.E. = standard error. N = 48 except where noted.

* Indicates statistically significant difference between MON 87427 and the conventional control ($\alpha=0.05$).

¹Reference range was calculated from the minimum and maximum mean values from among the 38 unique reference hybrids.

8. AGRONOMIC PRACTICES IN THE UNITED STATES

Assessment to consider whether the new characteristics in the plant will change how growers produce the crop; also includes a description of stewardship practices that growers can implement to sustainably use this introduced trait.



The U.S. Code of Federal Regulations, 7 CFR § 340.6(c)(4), requires that potential impacts to agricultural and cultivation practices be considered as part of plant pest risk assessments. This section is a summary of current agronomic practices in the U.S. for producing hybrid maize seed and, to a lesser extent, grain production. The innovation realized by MON 87427 will occur only on acres used for the production of hybrid maize seed. This section is included in this petition as a baseline to assess possible impacts to agricultural practices due to the introduction of MON 87427.

Hybridization is a fundamental concept used in maize breeding and production programs in the U.S. and most of the world. The fixation of alleles in pure lines (i.e., inbreds) causes a general reduction in maize vigor and productivity, but hybridization can improve vigor in the maize that is grown from the F1 hybrid seed² produced through crossing two inbred lines. Modern maize breeding is based on selecting inbred lines and producing crosses that possess desirable traits. Recent techniques such as marker-assisted selection can also reduce the time and cost required to achieve breeding goals (Yousef and Juvik, 2001). MON 87427 maize with tissue-selective glyphosate tolerance was developed to facilitate the production of viable hybrid maize seed. Use of MON 87427 and specifically timed glyphosate applications eliminates or greatly reduces the need for the manual and mechanical detasseling currently used in hybrid maize seed production.

This section provides a brief review and assessment of the plant pest potential of MON 87427 and its impact on agronomic practices as well as the environmental impact of the introduced CP4 EPSPS protein. USDA-APHIS has responsibility, under the Plant Protection Act (PPA) (7 U.S.C. § 7701-7772), to prevent the introduction and dissemination of plant pests into the U.S. Regulation 7 CFR § 340.6 provides that an applicant may petition APHIS to evaluate submitted data to determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article does not present a plant pest risk, the petition is granted, thereby allowing unrestricted introduction of the article.

According to the PPA, the definition of “plant pest” includes living organisms that can directly or indirectly injure, damage, or cause disease in any plant or plant product (7 U.S.C. § 7702[14]). The regulatory endpoint under the PPA for biotechnology-derived crop products is not zero risk, but rather a determination that deregulation of the article in question is not likely to pose a plant pest risk. Information in this petition related to plant pest risk characteristics includes: mode of action and changes to plant metabolism, composition, expression and characteristics of the gene product, potential for weediness of the regulated article, impacts to NTO, disease and pest susceptibilities, impacts on agronomic practices, impacts on the weediness of any other plant with which it can interbreed, as well as the potential for gene flow.

Monsanto knows of no study results or observations associated with MON 87427 or the CP4 EPSPS protein indicating that there would be an adverse environmental consequence from the introduction of MON 87427. MON 87427 produces the CP4 EPSPS protein in the vegetative and female reproductive tissues, rendering the leaf, stalk and root tissues and the tissues that develop into seed or grain in the maize plant tolerant to the herbicide glyphosate. However, limited to no CP4 EPSPS protein is expressed in the tapetum and pollen microspore cells in MON 87427, thus these tissues and pollen are not tolerant to the herbicide glyphosate. The CP4 EPSPS protein produced in MON 87427 is identical to the CP4 EPSPS protein present in Roundup Ready crop products that were previously granted a determination of nonregulated status by APHIS, and has been widely planted in the U.S. and globally. As demonstrated by field results and laboratory tests, the only phenotypic difference between MON 87427 and conventional maize is glyphosate tolerance during vegetative and female reproductive stages.

The data and information presented in this petition demonstrate that MON 87427 is unlikely to pose an increased plant pest risk or to have an adverse environmental consequence compared to conventional maize. This conclusion is reached based on multiple lines of evidence developed from a detailed characterization of the product compared to conventional maize, followed by risk assessment on detected differences.

9. PLANT PEST ASSESSMENT

Conclusion statement bringing together all of the pieces of the previous analyses and evaluations to conclude whether or not the GM plant will pose an increased plant pest risk.



DID YOU KNOW?

USDA in its evaluation of GM plants considers these elements:

- // impact of the inserted genetic material on plant pest potential as well as disease and pest susceptibility
- // potential impacts on non-target organisms (NTOs) beneficial to agriculture
- // potential for enhanced weediness of the GM plant
- // potential impact on the weediness of other plants with which the GM plant can interbreed
- // potential changes to agriculture or cultivation practices



10. ADVERSE CONSEQUENCES OF INTRODUCTION

Statement that we are not aware of any additional information not already described that could indicate adverse consequences from the general release of the GM plant.



REFERENCES

At the end of each submission, a list of all references cited is provided, including scientific articles, guidelines and protocols used, and regulations.

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Agrawal, P.K., B.D. Agrawal, P.V. Rao and J. Singh. 1998. Seed multiplication, conditioning, and storage. Pages 103-123 in Maize Seed Industries in Developing Countries. M.L. Morris (ed.). Lynne Rienner Publishers, Boulder, Colorado.

Al-Kaisi, M., M.H. Hanna and M. Tidman. 2003. Crop rotation considerations for 2004 management season rotation. Iowa State University, Ames, Iowa.
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

401 M Street, S.W.
Washington, D.C. 20460

Form Approved OMB No 2070-0060

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DATA MATRIX

Date August 16, 2002			EPA Reg No./File Symbol: 524-522		Page 1 of 7
Applicant's/Registrant's Name & Address Monsanto Company, 600 13th Street N.W., Suite 660, Washington, D.C 20005			Product: Bollgard® II Cotton		
Ingredient A <i>Bacillus thuringiensis</i> Cry2Ab2 protein and the genetic material necessary for its production in Bollgard® II cotton event 15985					
Guideline Reference Number	Guideline Study Name	MRID Number	Submitter	Status	Note
	Administrative Materials in Support of the Request for the Exemption from the Requirement of Tolerance for Glucuronidase (GUS) as a Plant-Incorporated Protectant Formulation Inert Ingredient.	44939407	Monsanto Company	OWN	
	Administrative Materials in Support of the Amendment for the Request for Exemption from the Requirement of Tolerance for Cry2Ab (Petition PP 7F4888).	44966600	Monsanto Company	OWN	
	Validated Method for Detection and Direct ELISA Analysis of Cry2Ab2 in Cottonseed An unpublished study conducted by Monsanto Company	Not yet assigned	Monsanto Company	OWN	Submitted 13-Apr-01
	Insect Resistance Management Plan for Bollgard II Cotton An unpublished study conducted by Monsanto Company	45545701	Monsanto Company	OWN	
	Public Interest Document Supporting the Registration of the Plant-Incorporated Protectant, <i>Bacillus thuringiensis</i> Cry2Ab Insect Control Protein, as Produced in Cotton (<i>Gossypium hirsutum</i> L)	Not yet assigned	Monsanto Company	OWN	Submitted 11-Dec-01
	Administrative Materials in Support of the Request for the Registration of the Plant-Incorporated Protectant, <i>Bacillus thuringiensis</i> Cry2Ab Insect Control Protein, as Produced in Corn (<i>Zea mays</i> L) and Cotton (<i>Gossypium hirsutum</i> L).	45086300	Monsanto Company	OWN	

DID YOU KNOW?

- // EPA regulates GM crops with traits that protect the crop from agricultural pests, also known as Plant-Incorporated-Protectants (PIPs).
- // EPA does not regulate herbicide tolerant GM crops.
- // The EPA then publishes an agency-generated summary of the risk assessment and regulatory decisions for the PIP.
- // These summaries can be found at [EPA's website](#).

DID YOU KNOW?

- // Instances where EPA determines that the PIP present in agricultural commodities involves no hazard to human or animal health, they grant an exemption from the requirement to set a maximum "residue" limit (MRL) or maximum level of the PIP.

This table is an example of the study index that is submitted to the EPA.

The essence of an EPA

submission is the study index (or also referred to as the Data Matrix), which lists all of the documents used by the EPA to assess the safety of the Plant-Incorporated-Protectants (PIP), establish an exemption from the requirement of a tolerance (maximum residue level), and issue a commercial registration for the PIP.