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Petition for the Determination of Nonregulated Status for MON 87427 Maize with Tissue-Selective Glyphosate Tolerance Facilitating the Production of Hybrid Maize Seed

The undersigned submits this petition under 7 CFR Part 340.6 to request that the Administrator make a determination that the article should not be regulated under 7 CFR Part 340

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RELEASE OF INFORMATION

Monsanto is submitting the information in this petition for review by the USDA as part of the regulatory process. By submitting this information, Monsanto does not authorize its release to any third party. In the event the USDA receives a Freedom of Information Act request, pursuant to 5 U.S.C., § 552, and 7 CFR Part 1, covering all or some of this information, Monsanto expects that, in advance of the release of the document(s), USDA will provide Monsanto with a copy of the material proposed to be released and the opportunity to object to the release of any information based on appropriate legal grounds, e.g., responsiveness, confidentiality, and/or competitive concerns. Monsanto understands that a copy of this information may be made available to the public in a reading room and by individual request as part of a public comment period. Except in accordance with the foregoing, Monsanto does not authorize the release, publication or other distribution of this information (including website posting) without Monsanto's prior notice and consent.

CERTIFICATION

The undersigned certifies that, to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination, and that it includes all relevant data and information known to the petitioner that are unfavorable to the petition.

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EXECUTIVE SUMMARY

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has responsibility under the Plant Protection Act (Title IV Pub. L. 106-224, 114 Stat. 438, 7 U.S.C. § 7701-7772) to prevent the introduction and dissemination of plant pests into the United States. APHIS 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 no longer should 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.

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 Part 340.

Product Description

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. CP4 EPSPS confers tolerance to the herbicide glyphosate. Tissue-selective expression of CP4 EPSPS protein in MON 87427 enables an extension of the use of glyphosate tolerant maize as a tool in hybrid maize seed production.

MON 87427 utilizes a specific promoter and intron combination (*e35S-hsp70*) to drive CP4 EPSPS protein expression in vegetative and female reproductive tissues, conferring tolerance to glyphosate in the leaves, stalk, and root tissues and tissues that develop into seed or grain and silks. This specific promoter and intron combination also results in limited or no production of CP4 EPSPS protein in two key male reproductive tissues: pollen microspores which develop into pollen grains, and tapetum cells that supply nutrients to the pollen. Thus, in MON 87427, male reproductive tissues critical for male gametophyte development are not tolerant to glyphosate. This allows glyphosate-treated MON 87427 containing inbred lines to serve as a female parent in the production of hybrid seed. Two glyphosate applications beginning just prior and/or during tassel development stages (approximate maize vegetative growth stages ranging from V8 to V13) will produce a male sterile phenotype through tissue-selective glyphosate tolerance, and will eliminate or greatly reduce the need for detasseling which is currently used in the production of hybrid maize seed. In a hybrid maize seed production system, the MON 87427 inbred plants, with glyphosate applied at tassel development timings will be pollinated by pollen donor (male) plants, resulting in viable hybrid maize seed carrying

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the gene for tissue-selective glyphosate tolerance. For weed control during seed production and in commercial fields, glyphosate may be applied to MON 87427 at vegetative stages as directed on Roundup® agricultural product labels, at the same rates used in previously deregulated Roundup Ready® corn 2 events (NK603 and MON 88017).

Only specifically timed glyphosate applications beginning just prior and/or during tassel development stages (approximate maize vegetative growth stages ranging from V8 to V13) will produce a male sterile phenotype through tissue-selective glyphosate tolerance in MON 87427. Glyphosate is a systemic herbicide that is readily translocated via the phloem in plants. Once glyphosate is in the phloem, it moves to areas of high meristematic activity, following a typical source to sink distribution. Pollen development in a maize plant takes approximately 4 weeks to complete. Early tassel growth stages start at the approximate maize vegetative growth stage V9, therefore glyphosate applications made at approximately this time allow maximum translocation of glyphosate to the male reproductive tissues, and selectively cause cell death in only those cells that are not tolerant to glyphosate (i.e. tapetum and pollen cells). Glyphosate applications made during early vegetative stages, consistent with the application timing specified in the current Roundup agricultural product label for weed control purposes, do not affect pollen production of MON 87427 because the sensitive male reproductive tissues are not actively developing at that time.

The benefits of MON 87427 in the production of hybrid seed include:

- *Increased Flexibility in Hybrid Seed Production*: Each year approximately 0.5 M acres used for hybrid maize seed production must be detasseled in order to meet commercial growers' hybrid maize seed needs and to meet established seed purity criteria in the U.S. The critical time period for detasseling is after the tassel has emerged but prior to pollen shed and silk emergence, and encompasses an average 3 - 4 day window. Current detasseling practices may require up to two passes with mechanical detasseling equipment and up to three passes if hand detasseling is used. Further complicating detasseling activity is the logistical planning required for moving enough labor and resources to the designated hybrid seed production fields at the appropriate time. Glyphosate applications to MON 87427 that will result in the male sterile phenotype through tissue-selective glyphosate tolerance will take place during approximate maize vegetative growth stages ranging from V8 to V13. The two glyphosate applications would take place during an approximate 14 day window within these growth stages, a much longer time period compared to an average 3 – 4 day window between tassel emergence and pollen shed and silk emergence. This timing accounts for significantly improved flexibility in hybrid seed production.

- *Economic Benefits for Hybrid Seed Producers:* Seed manufacturers continually seek ways to improve hybrid seed productivity and reduce the inputs and land area used to produce high quality hybrid seed. Agricultural field labor costs continue to significantly outpace inflation in the U.S. Compounding this increasing cost is population migration towards urban areas that is shrinking the

agricultural labor pool, thus reducing a reliable labor pool for this work. Costs associated with labor recruitment and deployments to perform detasseling are one of the single largest cost improvement opportunities in hybrid seed production. MON 87427 will decrease hybrid seed production costs primarily from a reduction in direct and associated labor costs.

Data and Information Presented Confirms the Lack of Plant Pest Potential of MON 87427 Compared to Conventional Maize

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. The food, feed and environmental safety of MON 87427 was confirmed based on multiple, well established lines of evidence:

- Conventional maize is a familiar crop that does not possess any of the attributes commonly associated with weeds, has a history of safe consumption, and serves as an appropriate basis of comparison for MON 87427.

- A detailed molecular characterization of the introduced DNA demonstrated a single, intact copy of the transgenic insert in a single locus within the maize genome.

- The CP4 EPSPS protein in MON 87427 is identical to the CP4 EPSPS protein produced in several other commercially available crops that have been reviewed by USDA and previously de-regulated (e.g. NK603 maize, MON 88017 maize, 40-3-2 soybean). The safety of CP4 EPSPS proteins present in biotechnology-derived crops has been thoroughly assessed, and is the subject of numerous publications. The mode of action of CP4 EPSPS protein and how it confers glyphosate tolerance has been extensively studied and is well documented in peer reviewed publications.

- A compositional assessment confirmed that MON 87427 grain and forage are compositionally equivalent to grain and forage of conventional maize.

- An extensive evaluation of MON 87427 phenotypic and agronomic characteristics and environmental interactions demonstrated MON 87427 has no increased plant pest potential compared to conventional maize.

- An assessment of potential impact to non-target organisms (NTO) and endangered species indicated that, under normal agricultural conditions, MON 87427 is unlikely to have adverse effects on these organisms, similar to conventional maize.

- Evaluation of MON 87427 using intended and current cultivation and management practices for maize concluded that deregulation of MON 87427 will not significantly impact maize agronomic practices or land use.

Maize is a Familiar Crop Lacking Weedy Characteristics and is an Appropriate Comparator to MON 87427

Maize is grown extensively throughout the world, and is the largest cultivated crop in the world followed by wheat (*Triticum sp*.) and rice (*Oryza sativa L*.) in total global metric ton production. In the U.S., maize is grown in almost all states and is the largest crop grown in terms of acreage planted and net value. Maize has been studied extensively, and the domestication of maize can be traced back to approximately 10,000 years ago in southern Mexico.

Maize is not listed as a weed in the major literature references on weeds, nor is it present on the lists of noxious weed species distributed by the federal government (7 CFR Part 360). In addition, maize has been grown throughout the world without any report that it is a serious weed. Maize is poorly suited to survive without human assistance and is not capable of surviving as a weed because of past selection in the domestication of maize. During domestication of maize, traits often associated with weediness, such as, seed dormancy, a dispersal mechanism, or the ability to establish reproducing populations outside of cultivation, have not been selected. Similarly, the history of hybrid breeding in the U.S. does not indicate there are any changes in characteristics of maize weediness to change the weediness profile of the crop. Although maize seed can overwinter into a crop rotation with soybeans, mechanical and chemical measures can be used to control volunteers. Some populations of wild annual and perennial species that could hybridize with MON 87427 are known to exist in the U.S., however key differences in several factors such as flowering time, geographical separation, and development timings make natural crosses in the U.S. highly unlikely.

Conventional control materials were developed for two generations of MON 87427 that were used in Regulatory studies. The conventional control materials included the original transformation line (LH198 × HiII), used for the molecular characterization; and the hybrid conventional control (LH198 × LH287) which has a similar genetic background to the hybrid MON 87427 test material (LH198 BC3F7 × LH287). The LH198 × LH287 hybrid was the conventional control used in the phenotypic, agronomic and environmental interactions assessment, compositional analysis, and protein expression analysis. Where appropriate, commercial reference maize materials were used to establish a range of variability or responses representative of commercial maize in the U.S.

Molecular Characterization Verified the Integrity and Stability of the Inserted DNA

MON 87427 was developed through *Agrobacterium*-mediated transformation of maize immature embryos from line LH198 × HiII utilizing plasmid vector PV-ZMAP1043.

PV-ZMAP1043 contains one T-DNA that is delineated by Left and Right border sequences. The T-DNA contains one expression cassette consisting of the *cp4 epsps* coding sequence under the regulation of the *e35S* promoter, the *hsp70* intron, the *CTP2* targeting sequence, and the *nos* 3′ non-translated region. After transformation, a single plant was selected and increased (MON 87427).

MON 87427 was subjected to an extensive molecular characterization. Southern blot analyses demonstrated that a single copy of the T-DNA sequence from PV-ZMAP1043 was integrated into the maize genome at a single locus. These analyses also demonstrated that there were no additional genetic elements, including backbone sequences, from PV-ZMAP1043 detected, linked or unlinked to the intact T-DNA present in MON 87427. The PCR and DNA sequence analyses performed on MON 87427 confirmed the organization of the elements within the insert, assessed potential rearrangements at the insertion site, and resulted in the complete DNA sequence of the T-DNA and adjacent maize genomic DNA sequence in MON 87427. Furthermore, Southern blot analysis demonstrated that the T-DNA insert in MON 87427 has been maintained through five breeding generations, thereby confirming the stability of the T-DNA in MON 87427. Finally, results from segregation analyses demonstrate heritability of the insert occurred as expected across multiple generations, which corroborates the molecular insert stability analysis and establishes the genetic behavior of the T-DNA in MON 87427 at a single chromosomal locus.

Data Confirm CP4 EPSPS Protein Safety

A multistep approach was used to characterize the CP4 EPSPS protein expressed in MON 87427 resulting from the genetic modification. This detailed characterization confirms the CP4 EPSPS protein is safe for human and animal consumption. The assessment involved: 1) confirmation of 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 CP4 EPSPS 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. CP4 EPSPS has no amino acid sequence similarities with known allergens, gliadins, glutenins, or protein toxins that can have adverse effects on mammals. Safety studies conducted with the CP4 EPSPS protein demonstrated that it degrades rapidly in simulated gastric and intestinal fluids, and does not cause any adverse effects to the health of mice when gavaged at high levels in an acute oral toxicity test. The safety assessment supports the conclusion that dietary exposure to CP4 EPSPS protein derived from MON 87427 poses no meaningful risk to human or animal health.

MON 87427 is Compositionally Equivalent to Conventional Maize

Detailed compositional analyses were conducted in accordance with the Organization for Economic Co-operation and Development (OECD) guidelines and determined that levels

of key nutrients, anti-nutrients and secondary metabolites in MON 87427 were comparable to levels present in the conventional control and several commercial references. The commercial references were used to establish the natural range of levels of the key nutrients, anti-nutrients, and secondary metabolites in commercial maize hybrids that have a history of safe consumption. The samples utilized for compositional analysis were obtained from three sites: Jefferson County, Iowa; Stark County, Illinois; and Jackson County, Arkansas. The sites were planted in a randomized complete block design with three blocks per site. In addition, the MON 87427 plots were treated with glyphosate herbicide. Nutrients assessed in this analysis included proximates (ash, carbohydrates by calculation, moisture, protein, and fat), acid detergent fiber (ADF), neutral detergent fiber (NDF), total dietary fiber, amino acids, fatty acids (C8-C22), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), and vitamins [folic acid, niacin, A (β-carotene), B_1, B_2, B_6, and E] in the grain, and proximates, ADF, NDF, calcium and phosphorus in forage. The anti-nutrients assessed in grain included phytic acid and raffinose. Secondary metabolites assessed in grain included furfural, ferulic acid, and p-coumaric acid.

Combined-site analyses were conducted to determine statistically significant differences (5% level of significance) between MON 87427 and the conventional control on both forage and grain samples. Statistical results from the combined-site data were reviewed using considerations relevant to safety and/or nutritional value. These considerations included assessments of: 1) the relative magnitude of the differences in the mean values of nutrient, anti-nutrient, and secondary metabolite components of MON 87427 and the conventional control, 2) whether the MON 87427 component mean value is within the range of natural variability of that component as represented by the 99% tolerance interval of commercial maize hybrids grown concurrently, 3) evaluation of the reproducibility of the significant (α=0.05) combined-site component differences at individual sites, and 4) assessing the difference within the context of natural variability of commercial maize composition published in the scientific literature and in the International Life Sciences Institute (ILSI) Crop Composition Database.

Analytical results confirmed that the composition of the forage and grain of MON 87427 is equivalent to that of the conventional control and within the natural variability of commercial reference hybrids grown concurrently. Of the 62 components statistically analyzed, 55 were not significantly different from the conventional control. Where differences between MON 87427 and the conventional control were observed in the combined-site analysis, these differences were not meaningful to food and feed safety and nutritional value and did not alter the conclusion that MON 87427 is compositionally equivalent to the conventional maize.

MON 87427 Does Not Change Maize Plant Pest Potential or Environmental Interactions

Plant pest potential of a biotechnology-derived crop is assessed from the basis of familiarity that the USDA recognizes as an important underlying concept in risk assessment. The concept of familiarity is based on the fact that the biotechnology-

derived plant is developed from a conventional plant hybrid or variety whose biological properties and plant pest potential are well known. Familiarity considers the biology of the plant, the introduced trait, the receiving environment, and the interactions among these factors. This provides a basis for comparative risk assessment between a biotechnology-derived plant and the conventional control. Thus, the phenotypic, agronomic, and environmental interaction assessment of MON 87427 included the conventional control as a comparator. This evaluation used a weight of evidence approach and considered statistical differences between MON 87427 and the conventional control with respect to reproducibility, magnitude, and directionality. The observations were taken on plants not treated with glyphosate in order to evaluate only the impact of the introduced trait in MON 87427. Comparison to a range of commercial references established the range of natural variability for maize, and provided a context from which to further evaluate any statistical differences. Characteristics assessed included: seed dormancy and germination, pollen morphology, and plant phenotypic observations and environmental interaction evaluations conducted in the field. Commercial references were used to establish a range of natural variability for each assessed characteristic in maize. The phenotypic, agronomic, and environmental interaction assessment demonstrated that MON 87427 is comparable to the conventional control. Thus, MON 87427 is unlikely to have increased weediness or plant pest potential compared to conventional maize.

Seed dormancy and germination characterization indicated that MON 87427 seed had dormancy and germination characteristics similar to seed of conventional maize. In particular in MON87427, the lack of hard seed, a well recognized seed characteristic associated with weediness, supports a conclusion of no increased weediness of MON 87427 compared to conventional maize resulting from germination and dormancy characteristics. For pollen characteristic assessments, there were no statistically significant differences (α=0.05) detected between MON 87427 and the conventional maize for pollen diameter. MON 87427 had statistically significant higher percent pollen viability than the conventional control (99.7 vs. 98.9%) and was slightly outside the reference range. However, the difference between MON 87427 and the conventional control for pollen viability was less than one percentage point and is not deemed biologically meaningful.

The field evaluation of phenotypic, agronomic, and environmental characteristics of MON 87427 also supports the conclusion that MON 87427 is not likely to have increased weediness or plant pest potential or an altered environmental impact compared to conventional maize. The evaluations were conducted at 16 replicated field sites across U.S. maize production regions. These assessments included 14 plant growth and development characteristics, as well as observations for plant responses to abiotic stressors and plant-disease and plant-arthropod interactions. The observed phenotypic characteristics were comparable between MON 87427 and the conventional control. Across sites, data show no statistically significant differences between MON 87427 and the conventional control for early stand count, days to 50% pollen shed and silking, stay green, ear height, plant height, dropped ears, stalk and root lodging, final stand count, grain moisture, test weight, and yield. One statistically significant difference was detected between MON 87427 and the conventional control in the combined-site

analysis. MON 87427 seedlings were less vigorous than the conventional control seedlings (2.7 vs. 2.4 rating on a 1-9 scale). This difference was small in magnitude and the mean value for seedling vigor for MON 87427 was within the natural variability of the commercial references grown with MON 87427 and the conventional control. This difference was not considered biologically meaningful in terms of increased pest potential or an altered environmental impact from MON 87427 compared to conventional maize.

In an assessment of abiotic stress response and disease damage, no numeric differences were observed between MON 87427 and the conventional control for any of the 172 comparisons for the assessed abiotic stressors or for any of the 210 comparisons for the assessed diseases among all observations across the sites.

In an assessment of arthropod damage, no differences were observed between MON 87427 and the conventional control for any of the 167 comparisons. Additionally, no statistically significant differences were detected across sites between MON 87427 and the conventional control for the quantitative evaluations of corn earworm damage or for European corn borer damage.

In an assessment of pest and beneficial arthropod abundance, no statistically significant differences were detected between MON 87427 and the conventional control for 191 out of 203 comparisons (including 98 pest arthropod comparisons and 105 beneficial arthropod comparisons) among the observations at the four sites where these evaluations were made. The mean pest or beneficial arthropod abundance values from MON 87427 were within the respective reference ranges for six of the 12 detected differences. For the remaining six differences, the mean abundance values for MON 87427 were outside of the reference range; however, these differences were not consistent across observations or across sites. These results are not indicative of a consistent response associated with the trait and are not biologically meaningful in terms of adverse environmental impacts of MON 87427 compared to the conventional control.

In summary, phenotypic, agronomic, and environmental interaction data were evaluated to characterize MON 87427, and to assess whether MON 87427 had an altered plant pest potential compared to the conventional control. Results from the phenotypic, agronomic, and environmental interactions assessment support the conclusion that MON 87427 does not possess weedy characteristics, increased susceptibility or tolerance to specific abiotic stress, diseases, or arthropods, or characteristics that would confer a plant pest risk or a significant environmental impact compared to conventional maize. Collectively, these results support the conclusion that MON 87427 is not likely to exhibit increased weediness or plant pest potential or an altered environmental impact compared to conventional maize.

MON 87427 Will Not Adversely Affect NTO or Threatened or Endangered Species

Evaluation of the impacts of MON 87427 on non-target organisms (NTO) is a component of the plant pest risk assessment. Since MON 87427 does not possess pesticide activity,

all organisms that interact with MON 87427 are considered to be NTO. The environmental assessment of MON 87427 indicated that it poses no adverse effect on NTO or endangered species using current and intended agricultural practices. The assessment indicates that the CP4 EPSPS protein found in MON 87427 did not unexpectedly alter plant-arthropod interactions, including beneficial arthropods, or alter disease susceptibility compared to the conventional control.

The safety of CP4 EPSPS proteins present in biotechnology-derived crops has been extensively assessed. A mouse gavage study demonstrated no acute oral toxicity and consequently the low potential for impact to terrestrial vertebrate NTO including threatened and endangered vertebrate species. In addition, the history of safe use of Roundup Ready crops and the ubiquitous presence of functionally identical EPSPS proteins in plants and microbes in the environment make it unlikely that the presence of CP4 EPSPS protein in MON 87427 will have a significant impact on water quality. Data from the 2008 U.S. phenotypic and agronomic study, and observational data on environmental interactions such as plant-disease interaction, arthropod damage and arthropod abundance, were collected at select sites for MON 87427 and conventional control. Results from this study support the conclusion of no adverse environmental impact from cultivation of MON 87427 to non-target arthropod populations. Taken together, these data support the conclusion that MON 87427 has no reasonable mechanism for harm to NTO, or impact on threatened and endangered species compared to the cultivation of conventional maize.

The potential for MON 87427 outcrossing to sexually compatible species is unlikely in the U.S. Maize and annual teosinte (*Zea mays* subsp. *mexicana*) are genetically compatible, wind-pollinated and, in areas of Mexico and Guatemala, hybridize when in close proximity to each other. However, teosinte is not present in the U.S. other than as an occasional botanical garden specimen. Differences in factors such as flowering time, geographical separation and development timings make natural crosses between maize and annual teosinte in the U.S. highly unlikely. In contrast with maize and teosinte, it is only with extreme difficulty and special techniques that maize and the closely related perennial species, *Tripsacum* (gamma grass) hybridize. Additionally, the offspring of the cross show varying levels of sterility and are typically genetically unstable. Further, the *Tripsacum* species that exist in the U.S. occur in areas not preferred for commercial maize production. Finally, *Tripsacum*-maize hybrids have not been observed in the field. Therefore, the environmental consequence of pollen transfer from MON 87427 to other wild plant species, including any threatened or endangered plant species, is considered negligible.

Deregulation of MON 87427 Will Not Significantly Impact Maize Agronomic Practices or Land Use

MON 87427 will be used to facilitate the production of viable hybrid maize seed and offers an alternative to mechanical and manual detasseling methods, and Cytoplasmic Male Sterile technology. The practices for the production of hybrid maize seed with MON 87427 are essentially unchanged with regard to current practices for hybrid maize

seed production, with the exception of the reduction in the use of mechanical detasseling and the use of glyphosate sprays during the approximate corn vegetative stages ranging from V8-V13 to produce the male sterile phenotype in (female) inbred plants. Hybrid maize seed production fields and hybrid maize cultivation fields are typically highly managed agricultural areas that can be expected to be dedicated to crop production for many years. Maize hybrids containing MON 87427 likely would be used in common rotations on land previously used for agricultural purposes. No significant impact would be expected following the introduction of MON 87427 on current cultivation and management practices for hybrid maize cultivation. Except for the expression of CP4 EPSPS in MON 87427, this product is no different from conventional maize in its agronomic, phenotypic, and ecological characteristics and has the same levels of resistance to insects and diseases as current commercial maize. Prior to the introduction of MON 87427, glyphosate has not been used in hybrid maize seed production fields because not all inbreds were glyphosate-tolerant. With the introduction of MON 87427, early applications of glyphosate for weed control could be used in hybrid maize seed production fields for Roundup Ready Corn 2 products because both the MON 87427 containing (female) inbred and the NK603 or MON 88017 containing (male) inbreds would be glyphosate-tolerant for in-season weed control as directed on Roundup® agricultural product labels. Additionally, two other glyphosate applications ranging from V8 through V13 will be used to induce the male sterile phenotype through tissue-selective glyphosate tolerance in MON 87427 in hybrid maize seed production fields. However, in regard to commercial cultivation of MON 87427-containing hybrids, glyphosate use rates, timings and recommendations for weed management will not be different than those recommended for the previously de-regulated Roundup Ready Corn 2 products (NK603 and MON 88017). Other than the applications of glyphosate, MON 87427 will be grown using the same agricultural inputs as other maize inbreds used in hybrid maize seed production. Based on these considerations, there is no apparent potential for significant impact on agronomic practices or land use.

Conclusion

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.

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ABBREVIATION AND DEFINITIONS[1]

AA	amino acid
ae/A	acid equivalent per acre
ai	active ingredient
ALS	Acetolactate synthase
ADF	acid detergent fiber
AOSA	Association of Official Seed Analysts
APHIS	Animal and Plant Health Inspection Service
BIO	Biotechnology Industry Organization
Bt	*Bacillus thuringiensis*
BSA	bovine serum albumin
CEQ	Council on Environmental Quality
CFR	Code of Federal Regulations
CMS	Cytoplasmic male sterility
CO_2	Carbon dioxide
COA	Certificate of Analysis
CP4 EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase - protein isolated from *Agrobacterium* species strain CP4
CTP2	chloroplast transit peptide
DHB	5-dihydroxybenzoic acid
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
dw	dry weight
E. coli	*Escherichia coli* bacteria
EDTA	Ethylenediaminetetraacetic Acid
EPA	Environmental Protection Agency
EPSPS	5-enolpyruvylshikimate-3-phosphate synthase enzyme
FA	fatty acid
FDA	Federal Drug Administration
FFDCA	Federal Food, Drug and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
fw	fresh weight
g	g-force
GE	Genetically Engineered
GLP	Good Laboratory Practice
HEPES	N-[2-(hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)]
IgG	Immunoglobulin G
kDa	KiloDalton
LB	Laemmli buffer
ILSI CCD	International Life Sciences Institute Crop Composition Database
LOQ	limit of quantitation
M	Million
MBu	Million bushels
MALDI-TOF MS	Matrix Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry
MMT	Million metric tonnes

[1] Alred, G.J., C.T. Brusaw, and W.E. Oliu. 2003. Handbook of Technical Writing, 7th ed., pp. 2-7. Bedford/St. Martin's, Boston, MA.

MW	molecular Weight
MWCO	Molecular Weight Cutoff
NDF	neutral detergent fiber
NEPA	National Environmental Policy Act
NFDM	non-fat dry milk
NIST	National Institute of Standards and Technology
NTO	Non-target organism
OECD	Organization for Economic Co-operation and Development
OSTP	Office of Science and Technology Policy
PBS	Phosphate Buffered Saline
PBST	Phosphate Buffered Saline Containing 0.05% (v/v) Tween-20
PEP	Phosphoenolpyruvate
PPA	Plant Protection Act
ppm	parts per million
PRESS	predicted residual sum of squares
PTH	Phenylthiohydantoin
PVDF	Polyvinylidene Difluoride
S3P	Shikimate-3-Phosphate
SOP	standard operating procedure
SDS-PAGE	Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis
T/C/R	test/control/reference
TDF	total dietary fiber
TES	Threatened and Endangered Species
Tm	melting temperature
U.S.	United States of America
USC	United States Code
USD	United States Dollars
USDA	United States Department of Agriculture
UTP	Uridine-5'-triphosphate
v/v	Volume to Volume ratio
w/v	Weight to Volume ratio

I. RATIONALE FOR THE DEVELOPMENT OF MON 87427

I.A. Basis for the Request for a Determination of Nonregulated Status under 7 CFR Part 340.6

The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has responsibility, under the Plant Protection Act (7 U.S.C. § 7701-7772) to prevent the introduction and dissemination of plant pests into the U.S. The APHIS 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 no longer should 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.

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.

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

Maize (*Zea mays* L.) is the largest crop grown in the U.S. in terms of acreage planted and net value. Planted maize acres in the U.S. have ranged from 78.3 to 93.5 million acres from 2005 to 2009 (USDA-NASS, 2010). Maize differs from other major U.S. crops, such as soybean or cotton, in that it is typically planted as a hybrid, and maize hybrids are utilized on nearly all maize production acres currently planted in the U.S. Significant use of hybrids in U.S. maize production dates to the 1930's (Wych, 1988). 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. The seed is also not genetically uniform, and segregating for a whole range of traits, thus making management and cultivation of this seed difficult in subsequent generations. Therefore, new hybrid seed is used each year for planting.

The seed supply used to plant the U.S. maize acreage is produced via hybrid seed production and occurs on approximately 0.5 M acres annually (Jugenheimer, 1976). Modern hybrid maize seed production is based on the use of two maize inbred parents, one designated as a female parent and one as a male parent. Hybrid seed production is accomplished through the combining of genetic material from one inbred parent with that of the other inbred parent. Specifically, pollen from the tassel (male flower) of the male parent is used to fertilize the ear (female flower) of the female parent. Maize is a monoecious plant, and there exists the practical opportunity to easily facilitate combining genetic material due to the separation of the male and female flowers, compared to other plant species that contain both male and female reproductive structures in the same

flower. The physical separation of the male and female flowers on maize make it well suited for hybrid seed production.

One issue inherent to the production of hybrid maize seed is that the female parent produces pollen at the same time as the male parent. Therefore, pollen from the female parent must be removed or eliminated in order to assure genetic transfer via pollen only from the male parent to the female parent. Pollen from the female parent is removed or eliminated in one of two ways in current hybrid maize seed production. The current primary option utilized for removal of pollen from the female parent during hybrid maize seed production is detasseling. Detasseling is accomplished by physically removing the male flower (tassels) from the female parent prior to pollen shed. Although detasseling is the primary option for removing pollen from the female parent, negative aspects associated with it include the need for a large labor pool to do physically demanding work under very tight (3-4 day) time constraints with the need for repeated observation to ensure that only the appropriate pollen is available for hybrid seed production. The other option for eliminating pollen from the female parent during hybrid maize seed production is through the use of Cytoplasmic Male Sterile (CMS) maize, which is a naturally occurring, maternally inherited trait in maize known to produce male sterile plants (Laughnan and Gabay-Laughnan, 1983). However a resource intensive breeding integration process is necessary to move CMS into a particular inbred background, and incomplete male sterility has been noted with CMS that necessitates detasseling (Wych, 1988).

Monsanto Company has developed MON 87427 maize with tissue-selective glyphosate tolerance to facilitate the production of viable hybrid maize seed. This technology allows for more efficient maize hybrid seed production compared to mechanical detasseling or the use of CMS, while producing seed of the same commercially acceptable standards. MON 87427 produces the CP4 EPSPS protein via the incorporation of a *cp4 epsps* coding sequence. Tissue-selective expression of the CP4 EPSPS protein in MON 87427 enables an extension of the use of glyphosate tolerant maize to include its use as a tool in hybrid maize seed production.

MON 87427 utilizes a specific promoter and intron combination (*e35S-hsp70*) to drive CP4 EPSPS protein expression in vegetative and female reproductive tissues, conferring tolerance to glyphosate in the leaves, stalk, and root tissues and tissues that develop into seed or grain and silks. Use of this specific promoter and intron combination also results in limited or no production of CP4 EPSPS protein in two key male reproductive tissues: pollen microspores which develop into pollen grains, and tapetum cells that supply nutrients to the pollen. (Goldberg, et al., 1993; Huang, et al., 2009). Thus, in MON 87427, male reproductive tissues critical for male gametophyte development are not tolerant to glyphosate. Both the *e35S* promoter and the *CaMV 35S* promoter, which is the promoter from which *e35S* originated (Kay, et al., 1987; Odell, et al., 1985), have demonstrated limited ability in certain crops to drive expression of a gene of interest in pollen previously (CaJacob, et al., 2004; Hamilton, et al., 1992).

Only specifically timed glyphosate applications beginning just prior and/or during tassel development stages (approximate maize vegetative growth stages ranging from V8 to V13) will produce a male sterile phenotype through tissue-selective glyphosate tolerance, and will eliminate or greatly reduce the need for detasseling which is currently used in the production of hybrid corn seed. Glyphosate is a systemic herbicide that is readily translocated via the phloem in plants (Devine, et al., 1993). Once glyphosate is in the phloem, it moves to areas of high meristematic activity, following a typical source to sink distribution (Devine et al., 1993). Pollen development in a maize plant takes approximately 4 weeks to complete (Ma, et al., 2008)). Early tassel development stages start at the approximate maize vegetative growth stage V9 (Ritchie, et al., 1997), therefore glyphosate applications made at approximately this time allow maximum translocation of glyphosate to the male reproductive tissues, and selectively cause cell death in only those cells that are not tolerant to glyphosate (i.e. tapetum and pollen cells). Glyphosate applications made during early vegetative stages, consistent with the application timing specified in the current Roundup agricultural product label for weed control purposes, do not affect pollen production of MON 87427 because the sensitive male reproductive tissues are not actively developing at that time. The tissue-selective glyphosate tolerance of MON 87427 allows glyphosate-treated MON 87427 to serve as a female parent inbred in the production of hybrid seed. Pollen from the corresponding male parent inbred line will fertilize MON 87427 resulting in hybrid maize seed carrying the gene for tissue-selective glyphosate tolerance.

The benefits of MON 87427 in the production of hybrid seed include:

- *Increased Flexibility in Hybrid Seed Production*: Each year approximately 0.5 M acres used for hybrid maize seed production must be detasseled in order to meet commercial growers' hybrid maize seed needs. The critical time period for detasseling is after the tassel has emerged but prior to pollen shed and silk emergence, and encompasses an average 3 - 4 day window. Current detasseling practices may require up to two passes with mechanical detasseling equipment and up to three passes if hand detasseling is used. Further complicating detasseling activity is the logistical planning required for moving enough labor and resources to the designated hybrid seed production fields at the appropriate time. Glyphosate applications to MON 87427 that will result in the male sterile phenotype through tissue-selective glyphosate tolerance will take place at approximate maize vegetative growth stages ranging from V8 to V13. The two glyphosate applications would take place during an approximate 14 day window within these growth stages, a much longer time period compared to an average 3 – 4 day window between tassel emergence and pollen shed and silk emergence. This timing accounts for significantly improved flexibility in hybrid seed production.

- *Economic Benefits for Hybrid Seed Producers:* Seed manufacturers continually seek ways to improve hybrid seed productivity and reduce the inputs and land area used to produce high quality hybrid seed. Agricultural field labor costs continue to significantly outpace inflation in the U.S. Compounding this increasing cost is population migration towards urban areas that is shrinking the

agricultural labor pool, thus reducing a reliable labor pool for this work. Costs associated with labor recruitment and deployments to perform detasseling are one of the single largest cost improvement opportunities in hybrid seed production. MON 87427 will decrease hybrid seed production costs primarily from a reduction in direct and associated labor costs.

When MON 87427 is present in hybrid seed used by growers for the production of corn grain, it does not impact agronomic performance.

I.C. Submissions to Other Regulatory Agencies

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.

I.C.1. Submission to FDA

MON 87427 falls within the scope of the 1992 U.S. Food and Drug Administration's (FDA) policy statement concerning regulation of products derived from new plant varieties, including those developed through biotechnology (U.S. FDA, 1992). In compliance with this policy, Monsanto has initiated a consultation with the FDA on the food and feed safety and nutritional assessment of MON 87427. Monsanto will be submitting a safety and nutritional assessment summary document to FDA in the near future.

I.C.2. Submissions to EPA

The EPA has authority over the use of pesticide substances under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), as amended (7 U.S.C § 136 *et seq.*). The new use pattern includes application of glyphosate beginning just prior and/or during tassel development stages (approximate maize vegetative growth stages ranging from V8 to V13), for which a new field residue study was performed and submitted to EPA in June 2010. Residues in both maize grain and stover were far below the existing tolerances of 5 and 100 ppm, respectively. However, residues in corn forage were above the current tolerance of 6 ppm. Therefore, Monsanto's June 2010 submission included a petition to increase the tolerance for glyphosate in maize forage from 6 to 13 ppm. No other revisions to glyphosate pesticide residue tolerances are needed, including animal products such as meat or milk. In June 2010, Monsanto also submitted amended Supplemental Labeling for Registration Numbers 524-537 (Roundup WeatherMAX) and 524-549 (Roundup PowerMAX), which modifies the current use pattern for glyphosate in hybrid maize seed production systems based on MON 87427. This use of glyphosate and these Supplemental Labels were first approved by EPA in April 2008. The amended labeling refines the use directions and removes the current grazing restriction, which is currently

required due to the regulated status of MON 87427 and the potential for maize forage glyphosate residues above the current tolerance. However, this potential is very low, because this use pattern will be practiced on approximately 0.5 M acres, even after full adoption, and only a fraction of such maize seed production acres are used as forage. Similarly, this use of glyphosate does not present any new environmental exposures scenarios not previously evaluated and deemed acceptable by EPA. Additional details regarding glyphosate and its use on MON 87427 are available in Appendix K.

I.C.3. Submissions to Foreign Government Agencies

To support commercial introduction of MON 87427 in the U.S., regulatory submissions will be made to countries that import significant quantities of maize or its processed fractions from the U.S. and have established regulatory approval processes in place. These will include submissions to a number of foreign government regulatory authorities, including, but not limited to: Canada, Japan, Mexico, Korea, Taiwan, Philippines, and Colombia, as well as to regulatory authorities in other maize importing countries with functioning regulatory systems. As appropriate, notifications of importation will be made to importing countries that do not have a formal approval process.

II. THE BIOLOGY OF MAIZE

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

Additional information on the biology of maize can also be found on the Australian Government Department of Health and Ageing (Office of the Gene Technology Regulator) web site (OGTR, 2008).

In addition, more information about the reproductive biology of maize, specifically on the process of pollen development and gametogenesis in maize, is provided in The Maize Handbook (Bedinger and Russell, 1994).

II.A. Maize as a Crop

Maize is grown in nearly all areas of the world and is the largest cultivated crop in the world followed by wheat (*Triticum* sp.) and rice (*Oryza sativa L.*) in total global metric ton production. In 2008, maize was harvested on over 160 million hectares (ha) (395 million acres) globally resulting in 822.7 million metric tonnes (MMT) of grain production (FAOSTAT, 2009). The U.S., China, European Union, Brazil, and Mexico were the top five producers of maize between 2008 and 2009 (USDA-FAS, 2010). In the U.S., maize is grown in almost all states with 307.14 MMT of maize grain produced in 2009 worth a market value of USD $48.6 billion (USDA-FAS, 2010; USDA-NASS, 2010).

In industrialized countries maize has two major uses: (1) as animal feed in the form of grain, forage or silage; and (2) as a raw material for wet- or dry-milled processed products such as high fructose maize syrup, oil, starch, glucose, dextrose and ethanol; by-products of the wet- and dry- mill processes can also be used as animal feed. These processed products are used as ingredients in many industrial applications and in human food products. Most maize produced in industrialized countries is used as animal feed or for industrial purposes, but maize remains an important food staple in many developing regions, especially sub-Saharan Africa and Central America, where it is frequently the mainstay of human diets (Morris, 1998).

Maize is a very familiar plant that has been rigorously studied due to its use as a staple food/feed and the economic opportunity it brings to growers. The domestication of maize likely occurred in southern Mexico between 7,000 and 10,000 years ago (Goodman and Galinat, 1988). While the putative parents of maize have not been recovered, it is likely that teosinte played an important role in contributing to the genetic background of maize. Although grown extensively throughout the world, maize is not considered a persistent weed or a plant that is difficult to control. Maize, as we know it today, cannot survive in the wild because the female inflorescence (the ear) is covered by a husk thereby restricting seed dispersal. The transformation from a wild, weedy species to one dependent on humans for its survival most likely evolved over a long period of time through plant breeding by the indigenous inhabitants of the Western Hemisphere. Today, virtually all the maize grown in the U.S. is a hybrid, a production practice that started in the 1930's (Wych, 1988). Maize hybrids are developed and used based on the positive yield increases and plant vigor associated with heterosis, also known as hybrid vigor.

Conventional plant breeding results in desirable characteristics in a plant through the unique combination of genes already present in the plant. However, there is a limit to genetic diversity with conventional plant breeding. Biotechnology, as an additional tool to conventional breeding, offers access to greater genetic diversity than conventional breeding alone, resulting in expression of highly desirable traits that are profitable to growers.

II.B. Characteristics of the Recipient Plant

The maize germplasm that was utilized as the recipient of the transgenes in MON 87427 was LH198 x HiII. This line was used because it responds well to transformation with *Agrobacterium* and tissue regeneration.

The LH198 inbred line was released in 1992 by Holden's Foundation Seeds, Inc of Williamsburg Iowa. LH198 is an inbred related to the stiff-stalk family and was derived from the cross (LH132 × B84) × LH132. LH132 is also a Holden's Foundation Seed inbred and B84 is an inbred released by Iowa State University.

The HiII inbred germplasm was specifically developed for use in maize transformation and is publicly available from the Maize Genetics Stock Center (MaizeGDB, 2010). The HiII germplasm was derived from the cross between two Stiff Stalk inbreds B73 and A188 (Armstrong, et al., 1991).

II.C. Maize as a Test System in Product Safety Assessment

In developing the data to support this petition, appropriate test materials were generated for the molecular characterization (Sections III and IV), protein characterization and expression analysis (Section V), composition analysis (Section VI), and phenotypic, agronomic and environmental interactions assessment (Section VII). Molecular characterization was conducted with the MON 87427 test material generation LH198 BC3F4 (Figure IV-6) that was used to initiate commercial breeding efforts. Protein characterization and expression analysis, composition analysis, and phenotypic,

agronomic and environmental interactions assessment were conducted with the MON 87427 test material generation LH198 BC3F7 × LH287 (Figure IV-6).

For purposes of evaluating food, feed and environmental safety, there are no practical differences between MON 87427 containing hybrids used for grain production, and MON 87427 inbred maize lines used for seed production. In both instances hybrids and inbreds express the CP4 EPSPS protein and hybrid maize lines contain the genetic material from both parental inbreds. The hybrid generation of MON 87427 (LH198 BC3F7 × LH287) was used for protein characterization and expression analysis, composition analysis, and phenotypic, agronomic and environmental interactions assessment; because it is representative of commercial hybrid maize, and thus represents the form of MON 87427 that will be most exposed to the environment, consumers, and livestock. This reasoning is based on the millions of acres of commercial maize production and the millions of tons of commodity maize grain produced from that acreage, compared to the far smaller number of acres for hybrid seed production and the minimal amount of grain from those acres that enter commodity maize stocks. Therefore, the food, feed and environmental safety evaluation that was conducted on MON 87427 hybrids is appropriate and equally applicable to the inbreds.

Conventional control materials were developed for use in the Regulatory studies along side the MON 87427 test materials. These conventional controls were non-transformed maize lines with similar germplasm backgrounds to MON 87427, but did not contain the *cp4 epsps* expression cassette, so that the effect of the genetic insert could be assessed in an unbiased manner. The conventional control materials included the original transformation line (LH198 × HiII), used for the molecular characterization; and the hybrid conventional control (LH198 × LH287) which has a similar genetic background to the hybrid MON 87427 test material (LH198 BC3F7 × LH287). The LH198 × LH287 hybrid was the conventional control used in the phenotypic, agronomic and environmental interactions assessment (Section VII), compositional analysis (Section VI), and protein expression analysis (Section V). Where appropriate, commercial reference maize materials (hereafter referred to as commercial references) were used to establish a range of variability or responses representative of commercial maize in the U.S. The commercial references used at each location were selected based on their availability and agronomic fit. Further descriptions of MON 87427, the conventional controls, and commercial references are provided in the Methods and Materials sections of this petition (Appendices B, D, and E).

III. DESCRIPTION OF THE GENETIC MODIFICATION

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.

III.A. Plasmid Vector PV-ZMAP1043

PV-ZMAP1043 was used in the transformation of maize to produce MON 87427 is shown in Figure III-1, and the elements included in this vector are described in Table III-1. PV-ZMAP1043 is approximately 8.9 kb and contains one T-DNA that is delineated by Left and Right Border sequences. The T-DNA contains one expression cassette consisting of the *cp4 epsps* coding sequence under the regulation of the *e35S* promoter, the *hsp70* intron, the *CTP2* targeting sequence, and the *nos* 3′ non-translated region.

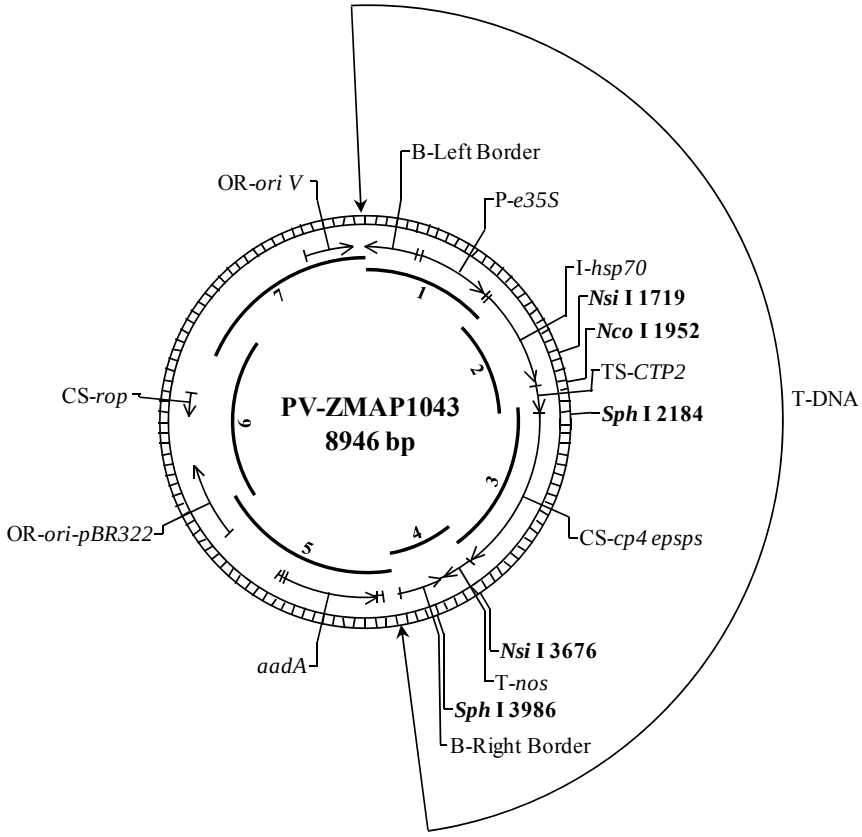
The backbone region of PV-ZMAP1043, located outside of the T-DNA, contains two origins of replication for maintenance of the plasmid vector in bacteria (*ori V*, *ori-pBR322*), a bacterial selectable marker gene (*aadA*), and a coding sequence for repressor of primer protein for maintenance of plasmid vector copy number in *E. coli* (*rop*). A description of the genetic elements and their prefixes (e.g., B-, P-, I-, TS-, CS-, T-, and OR-) in PV-ZMAP1043 is provided in Table III-1.

III.B. Description of the Transformation System

MON 87427 was developed through *Agrobacterium*-mediated transformation of immature maize embryos based on the method described by Sidorov and Duncan (2009), utilizing PV-ZMAP1043. Immature embryos were excised from a post-pollinated maize ear of LH198 × HiII. After co-culturing the excised immature embryos with *Agrobacterium* carrying the plasmid vector, the immature embryos were placed on selection medium containing glyphosate and carbenicillin disodium salt in order to inhibit the growth of untransformed plant cells and excess *Agrobacterium*. Once transformed callus developed, the callus was placed on media conducive to shoot and root development. Rooted R_0 plants with normal phenotypic characteristics were selected and transferred to soil for growth and further assessment.

The R_0 plants generated through the transformation process described above had already been exposed to glyphosate in the selection medium and demonstrated glyphosate tolerance. Starting from a single R_0 plant, LH198 was then used as the recurrent parent through four backcrossing generations. Backcross progeny generations were evaluated for tolerance to glyphosate using a rate of 0.75 lb ae/A (0.84 kg ae/ha), a representative commercial application rate and timing. Surviving plants were then selfed to produce

homozygous plants, which were identified through a quantitative polymerase chain reaction (PCR) analysis. MON 87427 was selected as the lead event based on superior phenotypic characteristics and comprehensive molecular profile. Regulatory studies on MON 87427 were initiated to further characterize the genetic insertion and the expressed protein, and to establish the food, feed, and environmental safety relative to commercial maize. The major steps involved in the development of MON 87427 are depicted in Figure III-2.



Probe	DNA Probe	Start Position (bp)	End Position (bp)	Total Length (~kb)
1	T-DNA Probe 1	1	1200	1.2
2	T-DNA Probe 2	1150	2150	1.0
3	T-DNA Probe 3	2100	3550	1.5
4	T-DNA Probe 4	3500	4192	0.7
5	Backbone Probe 5	4193	5942	1.8
6	Backbone Probe 6	5864	7368	1.5
7	Backbone Probe 7	7290	8946	1.7

Figure III-1. Circular Map of Plasmid Vector PV-ZMAP1043 Showing Probes 1-7
A circular map of the plasmid vector PV-ZMAP1043 used to develop MON 87427 is shown. Genetic elements and restriction sites used in Southern analyses (with positions relative to the size of the plasmid vector) are shown on the exterior of the map. The probes used in the Southern analyses are shown on the interior of the map. PV-ZMAP1043 contains a single T-DNA.

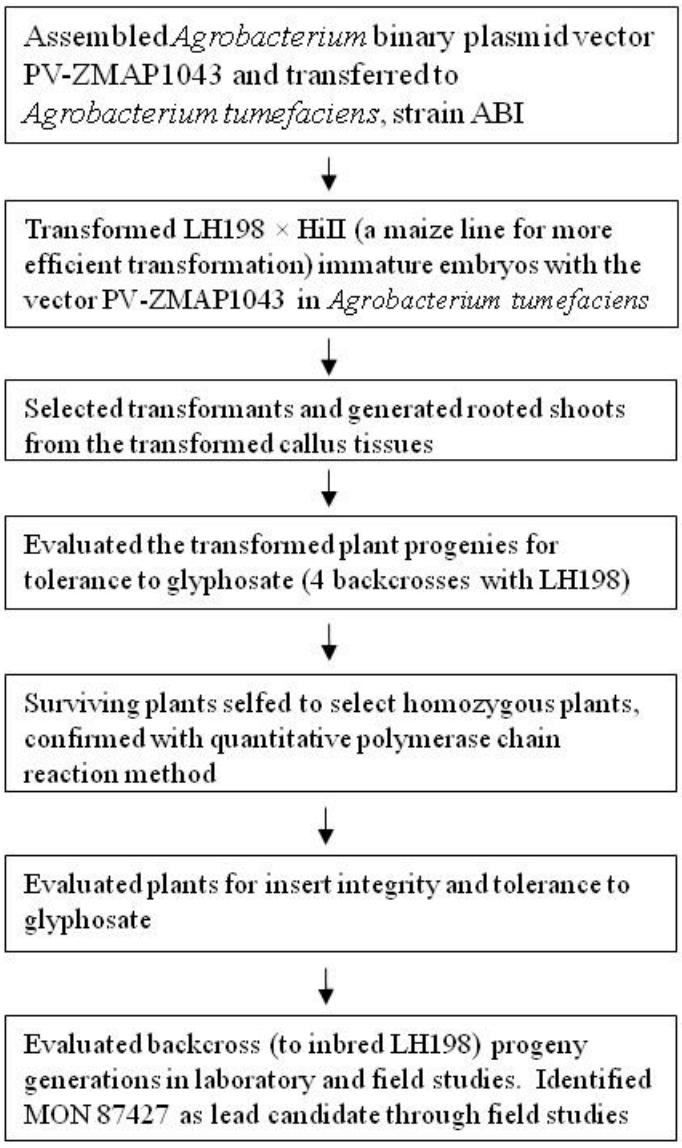


Figure III-2. Schematic of the Development of MON 87427

III.C. The *cp4 epsps* Coding Sequence and the CP4 EPSPS Protein (T-DNA)

The *cp4 epsps* expression cassette, also referred to as transfer DNA (T-DNA) in this petition, encodes a 47.6 kDa CP4 EPSPS protein consisting of a single polypeptide of 455 amino acids (Figure III-3) (Padgette, et al., 1996). The *cp4 epsps* coding sequence is the codon optimized coding sequence of the *aroA* gene from *Agrobacterium* sp. strain CP4 encoding CP4 EPSPS (Barry, et al., 2001; Padgette et al., 1996). The CP4 EPSPS protein is similar and functionally identical to endogenous plant EPSPS enzymes, but has a much reduced affinity for glyphosate, the active ingredient in Roundup agricultural herbicides, relative to endogenous plant EPSPS (Barry et al., 2001; Padgette et al., 1996).

III.D. Regulatory Sequences

The *cp4 epsps* coding sequence in MON 87427 is under the regulation of the *e35S* promoter, the *hsp70* intron, the *CTP2* targeting sequence, and the *nos* 3′ non-translated region. The *e35S* promoter, which directs transcription in plant cells, contains the duplicated enhancer region (Kay et al., 1987) from the cauliflower mosaic virus (CaMV) *35S* RNA promoter (Odell et al., 1985) The *hsp70* intron is the first intron from the maize heat shock protein 70 gene (Brown and Santino, 1997). The *CTP2* targeting sequence is the targeting sequence from the *ShkG* gene encoding the chloroplast transit peptide region of *Arabidopsis thaliana* EPSPS (Herrmann, 1995; Klee, et al., 1987) that directs transport of the CP4 EPSPS protein to the chloroplast. The *nos* 3' non-translated region is the 3' non-translated region of the *nopaline synthase (nos)* gene from *Agrobacterium tumefaciens* that terminates transcription and directs polyadenylation (Bevan, et al., 1983).

III.E. T-DNA Borders

PV-ZMAP1043 contains Right Border and Left Border regions (Figure III-1 and Table III-1) that were derived from *Agrobacterium tumefaciens* (Barker, et al., 1983; Depicker, et al., 1982; Zambryski, et al., 1982). The border regions each contain a 24-25 bp nick site that is the site of DNA exchange during transformation. The border regions separate the T-DNA from the plasmid backbone region and are involved in their efficient transfer into the maize genome.

III.F. Genetic Elements Outside of the T-DNA Borders

Genetic elements that exist outside of the T-DNA borders are those that are essential for the maintenance or selection of PV-ZMAP1043 in bacteria. The origin of replication *ori V* is required for the maintenance of the plasmid in *Agrobacterium* and is derived from the broad host plasmid RK2 (Stalker, et al., 1981). The origin of replication *ori-pBR322* is required for the maintenance of the plasmid in *E. coli* and is derived from the plasmid vector pBR322 (Sutcliffe, 1979). Coding sequence *rop* is the coding sequence of the repressor of primer (ROP) protein and is necessary for the maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989). The selectable marker *aadA* is a bacterial promoter and coding sequence for an enzyme from transposon Tn*7* that confers spectinomycin and streptomycin resistance (Fling, et al., 1985) in *E. coli* and *Agrobacterium* during molecular cloning. Because these elements are outside the border regions, they are not expected to be transferred into the maize genome. The absence of the backbone sequence in MON 87427 has been confirmed by Southern blot analyses (see Section IV.B).

Table III-1. Summary of Genetic Elements in Plasmid Vector PV-ZMAP1043

Genetic Element	Location in Plasmid Vector	Function (Reference)
T-DNA		
B[1]-Left Border	1-442	DNA region from *Agrobacterium tumefaciens* containing the Left Border sequence used for transfer of the T-DNA (Barker et al., 1983).
Intervening Sequence	443-483	Sequences used in DNA cloning
P[2]-*e35S*	484-1104	Promoter for the cauliflower mosaic virus (CaMV) 35S RNA (Odell et al., 1985) containing the duplicated enhancer region (Kay et al., 1987) that directs transcription in plant cells.
Intervening Sequence	1105-1125	Sequences used in DNA cloning
I[3]-*hsp70*	1126-1929	First intron from the maize heat shock protein 70 gene (Brown and Santino, 1997).
Intervening Sequence	1930-1953	Sequences used in DNA cloning
TS[4]-*CTP2*	1954-2181	Targeting sequence from the *ShkG* gene encoding the chloroplast transit peptide region of *Arabidopsis thaliana* EPSPS (Klee et al., 1987) that directs transport of the CP4 EPSPS protein to the chloroplast.
CS[5]-*cp4 epsps*	2182-3549	Codon-optimized coding sequence of the *aroA* gene from the *Agrobacterium* sp. strain CP4 encoding the CP4 EPSPS protein (Barry et al., 2001; Padgette et al., 1996)
Intervening Sequence	3550-3555	Sequences used in DNA cloning
T[6]-*nos*	3556-3808	3' non-translated region of the *nopaline synthase* (*nos*) gene from *Agrobacterium tumefaciens* that terminates transcription and directs polyadenylation (Bevan et al., 1983).
Intervening Sequence	3809-3835	Sequences used in DNA cloning
B-Right Border	3836-4192	DNA region from *Agrobacterium tumefaciens* containing the Right Border sequence used for transfer of the T-DNA (Depicker et al., 1982; Zambryski et al., 1982).

Table III-1 (continued). Summary of Genetic Elements in Plasmid Vector PV-ZMAP1043

Genetic Element	Location in Plasmid Vector	Function (Reference)
Vector Backbone		
Intervening Sequence	4193-4328	Sequences used in DNA cloning
aadA	4329-5217	Bacterial promoter, coding sequence, and 3′ untranslated region for an aminoglycoside-modifying enzyme, 3"(9)-*O*-nucleotidyltransferase from the transposon Tn*7* (Fling et al., 1985) that confers spectinomycin and streptomycin resistance.
Intervening Sequence	5218-5747	Sequences used in DNA cloning
OR[7]-*ori-pBR322*	5748-6336	Origin of replication from pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe, 1979).
Intervening Sequence	6337-6763	Sequences used in DNA cloning
CS-*rop*	6764-6955	Coding sequence for repressor of primer protein from the ColE1 plasmid for maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989).
Intervening Sequence	6956-8463	Sequences used in DNA cloning
OR-*ori V*	8464-8860	Origin of replication from the broad host range plasmid RK2 for maintenance of plasmid in *Agrobacterium* (Stalker et al., 1981).
Intervening Sequence	8861-8946	Sequences used in DNA cloning

[1] B, Border
[2] P, Promoter
[3] I, Intron
[4] TS, Targeting Sequence
[5] CS, Coding Sequence
[6] T, Transcription Termination Sequence
[7] OR, Origin of Replication

```
  1   MAQVSRICNG VQNPSLISNL SKSSQRKSPL SVSLKTQQHP RAYPISSSWG
 51   LKKSGMTLIG SELRPLKVMS SVSTACMLHG ASSRPATARK SSGLSGTVRI
101   PGDKSISHRS FMFGGLASGE TRITGLLEGE DVINTGKAMQ AMGARIRKEG
151   DTWIIDGVGN GGLLAPEAPL DFGNAATGCR LTMGLVGVYD FDSTFIGDAS
201   LTKRPMGRVL NPLREMGVQV KSEDGDRLPV TLRGPKTPTP ITYRVPMASA
251   QVKSAVLLAG LNTPGITTVI EPIMTRDHTE KMLQGFGANL TVETDADGVR
301   TIRLEGRGKL TGQVIDVPGD PSSTAFPLVA ALLVPGSDVT ILNVLMNPTR
351   TGLILTLQEM GADIEVINPR LAGGEDVADL RVRSSTLKGV TVPEDRAPSM
401   IDEYPILAVA AAFAEGATVM NGLEELRVKE SDRLSAVANG LKLNGVDCDE
451   GETSLVVRGR PDGKGLGNAS GAAVATHLDH RIAMSFLVMG LVSENPVTVD
501   DATMIATSFP EFMDLMAGLG AKIELSDTKA A
```

Figure III-3. Deduced Amino Acid Sequence of the CTP2 Targeting Sequence and CP4 EPSPS Protein

The transit peptide CTP2 for the *cp4 epsps* gene is underlined. Accumulation of the CP4 EPSPS protein is targeted to the chloroplasts using cleavable CTP2, the transit peptide of the *Arabidopsis thaliana* EPSPS protein. The amino acid sequence of the CP4 EPSPS protein was deduced from the full-length coding nucleotide sequence present in PV-ZMAP1043.

IV. CHARACTERIZATION OF THE GENETIC MODIFICATION

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.

Southern blot analyses were used to determine the number of copies and insertion sites of the integrated DNA and the presence or absence of plasmid vector backbone sequences. The Southern blot strategy was designed to ensure that all potential transgenic segments could be identified. The entire maize genome was assayed with probes that spanned the complete plasmid vector to detect the presence of the insertion as well as confirm the absence of any plasmid vector backbone sequences. This was accomplished by using probes that were less than 2 kb in length to ensure a high level of sensitivity. This high level of sensitivity was demonstrated for each blot by detection of a positive control added at 0.1 copy per genome equivalent. Two restriction enzymes were specifically chosen to fully characterize the T-DNA and detect any potential fragments of the T-DNA. This two enzyme design also maximizes the possibility of detecting an insertion elsewhere in the genome that could be overlooked if that band co-migrated with an expected band. One of the restriction enzymes had a cleavage site in the 5' flanking sequence, and the other had a cleavage site in the 3' flanking sequence. Together, the enzymes result in overlapping segments covering the entire insert. Therefore, at least one segment for each flank is of a predictable size and overlaps with another predictable size segment. This strategy confirms that the entire insert sequence is identified in a predictable hybridization pattern.

To determine the number of copies and insertion sites of the T-DNA, and the presence or absence of the plasmid vector backbone sequences, duplicated samples that consisted of equal amounts of digested DNA were run on the agarose gel. One set of samples was run for a longer period of time (long run) than the second set (short run). The long run allows for greater resolution of large molecular weight DNA, whereas the short run allows the detection of small molecular weight DNA. The molecular weight markers on the left of the figures were used to estimate the sizes of the bands present in the long run lanes of the Southern blots, and the molecular weight markers on the right of the figures were

used to estimate the sizes of bands present in the short run lanes of the Southern blots (Figures IV-2 to IV-5).

The DNA sequencing analyses complemented the Southern analyses. Southern blot results determined that MON 87427 contains a single copy of the T-DNA at a single insertion site. Sequencing of the insert and flanking genomic DNA confirmed the organization of the elements within the insert and determined the 5' and 3' insert-to-plant junctions, as well as the complete DNA sequence of the insert and adjacent maize genomic DNA. In addition, DNA sequencing analyses confirmed the DNA sequences flanking the 5′ and 3′ ends of the insert in MON 87427, each genetic element in the insert is intact, and the sequence of the insert matches the corresponding sequence in PV-ZMAP1043. Furthermore, the genomic organization at the insertion site was assessed by comparing the insert and flanking sequence to the insertion site in conventional maize, and identified a 41 base pair insertion adjacent to the 5' end of the MON 87427 insert, a 24 base pair insertion adjacent to the 3' end of the MON 87427 insert, and a 140 base pair deletion that occurred during integration of the T-DNA sequences.

The stability of the T-DNA present in MON 87427 across multiple generations was demonstrated by Southern blot fingerprint analyses. Genomic DNA from five generations of MON 87427 (Figure IV-6) was digested with one of the enzymes used for the insert and copy number analysis and was hybridized with two probes that detect restriction segments that encompass the entire insert. This fingerprint strategy consists of two border segments and one segment internal to the T-DNA that assess not only the stability of the insert, but also the stability of the DNA directly adjacent to the insert.

The results of these analyses of MON 87427 demonstrated that a single copy of the T-DNA was inserted at a single locus of the genome, and no additional genetic elements, including backbone sequences, from PV-ZMAP1043 were detected in MON 87427. Generational stability analysis demonstrated that an expected Southern blot fingerprint of MON 87427 was maintained through five generations of the breeding history, thereby confirming the stability of T-DNA in MON 87427. Results from segregation analyses showed heritability and stability of the insert occurred as expected across multiple generations, which corroborates the molecular insert stability analysis and establishes the genetic behavior of the T-DNA at a single chromosomal locus (Table IV-3).

The Southern blot analyses confirmed that the T-DNA reported in Figure IV-1 represents the only detectable insert in MON 87427. Figure IV-1 is a linear map depicting restriction sites within the insert as well as within the known maize genomic DNA immediately flanking the insert in MON 87427. The circular map of PV-ZMAP1043 annotated with the probes used in the Southern blot analysis is presented in Figure III-1. Based on the linear map of the insert and the plasmid map, a table summarizing the expected DNA segments for Southern analyses is presented in Table IV-1. The genetic elements integrated in MON 87427 are summarized in Table IV-2. The generations used in the generational stability analysis are depicted in the breeding history shown in Figure IV-6. Materials and methods used for the characterization of the insert in MON 87427 are found in Appendix B.

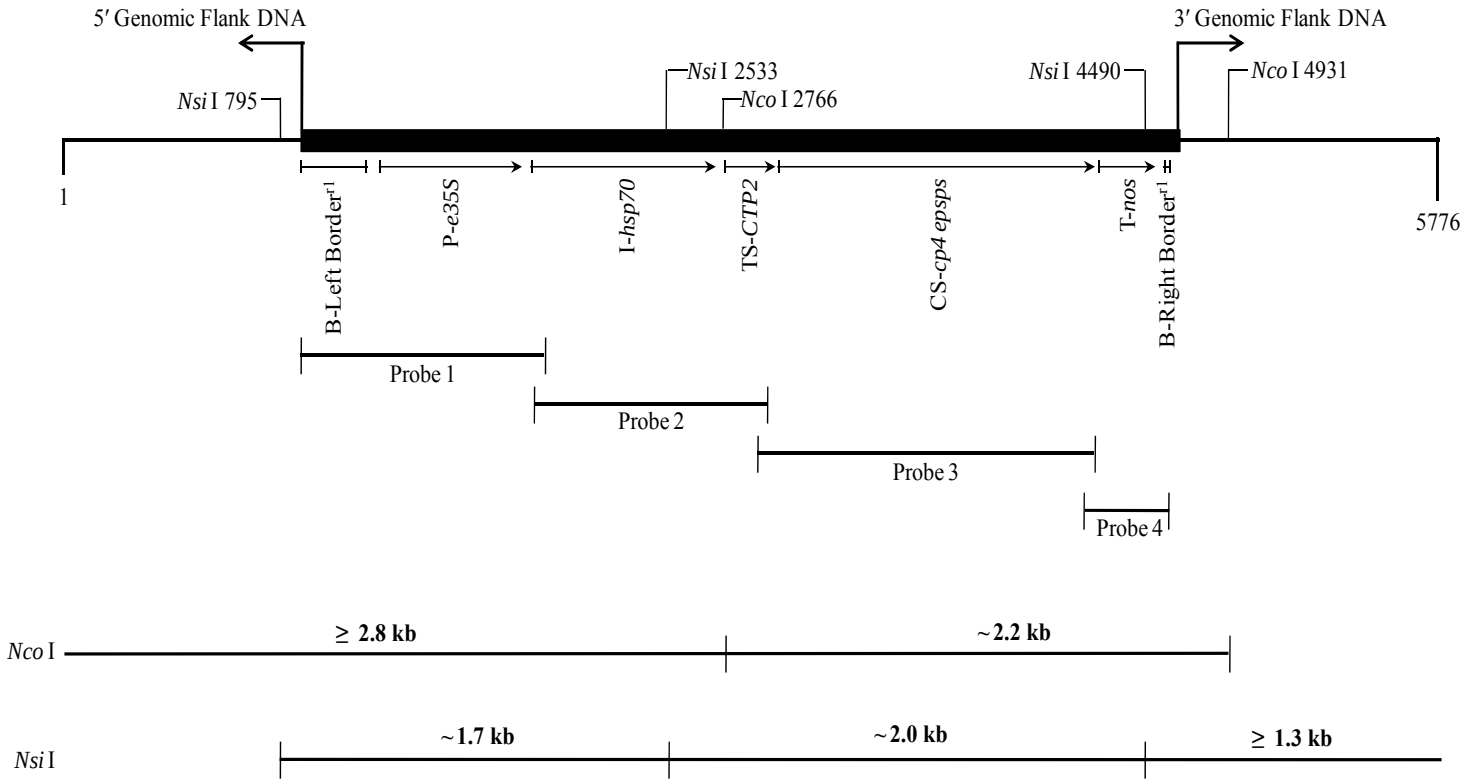


Figure IV-1. Schematic Representation of the Insert and Genomic Flanking Sequences in MON 87427

A linear map showing DNA derived from the T-DNA of PV-ZMAP1043 and integrated into MON 87427 is shown. Right-angled arrows indicate the ends of the integrated DNA and the beginning of maize genomic flanking sequence. Identified on the map are genetic elements within the insert, as well as restriction sites with positions relative to the size of the DNA sequence (genomic flank and insert) represented by the linear map for enzymes used in the Southern analyses. Also indicated are the relative sizes and locations of the T-DNA probes and the expected sizes of restriction segments identified by the probes. This schematic figure is not drawn to scale. Locations of genetic elements, restriction sites, and T-DNA probes are approximate. Probes are described in Figure III-1

Table IV-1. Summary Chart of the Expected DNA Segments Based on Hybridizing Probes and Restriction Enzymes Used in MON 87427 Analyses

Southern Blot Figure		**IV-2**	**IV-3**	**IV-4**	**IV-5**	**IV-7**
Probes Used		1, 4	2	3	5, 6, 7	1, 4
Probing Target	**Digestion Enzyme**	**Expected Band Sizes on Each Southern Blot**				
Plasmid Vector PV-ZMAP1043	*Sph* I	~7.1 kb ~1.8 kb	~7.1 kb	~7.1 kb ~1.8 kb	~7.1 kb	~7.1 kb ~1.8 kb
Probe Template Spikes[1]		~1.2 kb ~0.7 kb	~1.0 kb	~1.5 kb	~1.8 kb ~1.5 kb ~1.7 kb	~1.2 kb ~0.7 kb
MON 87427	*Nco* I	≥ 2.8 kb ~2.2 kb	≥ 2.8 kb ~2.2 kb	~2.2 kb	No band	--[2]
	Nsi I	~1.7 kb ~2.0 kb ≥ 1.3 kb	~2.0 kb ~1.7 kb	~2.0 kb	No band	~1.7 kb ~2.0 kb ≥ 1.3 kb

[1] probe template spikes were used as positive hybridization controls in Southern blot analyses
[2] '--' indicates that the particular restriction enzyme or the combination of the enzymes was not used in the analysis.

Table IV-2. Summary of Genetic Elements in MON 87427

Genetic Element	Location in Sequence	Function (Reference)
Sequence flanking 5' end of the insert	1-1003	DNA sequence adjacent to the 5' end of the insertion site
B[1]-Left Border[r1]	1004-1255	DNA region from *Agrobacterium tumefaciens* containing the Left Border sequence used for transfer of the T-DNA (Barker et al., 1983)
Intervening Sequence	1256-1296	Sequences used in DNA cloning
P[2]-*e35S*	1297-1917	Promoter for the cauliflower mosaic virus (CaMV) 35S RNA (Odell et al., 1985) containing the duplicated enhancer region (Kay et al., 1987) that directs transcription in plant cells
Intervening Sequence	1918-1938	Sequences used in DNA cloning
I[3]-*hsp70*	1939-2742	First intron from the maize heat shock protein 70 gene (Brown and Santino, 1997)
Intervening Sequence	2743-2766	Sequences used in DNA cloning
TS[4]-*CTP2*	2767-2994	Targeting sequence from the *ShkG* gene encoding the chloroplast transit peptide region of *Arabidopsis thaliana* EPSPS (Herrmann, 1995; Klee et al., 1987) that directs transport of the CP4 EPSPS protein to the chloroplast
CS[5]-*cp4 epsps*	2995-4362	Codon-optimized coding sequence of the *aroA* gene from the *Agrobacterium* sp. strain CP4 encoding the CP4 EPSPS protein (Barry et al., 2001; Padgette et al., 1996)
Intervening Sequence	4363-4368	Sequences used in DNA cloning
T[6]-*nos*	4369-4621	3' non-translated region of the *nopaline synthase* (*nos*) gene from *Agrobacterium tumefaciens* which terminates transcription and directs polyadenylation (Bevan et al., 1983)
Intervening Sequence	4622-4648	Sequences used in DNA cloning
B-Right Border[r1]	4649-4684	DNA region from *Agrobacterium tumefaciens* containing the Right Border sequence used for transfer of the T-DNA (Depicker et al., 1982; Zambryski et al., 1982)

Table IV-2 (continued). Summary of Genetic Elements in MON 87427

Genetic Element	Location in Sequence	Function (Reference)
Sequence flanking 3' end of the insert	4685-5776	DNA sequence adjacent to the 3' end of the insertion site

[1] B, Border
[2] P, Promoter
[3] TS, Targeting Sequence
[4] I, Intron
[5] CS, Coding Sequence
[6] T, Transcription Termination Sequence
[r1] Superscripts in Left and Right Borders indicate that the sequences in MON 87427 were truncated compared to the sequences in PV-ZMAP1043

IV.A. Southern Blot Analysis to Determine Insert and Copy Number of the T-DNA in MON 87427

The copy number and insertion sites of the T-DNA were assessed by digesting MON 87427 genomic DNA with the restriction enzymes *Nco* I or *Nsi* I and hybridizing Southern blots with probes that span the T-DNA (Figure III-1). Each restriction digest is expected to produce a specific banding pattern on the Southern blots (Table IV-1) and any additional integration sites would produce a different banding pattern with additional bands.

The restriction enzyme *Nco* I cuts once within the T-DNA and once within the known genomic DNA flanking the 3′ end of the T-DNA (Figure IV-1). Therefore, if T-DNA sequences were present at a single integration site in MON 87427, the digestion with *Nco* I was expected to generate two border segments with expected sizes of greater than 2.8 kb and ~2.2 kb (Figure IV-1 and Table IV-1). The greater than 2.8 kb restriction segment contains genomic DNA flanking the 5′ end of the insert, the Left Border, the *e35S* promoter, and the *hsp70* intron. The ~2.2 kb restriction segment contains the *CTP2* targeting sequence, the *cp4 epsps* coding sequence, the *nos* 3' non-translated sequence, the Right Border, and genomic DNA flanking the 3′ end of the insert.

The restriction enzyme *Nsi* I cuts twice within the T-DNA and once within the known genomic DNA flanking the 5′ end of the T-DNA (Figure IV-1). Therefore, if T-DNA sequences are present at a single integration site in MON 87427, the digestion with *Nsi* I was expected to generate two border segments with expected sizes of ~1.7 kb and greater than 1.3 kb, and one segment internal to the T-DNA insert with an expected size of ~2.0 kb (Figure IV-1 and Table IV-1). The ~1.7 kb restriction segment contains genomic DNA flanking the 5′ end of the insert, the Left Border, the *e35S* promoter, and a portion of the *hsp70* intron. The ~2.0 kb restriction segment contains a portion of the *hsp70* intron, the *CTP2* targeting sequence, the *cp4 epsps* coding sequence, and a portion of the *nos* 3' non-translated region.. The greater than 1.3 kb restriction segment contains a portion of the *nos* 3′ non-translated sequence, the Right Border, and genomic DNA flanking the 3′ end of the insert.

In the Southern blot analyses performed, each Southern blot contained a negative control and several positive controls. The conventional control LH198 × HiII was a non-transformed maize line that contained similar background genetics as MON 87427 (LH198 BC3F4) but did not contain the *cp4 epsps* expression cassette (Refer to Section II). Conventional control genomic DNA digested with either the restriction enzyme *Nco* I or *Nsi* I was used as a negative control to determine if the probes hybridized to any endogenous maize sequences. Conventional control genomic DNA digested with the appropriate restriction enzymes and spiked with either PV-ZMAP1043 DNA digested with the restriction enzyme *Sph* I, or probe template(s) served as positive hybridization controls. The positive hybridization control was spiked at 1 and 0.1 genome equivalents to demonstrate sufficient sensitivity of the Southern blot. Individual Southern blots were hybridized with the following probes: Probes 1 and 4, Probe 2, and Probe 3 (Figure III-1 and Table IV-1). The results of these analyses are shown in Figure IV-2 through Figure IV-4.

IV.A.1. Probes 1 and 4

Conventional control genomic DNA digested with *Nco* I (Figure IV-2, lane 1 and lane 8) and hybridized with Probe 1 and Probe 4 (Figure III-1) produced endogenous hybridization bands of ~6.1 kb and ~4.1 kb. Conventional control genomic DNA digested with *Nsi* I (Figure IV-2, lane 3 and lane 10) and hybridized with Probe 1 and Probe 4 (Figure III-1) produced endogenous hybridization bands of ~9.8 kb and ~4.3 kb. These signals were present in the lanes containing MON 87427 digested DNA as well, and most likely resulted from hybridization with the endogenous maize *hsp70* intron sequence, because Probe 1 contains a small portion of the *hsp70* intron (Figure III-1). Since the region of Probe 1 corresponding to the *hsp70* intron sequence was small, the hybridization signals were relatively weak, and are not specific to the inserted DNA in MON 87427.

PV-ZMAP1043 digested with the restriction enzyme *Sph* I and mixed with conventional control genomic DNA pre-digested with the restriction enzyme *Nco* I (Figure IV-2, lane 7) produced the two expected bands at ~7.1 kb and ~1.8 kb (Figure III-1 and Table IV-1) in addition to the endogenous hybridization bands listed above. Probe templates generated from PV-ZMAP1043 (Figure III-1) were mixed with conventional control genomic DNA pre-digested with the restriction enzyme *Nco* I (Figure IV-2, lane 5 and lane 6) produced the expected bands at ~1.2 kb and ~0.7 kb (Figure III-1 and Table IV-1) in addition to the endogenous hybridization bands listed above. These results indicate that the probes hybridized to their target sequences.

MON 87427 genomic DNA digested with the restriction enzyme *Nco* I and hybridized with Probe 1 and Probe 4 (Figure III-1) produced two bands in addition to the endogenous hybridization bands (Figure IV-2, lane 2 and lane 9) listed above. The ~5.5 kb band represents the 5' end of the inserted T-DNA and the adjacent flanking DNA, which correlates with the expected border segment size of greater than 2.8 kb (Figure IV-1). The ~2.2 kb band represents the 3' end of the inserted T-DNA and the adjacent DNA flanking the 3' end of the insert, which correlates with the expected border segment size of ~2.2 kb (Figure IV-1).

MON 87427 genomic DNA digested with *Nsi* I (Figure IV-2, lane 4 and lane 11) and hybridized with Probe 1 and Probe 4 produced three bands (Table IV-1) in addition to the endogenous hybridization bands listed above. The ~1.7 kb band represents the 5' end of the inserted T-DNA and a small amount of adjacent flanking DNA, which correlates with the expected border segment size of ~1.7 kb (Figure IV-1). The ~2.0 kb band contains an internal portion of the inserted DNA which correlates with the expected segment size of ~2.0 kb (Figure IV-1). The ~6.4 kb band represents the 3' end of the inserted T-DNA and the adjacent DNA flanking the 3' end of the insert, which correlates with the expected border segment size of greater than 1.3 kb (Figure IV-1).

No additional bands were detected using Probe 1 and Probe 4 other than those listed above. Based on the results presented in Figure IV-2, it was concluded that T-DNA sequences covered by Probe 1 and Probe 4 reside at a single integration locus in MON 87427.

IV.A.2. Probe 2

Conventional control genomic DNA digested with *Nco* I (Figure IV-3, lane 1 and lane 8) and hybridized with Probe 2 (Figure III-1) produced an endogenous hybridization band of ~4.1 kb. Conventional control genomic DNA digested with *Nsi* I (Figure IV-3, lane 3 and lane 10) and hybridized with Probe 2 (Figure III-1) produced endogenous hybridization bands of ~5.2 kb and ~4.2 kb. These signals were present in all lanes, and most likely resulted from hybridization with the endogenous maize *hsp70* intron sequence because Probe 2 encompasses the majority of the *hsp70* intron in PV-ZMAP1043 (Figure III-1). Since the region of Probe 2 corresponding to the *hsp70* intron sequence was large, the hybridization signals were relatively strong, but are considered to be endogenous background hybridization and are not specific to the inserted DNA in MON 87427.

PV-ZMAP1043 digested with the restriction enzyme *Sph* I and mixed with conventional control genomic DNA pre-digested with the restriction enzyme *Nco* I (Figure IV-3, lane 7) produced the expected band of ~7.1 kb (Figure III-1 and Table IV-1) in addition to the endogenous hybridization band listed above. Probe template generated from PV-ZMAP1043 (Figure III-1) was mixed with conventional control genomic DNA pre-digested with the restriction enzyme *Nco* I (Figure IV-3, lane 5 and lane 6) produced the expected band of~1.0 kb (Figure III-1 and Table IV-1) in addition to the endogenous hybridization band listed above. These results indicate that the probe hybridized to its target sequence.

MON 87427 genomic DNA digested with *Nco* I and hybridized with Probe 2 (Figure IV-3, lane 2 and lane 9) produced two bands in addition to the endogenous hybridization band listed above. The ~5.5 kb band represents the 5' end of the inserted T-DNA and the adjacent flanking DNA and correlates with the expected border segment size of greater than 2.8 kb (Figure IV-1). The ~2.2 kb band represents the 3' end of the inserted DNA and the adjacent DNA flanking the 3' end of the insert and correlates with the expected border segment size of ~2.2 kb (Figure IV-1).

MON 87427 genomic DNA digested with *Nsi* I (Figure IV-3, lane 4 and lane 11) and hybridized with Probe 2 produced two bands in addition to the endogenous hybridization bands listed above. The ~1.7 kb band represents the 5' end of the inserted T-DNA and a small amount of adjacent flanking DNA, which correlates with the expected border segment size of~1.7 kb (Figure IV-1). The ~2.0 kb band represents an internal portion of the inserted T-DNA, which correlates with the expected segment size of ~2.0 kb (Figure IV-1).

No additional bands were detected using Probe 2 other than those listed above. Based on the results presented in Figure IV-3, it was concluded that the T-DNA sequences covered by Probe 2 reside at a single integration locus in MON 87427.

IV.A.3. Probe 3

Conventional control genomic DNA digested with the restriction enzyme *Nco* I (Figure IV-4, lane 1 and lane 8) or *Nsi* I (Figure IV-4, lane 3 and lane 10) and hybridized with Probe 3 (Figure III-1) showed no detectable hybridization bands. PV-ZMAP1043 DNA digested with the restriction enzyme *Sph* I and mixed with conventional control genomic DNA pre-digested with the restriction enzyme *Nco* I (Figure IV-4, lane 7) produced one band at ~1.8 kb (Figure III-1 and Table IV-1). Although the other *Sph* I segment from PV-ZMAP1043 (~7.1 kb) contains a small portion of the Probe 3 sequence, it was not detected under these assay conditions. Probe template generated from PV-ZMAP1043 (Figure III-1) was mixed with conventional control genomic DNA pre-digested with the restriction enzyme *Nco* I (Figure IV-4, lane 5 and lane 6) produced the expected band at ~1.5 kb (Figure III-1 and Table IV-1). These results indicate that the probe hybridized to its target sequence.

MON 87427 genomic DNA digested with the restriction enzyme *Nco* I and hybridized with Probe 3 (Figure III-1) produced one band (Figure IV-4, lane 2 and lane 9) of ~2.2 kb. The ~2.2 kb band represents the 3' end of the inserted DNA and the adjacent DNA flanking the 3' end of the insert, which correlates with the expected border segment size of ~2.2 kb (Figure IV-1).

MON 87427 genomic DNA digested with the restriction enzyme *Nsi* I and hybridized with Probe 3 (Figure III-1) produced one band (Figure IV-4, lane 4 and lane 11) of ~2.0 kb. The ~2.0 kb band represents an internal portion of the inserted DNA, which correlates with the expected segment size of ~2.0 kb (Figure IV-1).

No additional bands were detected using Probe 3 other than those listed above. Based on the results presented in Figure IV-4, it was concluded that the sequence covered by Probe 3 resides at a single integration locus in MON 87427.

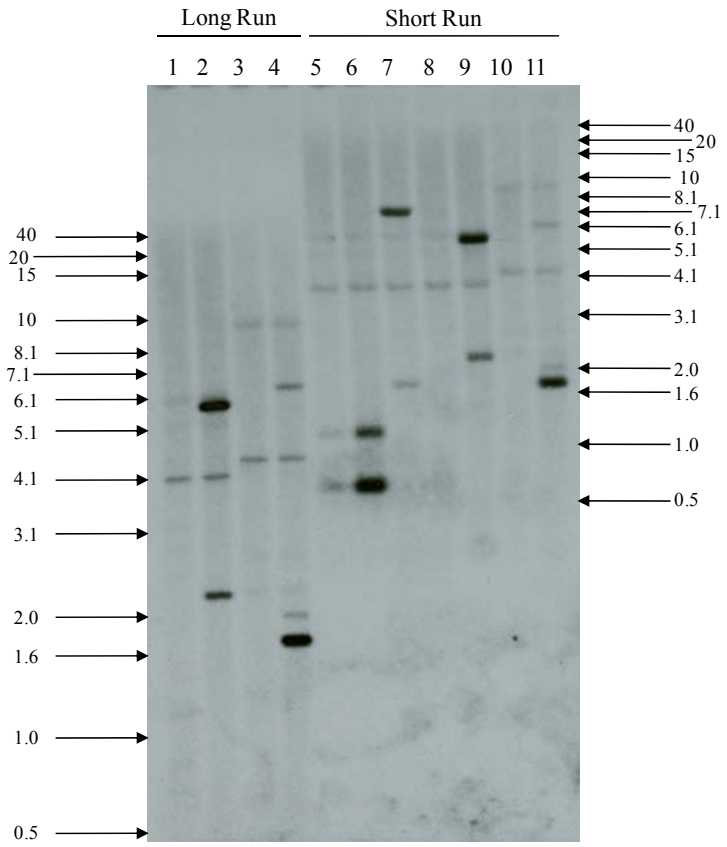


Figure IV-2. Southern Blot Analysis to Determine Insert and Copy Number of the T-DNA in MON 87427: Probe 1 and Probe 4

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

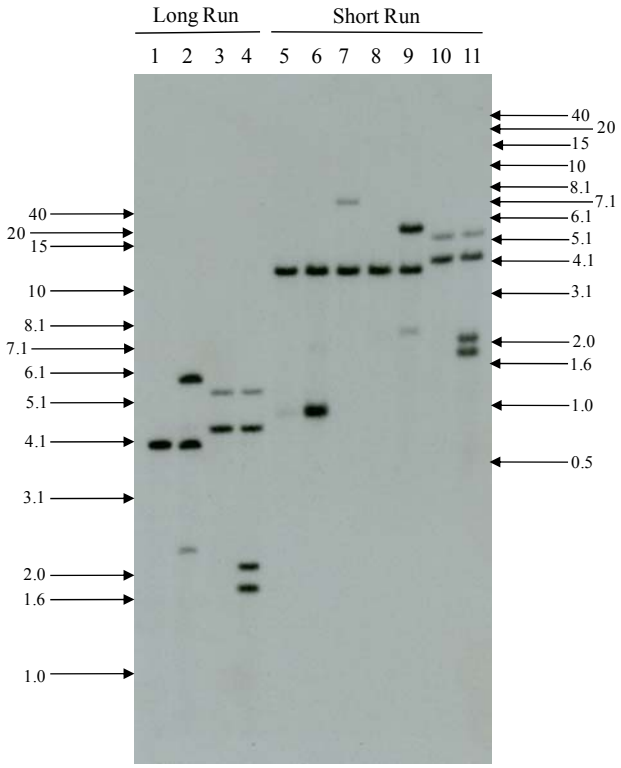


Figure IV-3. Southern Blot Analysis to Determine Insert and Copy Number of the T-DNA in MON 87427: Probe 2

The blot was hybridized with one ^{32}P-labeled probe that spanned a portion of the T-DNA sequence (Figure III-1, Probe 2). Each lane contains ~10 µg of digested genomic DNA isolated from maize seed. Lane designations are as follows:

Lane

1 Conventional control (*Nco* I)

2 MON 87427 (*Nco* I)

3 Conventional control (*Nsi* I)

4 MON 87427 (*Nsi* I)

5 Conventional control (*Nco* I) spiked with Probe 1 and Probe 4 [~0.1 genome equivalent]

6 Conventional control (*Nco* I) spiked with Probe 1 and Probe 4 [~1.0 genome equivalent]

7 Conventional control (*Nco* I) spiked with PV-ZMAP1043 (*Sph* I) [~1.0 genome equivalent]

8 Conventional control (*Nco* I)

9 MON 87427 (*Nco* I)

10 Conventional control (*Nsi* I)

11 MON 87427 (*Nsi* 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.

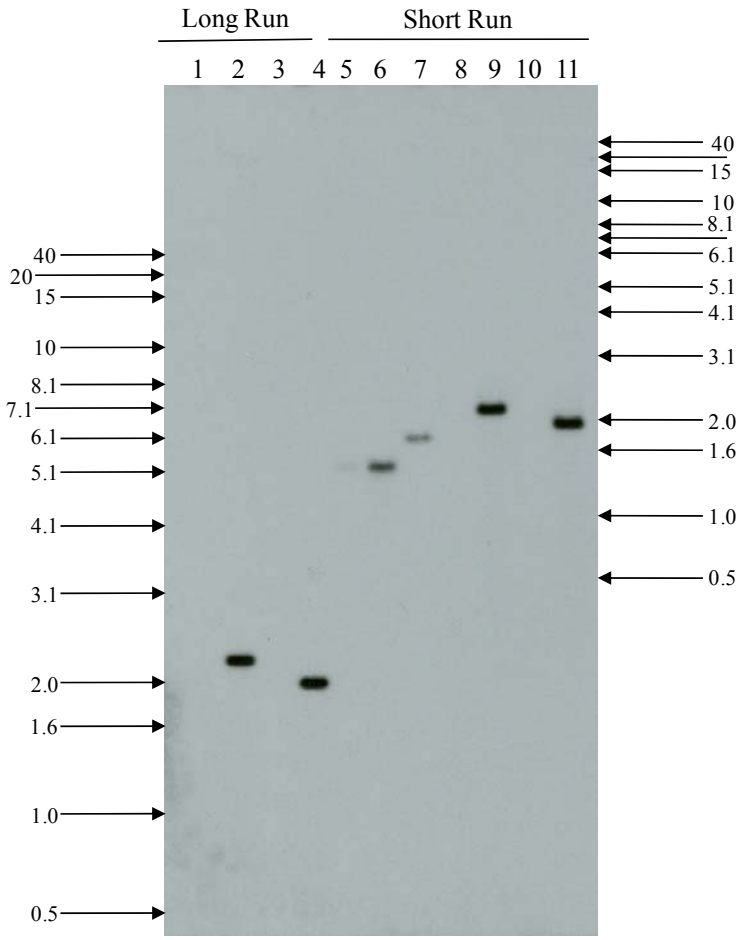


Figure IV-4 Southern Blot Analysis to Determine Insert and Copy Number of the T-DNA in MON 87427: Probe 3

The blot was hybridized with one ^{32}P-labeled probe that spanned a portion of the T-DNA sequence (Figure III-1, Probe 3). Each lane contains ~10 µg of digested genomic DNA isolated from maize seed. Lane designations are as follows:

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

IV.B. Southern Blot Analysis to Determine the Presence or Absence of Plasmid Vector PV-ZMAP1043 Backbone Sequences in MON 87427

To determine the presence or absence of PV-ZMAP1043 backbone sequences, MON 87427 and conventional control genomic DNA were digested with the restriction enzyme *Nco* I or *Nsi* I and the Southern blots were hybridized with overlapping probes spanning the entire backbone sequence of PV-ZMAP1043 (Figure III-1, Probe 5, Probe 6, and Probe 7). Digested PV-ZMAP1043 and probe templates generated from PV-ZMAP1043 were used as positive controls on the Southern blots. Approximately 1 genome equivalent of PV-ZMAP1043 digested with the restriction enzyme *Sph* I was mixed with pre-digested conventional control DNA. As an additional positive control, approximately 0.1 and 1 genome equivalent of probe templates (Figure III-1, Probe 5, Probe 6, and Probe 7) generated from PV-ZMAP1043 were mixed with pre-digested conventional control DNA. If backbone DNA sequences are present in MON 87427, then hybridizing with backbone probes should result in detectable bands. The results of this analysis are shown in Figure IV-5.

IV.B.1. Plasmid Vector Backbone Probes 5, 6, 7

Conventional control genomic DNA digested with *Nco* I (Figure IV-5, lane 1 and lane 10) or *Nsi* I (Figure IV-5, lane 3 and lane 12) and hybridized simultaneously with overlapping probes spanning the plasmid vector backbone of PV-ZMAP1043 (Figure III-1, Probe 5, Probe 6, and Probe 7) showed no detectable hybridization bands. PV ZMAP1043 digested with the restriction enzyme *Sph* I and mixed with conventional control genomic DNA pre-digested with the restriction enzyme *Nco* I (Figure IV-5, lane 9) produced one expected band of ~7.1 kb (Figure III-1 and Table IV-1). Probe templates generated from PV-ZMAP1043 (Figure III-1, Probe 5 and Probe 6) were mixed with conventional control genomic DNA pre-digested with the restriction enzyme *Nco* I (Figure IV-5, lane 5 and lane 6) produced two expected bands at ~1.8 kb and ~1.5 kb, respectively (Figure III-1 and Table IV-1). Probe template generated from PV-ZMAP1043 (Figure III-1, Probe 7) was mixed with conventional control genomic DNA pre-digested with the restriction enzyme *Nco* I (Figure IV-5, lane 7 and lane 8) produced the expected band at ~1.7 kb. These results indicate that the probes hybridized to their target sequences.

MON 87427 genomic DNA digested with *Nco* I (Figure IV-5, lane 2 and lane 11) or *Nsi* I (Figure IV-5, lane 4 and lane 13) and hybridized with Probe 5, Probe 6, and Probe 7 produced no detectable bands. Based on the results presented in Figure IV-5, it was concluded that MON 87427 contains no detectable backbone sequences from PV-ZMAP1043.

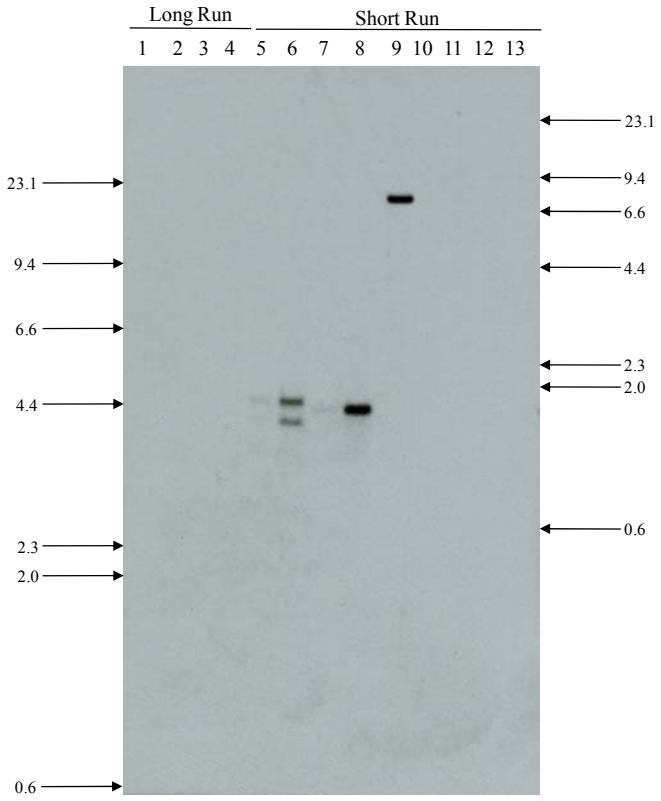


Figure IV-5 Southern Blot Analysis to Determine the Presence or Absence of Plasmid Vector PV-ZMAP1043 Backbone Sequences in MON 87427: Probes 5, 6, and 7

The blot was hybridized with three ^{32}P-labeled probes that overlapped the backbone sequence (Figure III-1, Probe 5, Probe 6, and Probe 7). Each lane contains ~10 µg of digested genomic DNA isolated from maize seed. Lane designations are as follows:

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

Arrows denote the size of the DNA, in kilobase pairs, obtained from the λDNA/*Hind* III Fragments (Invitrogen) on the ethidium bromide stained gel.

IV.C. Organization and Sequence of the Insert and Adjacent Genomic DNA in MON 87427

The organization of the elements within the T-DNA was confirmed using DNA sequence analysis. PCR primers were designed to amplify three overlapping regions of the genomic DNA that span the entire length of the insert (Figure B-1, Appendix B). The amplified PCR products were subjected to DNA sequencing analyses. The insert in MON 87427 is 3681 bp and matches the sequence of PV-ZMAP1043 as described in Table III-1.

IV.D. PCR and DNA Sequence Analyses to Examine the MON 87427 Insertion Site

PCR and sequence analysis were performed on genomic DNA extracted from MON 87427 and the conventional control to examine the insertion site in conventional maize. The PCR was performed with one primer specific to the genomic DNA sequence flanking the 5' end of the insert paired with a second primer specific to the genomic DNA sequence flanking the 3' end of the insert (Figure B-2, Appendix B). A sequence comparison between the PCR product generated from the conventional control and the sequence generated from the 5' and 3' flanking sequences of MON 87427 indicates there was a 41 base pair insertion adjacent to the 5' end of the MON 87427 insert, a 24 base pair insertion adjacent to the 3' end of the MON 87427 insert, and a 140 base pair deletion that occurred during integration of the T-DNA. Such changes are quite common during plant transformation; these changes presumably resulted from double-stranded break repair mechanisms in the plant during *Agrobacterium*-mediated transformation process (Salomon and Puchta, 1998).

IV.E. Southern Blot Analysis to Examine Insert Stability in Multiple Generations of MON 87427

In order to demonstrate the stability of the T-DNA present in MON 87427 through multiple generations, Southern blot analysis was performed using DNA obtained from five breeding generations of MON 87427. The breeding history of MON 87427 is presented in Figure IV-6, and the specific generations tested are indicated in the legend of Figure IV-7. The LH198 BC3F4 generation was used for the molecular characterization analyses shown in Figure IV-2 through Figure IV-5. To assess stability, four additional generations were evaluated by Southern blot analysis and compared to the fully characterized LH198 BC3F4 generation. The conventional control materials used for the generational stability analysis included LH198 × HiII, which included similar background genetics of the LH198 BC3F4 generation and represents the original transformation line, and LH198 × LH287, a hybrid with a similar germplasm background to the MON 87427 [LH198 BC3F7 × LH287] F1 hybrid. Genomic DNA isolated from each of the selected generations of MON 87427 and conventional controls was digested with the restriction enzyme *Nsi* I (Figure IV-1) and hybridized with Probe 1 and Probe 4 (Figure III-1). Probe 1 and Probe 4 will detect both border segments generated by the *Nsi* I digestion. Any instability associated with the T-DNA would be detected as novel bands on the Southern blot. The Southern blot has the same positive hybridization controls as described in Section IV.A. The results are shown in Figure IV-7.

IV.E.1. Probes 1 and 4

Conventional control genomic DNA digested with *Nsi* I (Figure IV-7) and hybridized with Probe 1 and Probe 4 (Figure III-1) produced hybridization signals resulting from endogenous targets residing in the maize genome. Each hybridization signal was produced in a conventional control lane, and a lane containing MON 87427 genomic DNA; therefore, these signals are considered to be endogenous background hybridization and are not specific to the inserted DNA in MON 87427. Conventional control LH198 × HiII genomic DNA (Figure IV-7, lane 4) digested with *Nsi* I and hybridized with Probe 1 and Probe 4 displayed an endogenous hybridization band of ~4.3 kb. Conventional control LH198 × LH287 genomic DNA (Figure IV-7, lane 9) digested with *Nsi* I and hybridized with Probe 1 and Probe 4 displayed the endogenous hybridization bands of ~4.4 kb and ~4.3 kb. The endogenous doublet hybridization bands in the conventional control LH198 × LH287 and MON 87427 [LH198 BC3F7 × LH287] F1 genomic DNA (Figure IV-7, lane 9 and lane 10), appeared faint on the film, although they were visible on a longer exposure.

PV-ZMAP1043 digested with the restriction enzyme *Sph* I and mixed with conventional control genomic DNA pre-digested with the restriction enzyme *Nsi* I (Figure IV-7, lane 3) produced the two expected bands at ~7.1 kb and ~1.8 kb (Figure III-1 and Table IV-1) in addition to the endogenous hybridization band. Probe templates generated from PV-ZMAP1043 (Figure III-1) were mixed with conventional control genomic DNA pre-digested with the restriction enzyme *Nsi* I (Figure IV-7, lane 1 and lane 2) produced the expected bands at ~1.2 kb and ~0.7 kb (Figure III-1 and Table IV-1) in addition to the endogenous hybridization band. These results indicate that the probes hybridized to their target sequences.

MON 87427 genomic DNA extracted from generations MON 87427 LH198 BC3F3, MON 87427 LH198 BC3F4, MON 87427 LH198 BC3F6, MON 87427 LH198 BC3F7, and MON 87427 [LH198 BC3F7 × LH287] F1, digested with *Nsi* I, and hybridized with Probe 1 and Probe 4 (Figure IV-7, lane 5, lane 6, lane 7, lane 8, and lane 10) produced three bands (Table IV-1) in addition to the endogenous hybridization bands listed above. The ~1.7 kb band represents the 5' end of the inserted T-DNA and a small amount of adjacent flanking DNA, which correlates with the expected border segment size of ~1.7 kb (Figure IV-1). The ~2.0 kb band contains an internal portion of the inserted T-DNA (Figure IV-1), which correlates with the expected segment size. The ~6.4 kb band represents the 3' end of the inserted T-DNA and the adjacent DNA flanking the 3' end of the insert, which correlates with the expected border segment size of greater than 1.3 kb (Figure IV-1). The fingerprint of the Southern signals from the four generations MON 87427 LH198 BC3F3, MON 87427 LH198 BC3F6, MON 87427 LH198 BC3F7, and MON 87427 [LH198 BC3F7 × LH287] F1 (Figure IV-7, lane 5, lane 7, lane 8, and lane 10) is consistent with that from the fully characterized generation MON 87427 LH198 BC3F4 (Figure IV-2, lane 4 and lane 11; Figure IV-7, lane 6). No unexpected bands were detected, indicating that MON 87427 contains one copy of the T-DNA that is stably maintained across multiple generations.

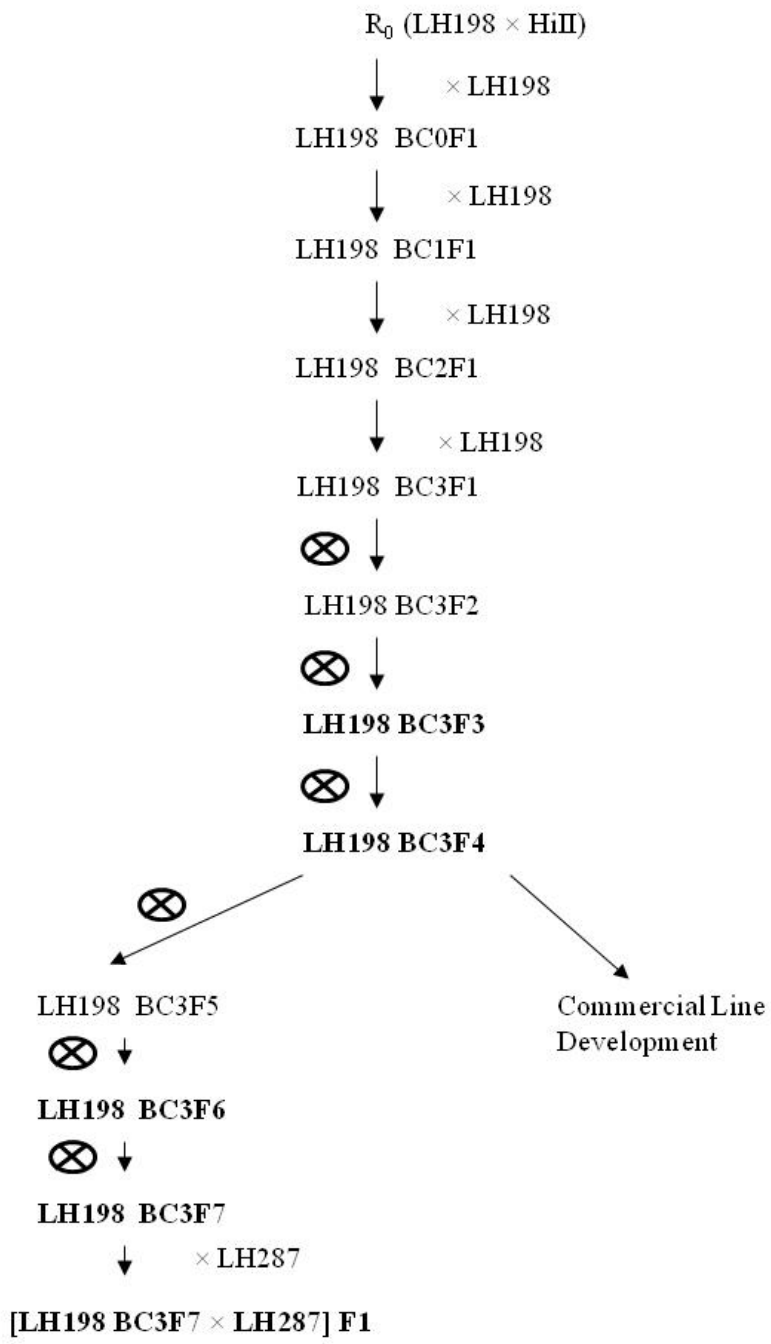


Figure IV-6. Breeding History of MON 87427
The LH198 BC3F4 generation was used for the molecular characterization of MON 87427. Generations used for generational stability are indicated in bold text. R_0 corresponds to the transformed plant. F# is the filial generation. ⊗ designates self-pollination. BC# is the backcross generation. The [LH198 BC3F7 × LH287] F1 generation was used for expression, composition and phenotypic, agronomic and environmental interaction analyses.

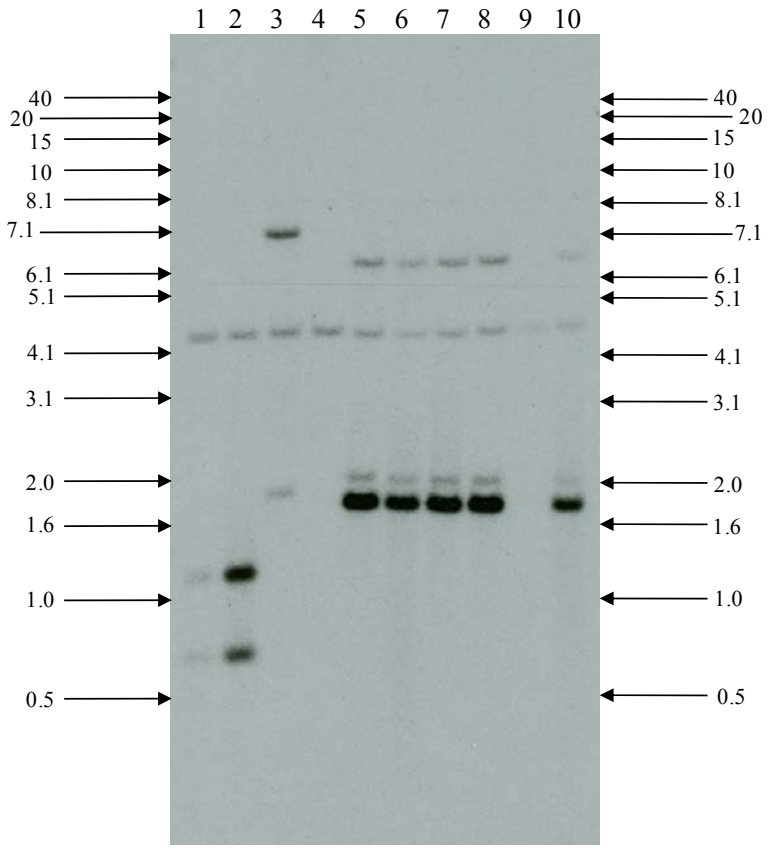


Figure IV-7. Southern Blot Analysis to Examine Insert Stability in Multiple Generations of MON 87427: Probes 1 and 4

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, with the exception of MON 87427 LH198 BC3F3, MON 87427 LH198 BC3F6, and MON 87427 LH198 BC3F7, which were isolated from maize leaf tissue. Lane designations are as follows:

Lane

1 Conventional control LH198 × HiII (*Nsi I*) spiked with Probe 1 and Probe 4 [~0.1 genome equivalent]
2 Conventional control LH198 × HiII (*Nsi* I) spiked with Probe 1 and Probe 4 [~1.0 genome equivalent]
3 Conventional control LH198 × HiII (*Nsi* I) spiked with PV-ZMAP1043 (*Sph* I) [~1.0 genome equivalent]
4 Conventional control LH198 × HiII (*Nsi* I)
5 MON 87427 (LH198 BC3F3) (*Nsi* I)
6 MON 87427 (LH198 BC3F4) (*Nsi* I)
7 MON 87427 (LH198 BC3F6) (*Nsi* I)
8 MON 87427 (LH198 BC3F7) (*Nsi* I)
9 Conventional control LH198 × LH287 (*Nsi* I)
10 MON 87427([LH198 BC3F7 × LH287] F1) (*Nsi* 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.

IV.F. Inheritance of the Genetic Insert in MON 87427

During development of MON 87427, segregation data were recorded to assess the heritability and stability of the *cp4 epsps* expression cassette present in MON 87427. Chi square analysis was performed over several generations to confirm the segregation and stability of the MON 87427 insert. The Chi square analysis is based on testing the observed segregation ratio to the expected segregation ratio according to Mendelian principles.

The MON 87427 breeding path for generating segregation data is described in Figure IV-8. The transformed R_0 plant was crossed several times with LH198 conventional maize through the LH198 BC3F1 generation. The LH198 BC0F1 generation consisted of five plants that were positive for the tissue-selective glyphosate tolerance trait. LH198 was then used as the recurrent parent through three backcrossing generations. Heterozygous LH198 BC3F1 plants were self-pollinated to produce LH198 BC3F2, which demonstrated the expected 3:1 (positive:negative) segregation ratio for the tissue-selective glyphosate tolerance trait. One surviving LH198 BC3F2 plant was identified and self-pollinated to produce LH198 BC3F3 plants, from which homozygous plants were identified and self-pollinated to produce LH198 BC3F4 plants. Endpoint Taqman analysis was used to confirm homozygosity of both LH198 BC3F3 and LH198 BC3F4 generations.

LH198 BC3F4 seed was used in trait integration and further commercial line development and was crossed with a recurrent parent (RP) that did not contain the *cp4 epsps* expression cassette to produce [RP × LH198 BC3F4] BC0F1 heterozygous seed. The resulting [RP × LH198 BC3F4] BC0F1 plants were crossed with the same recurrent parent to produce BC1F1 seed. The subsequent BC1F1 plants were tested for the presence of the CP4 EPSPS protein by glyphosate spray treatment. Surviving BC1F1 plants were again crossed with the same recurrent parent to produce BC2F1 seed. The subsequent BC2F1 plants were tested for the presence of the CP4 EPSPS protein by glyphosate spray treatment, and then self-pollinated to produce BC2F2 seed. The BC2F2 plants were also tested for the presence of the CP4 EPSPS protein by glyphosate application, and demonstrated the expected 3:1 segregation ratio for the MON 87427 trait. The heritability of the tissue-selective glyphosate tolerance trait and *cp4 epsps* expression cassette in MON 87427 was demonstrated in the BC1F1, BC2F1, and BC2F2 generations.

A Chi-square (χ^2) analysis was used to compare the observed segregation ratios to the expected ratios according to Mendelian inheritance principles. The Chi-square was calculated as:

$$\chi 2 = \sum [(| o - e |)2 / e]$$

where o = observed frequency of the genotype or phenotype and e = expected frequency of the genotype or phenotype. The level of statistical significance was predetermined to be 5% ($p \leq 0.05$). The results of the χ^2 analysis of the segregating progeny of MON 87427 are presented in Table IV-3. The χ^2 values in the BC1F1, BC2F1 and BC2F2 generations

indicated no statistically significant difference between the observed and expected 1:1, 1:1, and 3:1 (positive:negative) segregation ratios, respectively, for the tissue-selective glyphosate tolerance trait in MON 87427. The observed segregation ratios in the BC1F1, BC2F1, and BC2F2 generations confirm that the tissue-selective glyphosate tolerance trait in MON 87427 was fixed in the earlier LH198 BC3F4 generation that was used to initiate commercial inbred line development. These results support the conclusion that the *cp4 epsps* expression cassette responsible for the tissue-selective glyphosate tolerance trait in MON 87427 resides at a single locus within the maize genome and is inherited according to Mendelian inheritance principles. These results are also consistent with the molecular characterization data that indicate MON 87427 contains a single intact copy of the *cp4 epsps* expression cassette that was inserted into the maize genome at a single locus.

Table IV-3. Segregation of the Tissue-selective Glyphosate Tolerance Trait During the Development of MON 87427

Generation	Number of plants	Observed Positives	Observed Negatives	Expected Positives	Expected Negatives	χ^2	Probability
BC1F1	238	109	129	119	119	1.6807	>0.05
BC2F1[1]	290	145	145	145	145	0	>0.05
BC2F2[2]	1107	820	287	830	277	0.5062	>0.05

The plants were evaluated for the presence or absence of the glyphosate tolerance phenotype.
[1, 2] The BC1F1 and BC2F2 generations listed here are those from the trait integration breeding pathway as shown in Figure IV-8.

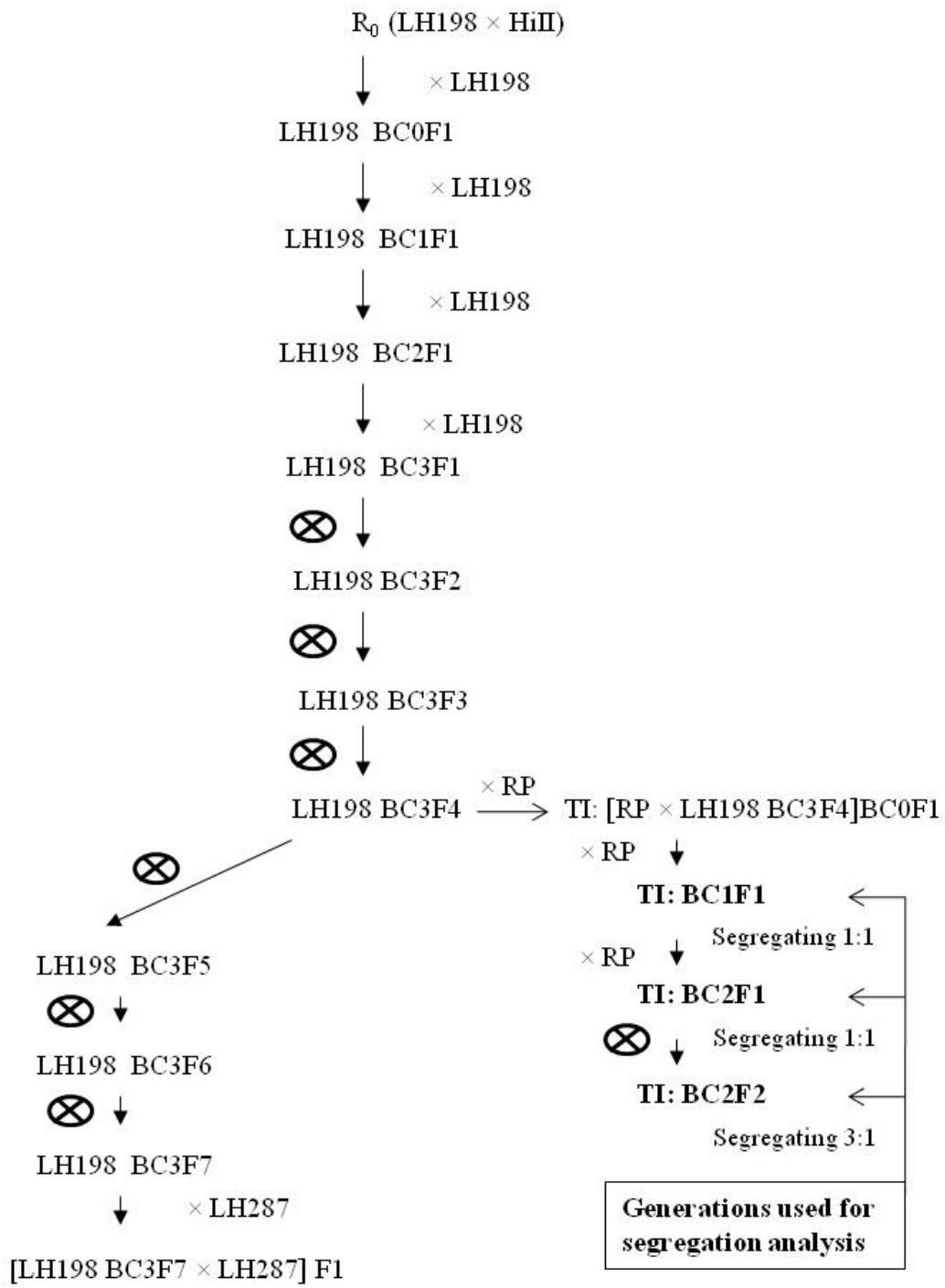


Figure IV-8. Breeding Path for Generating Segregation Data MON 87427
Chi square analysis conducted on segregation data from the BC1F1, BC2F1, and the BC2F2 generations (shown above in bold). R_0 corresponds to the transformed plant. F# is the filial generation. ⊗ designates self-pollination. BC# is the backcross generation, and TI corresponds to trait integration for commercial seed development.

IV.G. Characterization of the Genetic Modification Summary and Conclusion

Molecular characterization of MON 87427 by Southern blot analyses demonstrated that a single copy of the T-DNA sequence from PV-ZMAP1043 was integrated into the maize genome at a single locus. There were no additional genetic elements, including backbone sequences, from PV-ZMAP1043 detected, linked or unlinked to the intact T-DNA present in MON 87427.

The PCR and DNA sequence analyses performed on MON 87427 confirmed the organization of the elements within the insert, assessed potential rearrangements at the insertion site, and resulted in the complete DNA sequence of the T-DNA and adjacent maize genomic DNA sequence in MON 87427. Analysis of the T-DNA insertion site indicates that there was a 140 bp deletion of genomic DNA at the insertion site in MON 87427. Additionally, a 41 bp insertion was identified in the 5′ flanking sequence of MON 87427, and a 24 bp insertion was identified in the 3′ flanking sequence of MON 87427.

Generational stability analysis by Southern blot demonstrated that MON 87427 has been maintained through five breeding generations, thereby confirming the stability of the T-DNA in MON 87427. Results from segregation analyses show heritability and stability of the insert occurred as expected across multiple generations, which corroborates the molecular insert stability analysis and establishes the genetic behavior of the T-DNA in MON 87427 at a single chromosomal locus.

V. CHARACTERIZATION OF THE CP4 EPSPS PROTEIN PRODUCED IN MON 87427

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.

V.A. Identity and Function of the CP4 EPSPS Protein from MON 87427

The enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), catalyzes one of the enzymatic steps of the shikimic acid pathway, and is the target for the broad spectrum herbicide glyphosate (Haslam, 1993; Herrmann and Weaver, 1999; Kishore, et al., 1988; Steinrucken and Amrhein, 1980). The EPSPS family of enzymes is ubiquitous to plants and microorganisms. EPSPS proteins have been isolated from both plant and microbial sources, and their properties have been extensively studied (Harrison, et al., 1996; Haslam, 1993; Klee et al., 1987; Schonbrunn, et al., 2001; Steinrucken and Amrhein, 1984). The shikimate pathway and the EPSPS protein are absent in mammals, fish, birds, reptiles, and insects (Alibhai and Stallings, 2001). The bacterial and plant enzymes are mono-functional with a molecular weight of 44-51 kDa (Franz, et al., 1997b; Kishore et al., 1988). EPSPS proteins catalyze the transfer of the enolpyruvyl group from phosphoenolpyruvate (PEP) to the 5-hydroxyl of shikimate-3-phosphate (S3P), thereby yielding inorganic phosphate and 5-enolpyruvylshikimate-3-phosphate (EPSP) (Alibhai and Stallings, 2001). Shikimic acid is a substrate for the biosynthesis of the aromatic amino acids (phenylalanine, tryptophan and tyrosine) and other aromatic molecules. It has been estimated that aromatic molecules, all of which are derived from shikimic acid, represent 35% or more of the dry weight of a plant (Franz et al., 1997b).

The EPSPS transgene in MON 87427 is derived from *Agrobacterium* sp. strain CP4 (*cp4 epsps*). The *cp4 epsps* coding sequence encodes a 47.6 kDa EPSPS protein consisting of a single polypeptide of 455 amino acids (Padgette et al., 1996). The CP4 EPSPS protein is similar and functionally identical to endogenous plant EPSPS enzymes, but has a much reduced affinity for glyphosate, the active ingredient in Roundup agricultural herbicides, relative to endogenous plant EPSPS (Padgette et al., 1996). In conventional plants, glyphosate blocks the biosynthesis of EPSP, thereby depriving plants of essential amino acids (Haslam, 1993; Steinrucken and Amrhein, 1980). In Roundup Ready plants, which are tolerant to Roundup agricultural herbicides, requirements for aromatic amino acids and other metabolites are met by the continued action of the CP4 EPSPS enzyme in the

presence of glyphosate (Padgette et al., 1996). The CP4 EPSPS protein expressed in MON 87427 is identical to the CP4 EPSPS proteins in other Roundup Ready crops including Roundup Ready soybeans and Roundup Ready 2 Yield soybeans (Figure V-1), as well as the CP4 EPSPS in Roundup Ready corn 2, Roundup Ready canola, Roundup Ready sugar beet, and Roundup Ready cotton (alignments not shown).

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MON 87427  -LHGASSRPATARKSSGLSGTVRIPGDKSISHRSFMFGGLASGETRITGL    50
40-3-2     -LHGASSRPATARKSSGLSGTVRIPGDKSISHRSFMFGGLASGETRITGL    50
MON 89788  -LHGASSRPATARKSSGLSGTVRIPGDKSISHRSFMFGGLASGETRITGL    50

MON 87427  LEGEDVINTGKAMQAMGARIRKEGDTWIIDGVGNGGLLAPEAPLDFGNAA   100
40-3-2     LEGEDVINTGKAMQAMGARIRKEGDTWIIDGVGNGGLLAPEAPLDFGNAA   100
MON 89788  LEGEDVINTGKAMQAMGARIRKEGDTWIIDGVGNGGLLAPEAPLDFGNAA   100

MON 87427  TGCRLTMGLVGVYDFDSTFIGDASLTKRPMGRVLNPLREMGVQVKSEDGD   150
40-3-2     TGCRLTMGLVGVYDFDSTFIGDASLTKRPMGRVLNPLREMGVQVKSEDGD   150
MON 89788  TGCRLTMGLVGVYDFDSTFIGDASLTKRPMGRVLNPLREMGVQVKSEDGD   150

MON 87427  RLPVTLRGPKTPTPITYRVPMASAQVKSAVLLAGLNTPGITTVIEPIMTR   200
40-3-2     RLPVTLRGPKTPTPITYRVPMASAQVKSAVLLAGLNTPGITTVIEPIMTR   200
MON 89788  RLPVTLRGPKTPTPITYRVPMASAQVKSAVLLAGLNTPGITTVIEPIMTR   200

MON 87427  DHTEKMLQGFGANLTVETDADGVRTIRLEGRGKLTGQVIDVPGDPSSTAF   250
40-3-2     DHTEKMLQGFGANLTVETDADGVRTIRLEGRGKLTGQVIDVPGDPSSTAF   250
MON 89788  DHTEKMLQGFGANLTVETDADGVRTIRLEGRGKLTGQVIDVPGDPSSTAF   250

MON 87427  PLVAALLVPGSDVTILNVLMNPTRTGLILTLQEMGADIEVINPRLAGGED   300
40-3-2     PLVAALLVPGSDVTILNVLMNPTRTGLILTLQEMGADIEVINPRLAGGED   300
MON 89788  PLVAALLVPGSDVTILNVLMNPTRTGLILTLQEMGADIEVINPRLAGGED   300

MON 87427  VADLRVRSSTLKGVTVPEDRAPSMIDEYPILAVAAAFAEGATVMNGLEEL   350
40-3-2     VADLRVRSSTLKGVTVPEDRAPSMIDEYPILAVAAAFAEGATVMNGLEEL   350
MON 89788  VADLRVRSSTLKGVTVPEDRAPSMIDEYPILAVAAAFAEGATVMNGLEEL   350

MON 87427  RVKESDRLSAVANGLKLNGVDCDEGETSLVVRGRPDGKGLGNASGAAVAT   400
40-3-2     RVKESDRLSAVANGLKLNGVDCDEGETSLVVRGRPDGKGLGNASGAAVAT   400
MON 89788  RVKESDRLSAVANGLKLNGVDCDEGETSLVVRGRPDGKGLGNASGAAVAT   400

MON 87427  HLDHRIAMSFLVMGLVSENPVTVDDATMIATSFPEFMDLMAGLGAKIELS   450
40-3-2     HLDHRIAMSFLVMGLVSENPVTVDDATMIATSFPEFMDLMAGLGAKIELS   450
MON 89788  HLDHRIAMSFLVMGLVSENPVTVDDATMIATSFPEFMDLMAGLGAKIELS   450

MON 87427  DTKAA                                               455
40-3-2     DTKAA                                               455
MON 89788  DTKAA                                               455
```

Figure V-1. Amino Acid Sequence Alignment of CP4 EPSPS Protein from MON 87427 and comparison to deregulated Event 40-3-2 and MON 89788
The amino acid sequence alignment for CP4 EPSPS protein expressed in MON 87427, Roundup Ready soybean event 40-3-2 and Roundup Ready 2 Yield soybean (MON 89788) are shown. The proteins share 100% amino acid sequence identity. The N-terminal sequence observed in all cases showed that the methionine at position 1 has been removed.

V.B. Characterization and Equivalence of the Full Length CP4 EPSPS Protein from MON 87427

The safety assessment of crops derived through biotechnology includes characterization of the introduced protein produced from the inserted DNA, confirmation of its functional and physicochemical properties, and confirmation of the safety of the protein. The level of CP4 EPSPS protein produced in MON 87427 grain, the intended article of commerce, is too low to allow purification of sufficient quantities for use in subsequent safety assessment studies. Therefore, it was necessary to produce the protein in a high-expressing recombinant host system in order to obtain sufficient quantities of the CP4 EPSPS protein. CP4 EPSPS protein was produced in *E. coli*, and subsequently purified and characterized. A small quantity of the CP4 EPSPS protein was also purified from harvested MON 87427 grain. The equivalence of the physicochemical characteristics and functional activity between the MON 87427-produced and *E. coli*-produced CP4 EPSPS proteins was confirmed by a panel of analytical techniques, including: 1) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to establish equivalence of the apparent molecular weight between MON 87427-produced protein and the *E. coli*-produced reference standard protein, 2) western blot analysis to establish immunoreactive equivalence between MON 87427-produced protein and the *E. coli*-produced reference protein using an anti-CP4 EPSPS polyclonal antibody, 3) N-terminal sequence analysis, and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to generate a tryptic peptide map of MON 87427-produced CP4 EPSPS protein to establish the identity of the purified proteins, 4) CP4 EPSPS enzymatic activity analysis to demonstrate functional equivalence between MON 87427-produced and the *E. coli*-produced reference protein, and (5) glycosylation analysis to establish equivalent glycosylation status between MON 87427-produced and *E. coli*-produced reference protein. The details of the materials, methods, and results are described in Appendix C while the conclusions are summarized as follows.

A comparison of the MON 87427-produced CP4 EPSPS protein to the *E. coli*-produced CP4 EPSPS reference protein confirmed the identity of the MON 87427-produced CP4 EPSPS protein and established the equivalence of the plant produced protein to the *E. coli*-produced CP4 EPSPS reference protein. The molecular weights of the MON 87427-produced and *E. coli*-produced CP4 EPSPS proteins were estimated by SDS-PAGE. The results of SDS-PAGE demonstrated that the proteins migrated identically, indicating that the CP4 EPSPS proteins from both sources are equivalent in their molecular weight. The electrophoretic mobility and immunoreactive properties of the MON 87427-produced CP4 EPSPS protein were shown to be equivalent to those of the *E. coli*-produced CP4 EPSPS reference protein by immunoblot. The N-terminus of the MON 87427-produced CP4 EPSPS protein was consistent with the predicted amino acid sequence translated from the *cp4 epsps* coding sequence, and the MALDI-TOF mass spectrometry analysis yielded peptide masses consistent with the expected peptide masses from the translated CP4 EPSPS coding sequence. The MON 87427-produced CP4 EPSPS protein and *E. coli*-produced CP4 EPSPS reference protein were also found to be equivalent based on the functional activities and the lack of glycosylation. Taken together, these data provide a detailed characterization of the CP4 EPSPS protein isolated from MON 87427 and establish its equivalence to the *E. coli*-produced CP4 EPSPS

reference protein. Furthermore, since CP4 EPSPS proteins isolated from other Roundup Ready crops have been previously demonstrated to be equivalent to the *E. coli*-produced CP4 EPSPS reference protein, by inference, the MON 87427-produced CP4 EPSPS protein is equivalent to the CP4 EPSPS proteins expressed in other Roundup Ready crops, all of which have been deregulated by USDA-APHIS.

V.C. Expression Levels of CP4 EPSPS Protein in MON 87427

CP4 EPSPS protein levels in various tissues of MON 87427 relevant to the risk assessment were determined by a validated enzyme-linked immunosorbent assay (ELISA). Tissues of MON 87427 were collected from three replicates during the 2008 growing season from the following five field sites in the U.S.: Jackson County, Arkansas; Jefferson County, Iowa; Stark County, Illinois; Parke County, Indiana; and York County, Nebraska. These field sites were representative of maize producing regions suitable for commercial production. Over-season leaf (OSL1-4), grain, pollen, silk, forage, stover, over-season root (OSR1-4), forage-root, senescent root and over-season whole plant (OSWP1-4) tissue samples were collected from each replicated plot at all field sites.

CP4 EPSPS protein levels were determined in all nineteen tissue types. The results obtained from ELISA analysis are summarized in Table V-1 and the details of the materials and methods are described in Appendix D. CP4 EPSPS protein levels in MON 87427 were determined in all tissue types across all five sites with a range from below the limit of detection (LOD) to 940 µg/g dwt. The mean CP4 EPSPS protein levels across the five sites were highest in OSL (ranging from OSL3 290 µg/g dwt to OSL1 680 µg/g dwt), followed by OSWP (ranging from OSWP4 240 µg/g dwt to OSWP1 500 µg/g dwt), OSR (ranging from OSR3 73 µg/g dwt to OSR1 140 µg/g dwt), forage (120 µg/g dwt), silk (100 µg/g dwt), forage root (72 µg/g dwt), senescent root (72 µg/g dwt), stover (43 µg/g dwt), and grain (4.2 µg/g dwt). CP4 EPSPS protein levels in MON 87427 pollen across the sites were either <LOD, had a very low level just above LOQ (mean of 0.87 µg/g dwt) of CP4 EPSPS protein, or were not able to be determined (Table V-1). These varying results were possibly due to the presence of anthers in the pollen tissue samples.

The CP4 EPSPS protein expression data from MON 87427 is consistent with the MON 87427 product concept. As discussed in Section I, MON 87427 utilizes a specific promoter and intron combination (*e35S-hsp70*) to drive CP4 EPSPS protein expression in vegetative and female reproductive tissues, conferring tolerance to glyphosate in these tissues. The specific promoter and intron combination used in MON 87427 also drives little or no CP4 EPSPS protein production in the tapetum and microspore cells of pollen, thus these cells in MON 87427 are not tolerant to glyphosate. CP4 EPSPS protein was quantified in the vegetative (leaf, whole plant, forage, stover, and root) and female reproductive tissues (Table V-1). The low concentration of CP4 EPSPS protein found in pollen samples might be attributed to the presence of anther tissue collected with the pollen from MON 87427. Alternatively, a low amount of CP4 EPSPS protein in MON 87427 pollen may be inherent to this product due to the use of the e35S promoter (CaJacob et al., 2004).

Table V-1. Summary of CP4 EPSPS Protein Levels in Maize Tissues from MON 87427 Grown in 2008 U.S. Field Trials

Tissue Type[1]	Development Stage[2]	Days after planting (DAP)	Mean (SD) Range (µg/g fwt)[3]	Mean (SD) Range (µg/g dw)[4]	LOD/LOQ[5] (µg/g fwt)
OSL1	V2-V5	23-28	100 (21) 75 – 140	680 (170) 400 – 940	0.069/0.137
OSL2	V6-V8	32-46	83 (25) 30 –110	410 (130) 130 – 560	0.069/0.137
OSL3	V10-V12	41-67	61 (19) 35 –95	290 (74) 210 – 410	0.069/0.137
OSL4	VT	54-73	95 (30) 17 – 140	370 (120) 70 – 520	0.069/0.137
Grain	R6	118-182	3.6 (0.73) 2.6 – 5.3	4.2 (0.89) 2.8 – 6.2	0.16/0.228
Pollen[6]	At Pollination	58-81	< LOD (NA) 0.49 (0.36) 0.18 – 1.1	< LOD (NA) 0.87 (0.70) 0.25 – 2.2	0.099/0.137
Silk	During Pollination	58-76	9.4 (0.97) 8.1 – 11	100 (12) 90 – 120	0.121/0.137
Forage	R5	83-116	38 (14) 8.3 – 57	120 (48) 21 – 200	0.069/0.137
Stover	R6	124-180	14 (6.3) 5.9 – 26	43 (27) 13 – 98	0.069/0.137
OSR1	V2-V5	22-28	18 (5.3) 8.1 – 27	140 (46) 58 – 210	0.033/0.068
OSR2	V6-V8	32-46	16 (6.8) 8.3 –29	110 (62) 48 – 240	0.033/0.068
OSR3	V10-V12	41-67	12 (4.3) 4.9 –19	73 (28) 22 – 110	0.033/0.068

Table V-1. (continued) Summary of CP4 EPSPS Protein Levels in Maize Tissues from MON 87427 Grown in 2008 U.S. Field Trials

Tissue Type[1]	Development Stage[2]	Days after planting (DAP)	Mean (SD) Range (µg/g fwt)[3]	Mean (SD) Range (µg/g dwt)[4]	LOD/LOQ[5] (µg/g fwt)
OSR4	VT	54-73	15 (5.7) 5.6 – 23	83 (36) 23 – 140	0.033/0.068
Forage-Root	R5	83-116	15 (5.2) 8.6 – 24	72 (23) 39 – 100	0.033/0.068
Senescent Root	R6	124-180	16 (8.3) 5.9 – 29	72 (37) 26 – 130	0.033/0.068
OSWP1	V2-V5	22-28	50 (8.3) 37 – 66	500 (190) 310 – 840	0.069/0.137
OSWP2	V6-V8	32-46	46 (7.6) 33 – 58	360 (42) 300 – 420	0.069/0.137
OSWP3	V10-V12	41-67	43 (7.1) 28 – 56	380 (78) 230 – 500	0.069/0.137
OSWP4	VT	54-73	37 (6.3) 23 – 47	240 (42) 160 – 340	0.069/0.137

[1]OSL= over-season leaf; OSR= over-season root; OSWP= over-season whole plant.
[2]The maize development stage each tissue was collected (Ritchie et al., 1997).
[3]Protein levels are expressed as the arithmetic mean and standard deviation (SD) as microgram (µg) of protein per gram (g) of tissue on a fresh weight basis (fwt). The means, SD, and ranges (minimum and maximum values) were calculated for each tissue across all sites (n=14 for all tissues, except forage root where n=11). NA: Not Applicable.
[4]Protein levels are expressed as the arithmetic mean and standard deviation (SD) as microgram (µg) of protein per gram (g) of tissue on a dry weight basis (dwt). The dry weight values were calculated by dividing the µg/g fwt by the dry weight conversion factor obtained from moisture analysis data. NA: Not Applicable.
[5]LOQ=limit of quantitation; LOD=limit of detection.
[6]CP4 EPSPS protein levels in MON 87427 pollen across the sites were either < LOD µg/g dwt (n=6), or had a very low level of CP4 EPSPS (n=6).

V.D. Assessment of Potential Allergenicity of the CP4 EPSPS Protein

The allergenic potential of an introduced protein is assessed by comparing the biochemical characteristics of the introduced protein to biochemical characteristics of known allergens (Codex Alimentarius, 2003). A protein is not likely to be associated with allergenicity if: 1) the protein is from a non-allergenic source; 2) the protein represents a very small portion of the total plant protein; 3) the protein does not share structural similarities to known allergens based on the amino acid sequence; and 4) the protein is rapidly digested in mammalian gastrointestinal systems. The CP4 EPSPS protein has been assessed for its potential allergenicity according to these safety assessment guidelines.

1) The CP4 EPSPS protein originates from *Agrobacterium sp.* strain CP4 an organism that has not been reported to be a source of known allergens.

2) The CP4 EPSPS protein represents no more than 0.005% of the total protein in the seed of MON 87427.

3) Bioinformatics analyses demonstrated that the CP4 EPSPS protein does not share amino acid sequence similarities with known allergens and, therefore, is highly unlikely to contain immunologically cross-reactive allergenic epitopes.

4) Finally, *in vitro* digestive fate experiments conducted with the CP4 EPSPS protein demonstrate that the protein is rapidly digested in simulated gastric fluid (SGF) and in simulated intestinal fluid (SIF).

Taken together, these data support the conclusion that the CP4 EPSPS protein does not pose a significant allergenic risk to humans or animals.

V.E. Safety Assessment Summary of the CP4 EPSPS Protein

Numerous factors have been considered in the safety assessment of the CP4 EPSPS protein and a comprehensive food, feed, and environmental safety assessment of CP4 EPSPS protein was conducted. The results are summarized below along with the conclusions reached from the assessment.

V.E.1. The donor organism has a history of safe use.

The donor organism, *Agrobacterium sp.*, strain CP4 is not known for human or animal pathogenicity, and is not commonly allergenic (FAO/WHO, 1991). *Agrobacterium* sp. strain CP4 has been previously reviewed as a part of the safety assessment of the donor organism during Monsanto consultations with the FDA regarding Roundup Ready soybean (1994), Roundup Ready canola (1995), Roundup Ready cotton (1995), Roundup Ready 2 corn (1996), Roundup Ready sugar beet (1998), and Roundup Ready Flex cotton (2005). Further, the Environmental Protection Agency has established an exemption from the requirement of a tolerance for residues of CP4 EPSPS protein and the genetic material necessary for its production in all plants (U.S. EPA, 1996).

V.E.2. EPSPS proteins are common in food and feeds.

The CP4 EPSPS protein present in MON 87427 is similar to EPSPS proteins consumed in a variety of food and feed sources. CP4 EPSPS protein is homologous to EPSPS proteins naturally present in plants, including food crops (e.g., soybean and maize) and fungal and microbial food sources such as baker's yeast (*Saccharomyces cerevisiae*), all of which have a history of safe human consumption (Harrison et al., 1996; Padgette et al., 1996). The similarity of the CP4 EPSPS protein to EPSPS proteins in a variety of foods supports extensive human consumption of the family of EPSPS proteins and the lack of health concerns. The ubiquitous presence of homologous EPSPS enzymes in food crops and common microorganisms establishes that EPSPS proteins, and their enzyme activity, pose no hazards for human and animal consumption.

V.E.3. The CP4 EPSPS protein catalyzes a specific enzyme reaction.

EPSPS exerts its functions in the shikimate pathway that is integral to aromatic amino acid biosynthesis in plants and microorganisms (Levin and Sprinson, 1964; Steinrucken and Amrhein, 1980). Therefore, this enzyme and its activity are found widely in food and feed derived from plant and microbial sources. Genes for numerous EPSPS proteins have been cloned (Padgette et al., 1996) and the catalytic domains of this group of proteins are conserved. Bacterial EPSPS proteins have been well characterized with respect to their three dimensional X-ray crystal structures (Stallings, et al., 1991) and detailed kinetic and chemical mechanisms (Anderson and Johnson, 1990).

V.E.4. The CP4 EPSPS protein is not homologous to a known allergens or toxins.

The CP4 EPSPS protein does not share amino acid sequence similarities with known allergens or protein toxins that have adverse effects to mammals. This has been demonstrated by extensive assessment with bioinformatic tools, such as the FASTA sequence alignment tool and eight-amino acid sliding window search. An amino acid sequence is considered to have allergenic potential if it has an exact sequence identity of at least eight linearly contiguous amino acids with a potential allergen epitope (Hileman, et al., 2002; Metcalfe, et al., 1996). Using a sliding window of less than eight amino acids can produce matches containing significant uncertainty depending on the length of the query sequence (Silvanovich, et al., 2006) and are not useful to the allergy assessment process (Thomas, et al., 2005).

V.E.5. The CP4 EPSPS protein is labile in in vitro digestion assays.

The CP4 EPSPS protein is readily digestible in simulated gastric (SGF) and simulated intestinal fluids (SIF) (Harrison et al., 1996). Rapid degradation of the full-length CP4 EPSPS protein in SGF and SIF reduces the exposure of CP4 EPSPS protein to epithelial cells of the small intestine in a biologically active form.

V.E.6. The CP4 EPSPS protein is not acutely toxic.

An acute oral toxicology study with mice was conducted with a CP4 EPSPS protein (Harrison et al., 1996) that was shown to be physicochemically and functionally equivalent to the CP4 EPSPS protein produced in MON 87427. Results indicate that the CP4 EPSPS protein did not cause any adverse effects in mice, therefore the No Observable Adverse Effect Level (NOAEL) for CP4 EPSPS is considered to be 572 mg/kg, the highest dose level tested.

A common approach used to assess potential health risks for potentially toxic materials is to calculate a Margin of Exposure (MOE) between the lowest NOAEL from an appropriate animal toxicity study and an estimate of human exposure. Since no evidence of mammalian toxicity has been reported for CP4 EPSPS, a dietary risk assessment would normally not be considered necessary. Nevertheless, a dietary risk assessment was still conducted in order to provide further assurances of safety by calculating a Margin of Exposure (MOE) between the acute mouse NOAEL for CP4 EPSPS protein and 95th percentile consumption estimates of acute dietary exposure determined using the Dietary Exposure Evaluation Model (DEEM-FCID version 2.03, Exponent Inc.), which utilizes food consumption data from the 1994-1996 and 1998 USDA Continuing Survey of Food Intakes by Individuals [CSFII; includes vegetable maize, popcorn, and commercial maize (flour, meal, bran and starch)] . The MOEs for acute dietary intake of the CP4 EPSPS protein were estimated to be at least 70,000 and 31,000 for the general population and children (1-6 yrs), respectively. Actual MOEs will likely be much higher because: 1) the NOAEL used in this calculation was the highest dose tested and might have been higher if higher doses had been tested and 2) as described in section V.E.5, CP4 EPSPS is rapidly digested, further minimizing exposure. These very large MOEs indicate that there is no meaningful risk to human health from dietary exposure to the CP4 EPSPS protein produced by MON 87427.

Furthermore, as recently concluded by EFSA: “The effect of processing on the constituents of maize containing the CP4 EPSPS protein is not expected to be different compared to that on conventional maize” (EFSA, 2009). It should be noted that a multitude of processes are used in maize processing, including temperature treatments, hydrolyses, soaking in slightly acidic water, and drying. Maize processing is likely to enhance the degradation and/or denaturation of CP4 EPSPS significantly reducing human dietary exposure to the functionally active protein.

The potential CP4 EPSPS protein exposure to animals from consumption of MON 87427 in feeds was evaluated by calculating an estimate of daily dietary intake (DDI). The highest percentage of CP4 EPSPS protein (g/kg bwt) per total protein consumed was in the dairy cow, 0.036% (g/g) of the total dietary protein intake (0.00218 g CP4 EPSPS/kg bwt divided by 6 g dietary protein which is the total dietary protein intake for the cow). The chicken and pig percentages of the CP4 EPSPS protein consumed as part of the daily protein intake are much less than for the dairy cow. At the most, poultry, swine and lactating dairy cattle would be consuming less than 0.4 mg/g of their total protein intake as CP4 EPSPS protein from MON 87427. Therefore, there is minimal exposure to MON 87427 CP4 EPSPS in relation to the total protein consumed.

V.F. Conclusion of CP4 EPSPS Protein Characterization and Safety

The Environmental Protection Agency has established an exemption from the requirement of a tolerance for residues of CP4 EPSPS protein and the genetic material necessary for its production in all plants (U.S. EPA, 1996). This exemption was based on a safety assessment that included rapid digestion in simulated mammalian gastrointestinal fluids, lack of homology to toxins and allergens, and lack of toxicity in an acute oral mouse gavage study. Because the MON 87427-produced CP4 EPSPS protein is equivalent to the exempted CP4 EPSPS protein a similar conclusion can be reached that the MON 87427-produced CP4 EPSPS protein is safe for human and animal consumption.

Using the guidance provided by the FDA in its 1992 Policy Statement (U.S. FDA, 1992) regarding the evaluation of New Plant Varieties, a conclusion of "no concern" has been reached for the donor organism and the CP4 EPSPS protein. The protein safety data presented herein support the conclusion that food and feed products containing MON 87427 or derived from MON 87427 are as safe as maize currently on the market for human and animal consumption.

VI. COMPOSITIONAL ASSESSMENT OF MON 87427

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).

Compositional equivalence between biotechnology-derived and conventional crops provides an "equal or increased assurance of the safety of foods derived from genetically modified plants" (OECD, 2002). The OECD consensus documents emphasize quantitative measurements of essential nutrients, known anti-nutrients and secondary metabolites. This is based on the premise that such comprehensive and detailed analyses will most effectively discern any compositional changes that imply potential safety and anti-nutritional concerns. Levels of the components in forage and grain of the biotechnology-derived crop are compared to: 1) corresponding levels in a conventional comparator , grown concurrently, under field conditions, and 2) natural ranges generated from an evaluation of commercial references grown concurrently and from data published in the scientific literature.

The comparison to data published in the literature places any potential differences between the assessed crop and its comparator in the context of the natural variation in the concentrations of crop nutrients, anti-nutrients, and secondary metabolites.

VI.A. Compositional Equivalence of MON 87427 Forage and Grain to Conventional Maize

Compositional analysis of MON 87427 and comparison to the conventional control (LH198 × LH287) and commercial references demonstrated that MON 87427 is compositionally equivalent to conventional maize. Forage and grain samples were collected from MON 87427 and the conventional control from a 2008 U.S. field production. The background genetics of the conventional control were similar to that of MON 87427, but it did not contain the *cp4 epsps* expression cassette. Four different commercial references were included at each site of the field production to provide data

on natural variability of each compositional component analyzed. The samples utilized for compositional analysis were obtained from three sites: Jefferson County, Iowa, Stark County, Illinois, and Jackson County, Arkansas. The sites were planted in a randomized complete block design with three blocks per site. MON 87427, the conventional control, and commercial references were treated with conventional weed control programs. In addition, MON 87427 plots were treated with glyphosate herbicide.

Compositional analyses were conducted to assess whether levels of key nutrients, anti-nutrients, and secondary metabolites in MON 87427 were equivalent to levels in the conventional control and to the composition of commercial references. A description of nutrients, anti-nutrients, and secondary metabolites present in maize is provided in the OECD consensus document on compositional considerations for maize (OECD, 2002). Nutrients assessed in this analysis included proximates (ash, carbohydrates by calculation, moisture, protein, and fat), acid detergent fiber (ADF), neutral detergent fiber (NDF), total dietary fiber (TDF), amino acids, fatty acids (C8-C22), vitamins [A (β-carotene), B_1, B_2, B_6, E, niacin, and folic acid], and minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc) in the grain, and proximates, ADF, NDF, calcium and phosphorus in forage. The anti-nutrients assessed in grain included phytic acid and raffinose. Secondary metabolites assessed in grain included furfural, ferulic acid, and p-coumaric acid. In all, 78 different analytical components were measured (9 in forage, 69 in grain). Of these, 16 components (15 nutrients and one anti-nutrient) in grain had more than 50% of the observations below the assay limit of quantitation (LOQ) and, as a result, were excluded from the statistical analysis. Therefore, 62 components were statistically assessed using a mixed model analysis of variance method. Values for all components were expressed on a dry weight basis with the exception of moisture, which was expressed as percent fresh weight and fatty acids, which were expressed as percent of total FA.

For MON 87427, four statistical comparisons to the conventional control were conducted for each component. One comparison was based on compositional data combined across all three field sites (combined-site analysis) and three separate comparisons were conducted on data from each of the individual field sites. Statistically significant differences were identified at a 5% level of significance (α=0.05). Data from the commercial references were combined across all sites and used to calculate a 99% tolerance interval for each compositional component to define the natural variability of each component in maize hybrids that have a history of safe consumption and that were grown concurrently with MON 87427 and the conventional control.

For the combined-site analysis, significant differences in nutrient, anti-nutrient, and secondary metabolite components were further evaluated using considerations relevant to the safety and nutritional quality of MON 87427 when compared to the conventional counterpart with a history of safe consumption: 1) the relative magnitude of the differences in the mean values of nutrient, anti-nutrient, and secondary metabolite components of MON 87427 and the conventional control, 2) whether the MON 87427 component mean value is within the range of natural variability of that component as represented by the 99% tolerance interval of commercial references grown concurrently, 3) evaluation of the reproducibility of the significant (α=0.05) combined-site component

differences at individual sites, and 4) assessing the difference within the context of natural variability of commercial maize composition published in the scientific literature and in the International Life Sciences Institute (ILSI) Crop Composition Database (ILSI, 2009).

This analysis provides a comprehensive comparative assessment of the levels of key nutrients, anti-nutrients, and secondary metabolites in grain and of key nutrients in forage of MON 87427 and the conventional control, discussed in the context of natural variability in commercial maize. Results of the comparison indicate that the composition of the forage and grain of MON 87427 is equivalent to that of the conventional control and within the natural variability of commercial references.

VI.A.1. Nutrient Levels in Maize Grain

Grain was analyzed for 64 compositional nutrients including: protein, moisture, fat, ash, carbohydrates, ADF, NDF, TDF, amino acids (18), fatty acids (22), vitamins [A (β-carotene), B_1, B_2, B_6, E, niacin, folic acid], and minerals (9). Fifteen nutrients were below the limit of quantitation. In the combined-site analysis of grain, no significant differences were observed between MON 87427 and the conventional control for 43 nutrients. Significant differences included mean values for 16:0 palmitic acid, 18:0 stearic acid, 18:1 oleic acid, 18:2 linoleic acid, 20:0 arachidic acid, and total fat (Tables VI-1 and VI-2).

The significant differences in nutrients were evaluated using considerations relevant to the nutritional quality of MON 87427 when compared to the conventional control:

1) All nutrient component differences observed in the combined-site analysis, whether reflecting increased or decreased MON 87427 mean values with respect to the conventional control, were small. Relative magnitudes of differences (mean difference as % of control) ranged from 1.96% to 5.09%.

2) MON 87427 mean values for these nutrient components were within the 99% tolerance interval established from the commercial references grown concurrently. Therefore, the MON 87427 mean values were within the range of natural variability in commercial maize hybrids with a history of safe consumption (Tables VI-1 and VI-2).

3) Assessment of reproducibility for the combined-site significant differences at the three individual sites demonstrated significant differences (α=0.05) for 18:0 stearic acid and 20:0 arachidic acid at one individual site and significant differences for 16:0 palmitic acid, 18:1 oleic acid, and 18:2 linoleic acid at all three sites. No significant difference was observed for total fat at any of the individual sites. Individual site mean values of MON 87427 for all nutrient components with significant differences fell within the 99% tolerance interval established from the commercial references grown concurrently and were, therefore, within the range of natural variability of that component in commercial maize hybrids with a history of safe consumption.

4) All of the compositional components identified as significantly different from the conventional control were within the natural variability of these components in commercial maize composition as published in the scientific literature and available in the ILSI Crop Composition Database (ILSI, 2009); Table VI-6).

The six combined-site significant differences (α=0.05) between MON 87427 and the conventional control were attributable to five fatty acids (all expressed as percent total FA) and total fat. The relative magnitude of differences between the mean values for MON 87427 and conventional control were small in the combined-site analysis for 16:0 palmitic acid (3.52% increase), 18:0 stearic acid (3.67% increase), 18:1 oleic acid, (3.22% increase), 18:2 linoleic acid (1.96% decrease), 20:0 arachidic acid (4.00% increase) and total fat (5.09% decrease) and at the three individual sites (all were approximately 5% or less) (Tables VI-2, E-3, E-7, and E-11). The observed significant differences between MON 87427 and conventional control for 16:0 palmitic acid, 18:1 oleic acid, 18:2 linoleic acid 18:0 stearic acid, 20:0 arachidic acid, and total fat are markedly less than differences in hybrids developed through conventional breeding (Harrigan, et al., 2009; Reynolds et al., 2005). Harrigan, et al. (2009) and the ILSI Crop Composition Database (ILSI, 2009) highlight the extensive natural variability in compositional component levels in maize, as presented in Table VI-6. All of the compositional components identified as significantly different from the conventional control were within the natural variability of these components in maize based upon published literature data and the ILSI-CCD (Table VI-6). Therefore, these significant differences are not meaningful to food and feed safety and nutrition.

In summary, the statistical analysis identified six significant differences that were all small in magnitude. Of these significant differences, only 16:0 palmitic acid, 18:1 oleic acid, and 18:2 linoleic acid were observed as consistently at all of the individual sites. All of the components identified as significantly different were within the natural variability of commercial maize defined by the 99% tolerance interval and published literature ranges. These findings support the conclusion that with regard to nutrients in grain, MON 87427 is compositionally equivalent to conventional maize.

VI.A.2. Anti-Nutrient Levels in Maize Grain

Maize grain contains two main anti-nutrients according to OECD (OECD, 2002), phytic acid and raffinose. Phytic acid is present in maize grain, where it chelates mineral nutrients, including calcium, magnesium, potassium, iron, and zinc, rendering them biologically unavailable to mono-gastric animals consuming the grain (Liener, 2000). Raffinose is a low molecular weight non-digestible carbohydrate present in maize grain that is considered to be an anti-nutrient due to the gas production and resulting flatulence caused by consumption (Liener, 2000).

In the combined-site analysis, a statistically significant difference (α=0.05) between MON 87427 and conventional control (Tables VI-1 and VI-3) was identified for phytic acid. No significant difference was observed for raffinose.

1) The phytic acid component difference observed in the combined-site analysis was small in relative magnitude, a decrease of 5.92% in MON 87427 with respect to the conventional control.

2) The MON 87427 mean phytic acid value from the combined-site analysis was within the 99% tolerance interval established from the commercial references grown concurrently and was therefore within the range of natural variability of this component in commercial maize hybrids with a history of safe consumption (Tables V1-1 and V1-3).

3) No significant differences for phytic acid were observed at any of the individual sites. Mean values for phytic acid in MON 87427 at the individual sites were within the 99% tolerance interval established from the commercial references.

4) The difference in phytic acid was also within the range of the natural variability of commercial maize composition as published in the scientific literature and available in the ILSI Crop Composition Database (ILSI, 2009).

In summary, the statistical analyses found a significant difference in phytic acid that was small in magnitude and not consistently observed at all of the individual sites. The mean phytic acid values for MON 87427 different were within the natural variability of commercial maize defined by the 99% tolerance interval and published literature ranges. Thus, an evaluation of anti-nutrient components in grain support the conclusion that MON 87427 is compositionally equivalent to conventional maize.

VI.A.3. Secondary Metabolites in Maize Grain

Maize grain contains three main secondary metabolites according to OECD, furfural, ferulic acid, and p-coumaric acid (OECD, 2002). The non-starch polysaccharide pentosans are a major source of furfural (Adams, et al., 1997). Ferulic acid and p-coumaric acid are derived from the aromatic amino acids, phenylalanine and tyrosine (Buchanan, et al., 2000), and serve as precursors for a large group of phenylpropanoid compounds. There were no combined-site significant differences (α=0.05) observed in secondary metabolites when the grain mean values from MON 87427 were compared to the conventional control and furfural was not detected in MON 87427, the conventional control, or commercial references. Thus, an evaluation of secondary metabolite components in grain support the conclusion that MON 87427 is compositionally equivalent to conventional maize.

VI.A.4. Nutrient Levels in Maize Forage

Maize forage was analyzed for nine compositional nutrients (protein, moisture, fat, ash, carbohydrates, ADF, NDF, calcium, and phosphorus). There were no combined-site significant differences (α=0.05) observed when the forage mean values from MON 87427 were compared to the conventional control. Thus, an evaluation of nutrient components in forage support the conclusion that MON 87427 is compositionally equivalent to conventional maize.

Table VI-1. Summary of Differences (α=0.05) for the Comparison of Maize Component Levels for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean[3]	Control[4] Mean	Mean Difference (Test minus Control)		Test Range	Commercial Tolerance Interval[5]
			Mean Difference (% of Control)	Significance (p-Value)		
Statistical Differences Observed in Combined-Site Analysis						
Grain Proximate (% dw)						
Total Fat	3.50	3.69	-5.09	0.036	3.13 - 3.83	2.12, 5.35
Grain Fatty Acid (% Total FA)						
16:0 Palmitic	10.91	10.54	3.52	<0.001	10.44 - 11.52	6.42, 15.23
18:0 Stearic	1.97	1.90	3.67	0.038	1.81 - 2.17	0.87, 2.88
18:1 Oleic	24.28	23.52	3.22	0.010	22.84 - 26.62	11.30, 43.27
18:2 Linoleic	60.84	62.06	-1.96	0.002	57.61 - 62.70	41.35, 74.78
20:0 Arachidic	0.42	0.41	4.00	0.005	0.37 - 0.48	0.15, 0.67
Grain Anti-nutrient (% dw)						
Phytic Acid	0.96	1.02	-5.92	0.008	0.87 - 1.04	0.73, 1.23

Table VI-1 (continued). Summary of Differences (α=0.05) for the Comparison of Maize Component Levels for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean[3]	Control[4] Mean	Mean Difference (Test minus Control)		Test Range	Commercial Tolerance Interval[5]
			Mean Difference (% of Control)	Significance (p-Value)		
Statistical Differences Observed in More than One Individual Site						
Grain Fatty Acid (% total FA)						
16:0 Palmitic Site ARNE	11.49	10.99	4.53	<0.001	11.47 - 11.52	6.42, 15.23
16:0 Palmitic Site IARL	10.72	10.44	2.66	0.007	10.58 - 10.85	6.42, 15.23
16:0 Palmitic Site ILWY	10.54	10.21	3.25	<0.001	10.44 - 10.65	6.42, 15.23
18:1 Oleic Site ARNE	26.34	25.35	3.93	<0.001	26.16 - 26.62	11.30, 43.27
18:1 Oleic Site IARL	22.91	21.95	4.41	0.002	22.84 - 22.98	11.30, 43.27
18:1 Oleic Site ILWY	23.58	23.24	1.44	0.043	23.29 - 23.78	11.30, 43.27
18:2 Linoleic Site ARNE	57.94	59.56	-2.72	<0.001	57.61 - 58.13	41.35, 74.78
18:2 Linoleic Site IARL	62.57	63.90	-2.09	<0.001	62.49 - 62.70	41.35, 74.78

Table VI-1 (continued). Summary of Differences (α=0.05) for the Comparison of Maize Component Levels for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean[3]	Control[4] Mean	Mean Difference (Test minus Control) Mean Difference (% of Control)	Mean Difference (Test minus Control) Significance (p-Value)	Test Range	Commercial Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Grain Fatty Acid (% total FA)						
18:2 Linoleic Site ILWY	62.01	62.72	-1.13	0.005	61.68 - 62.32	41.35, 74.78
Grain Amino Acid (% dw)						
Methionine Site ARNE	0.29	0.27	6.48	0.043	0.28 - 0.29	0.11, 0.29
Methionine Site IARL	0.23	0.25	-7.29	0.018	0.22 - 0.23	0.11, 0.29
Grain Fatty Acid (% total FA)						
18:3 Linolenic Site ARNE	1.15	1.19	-3.92	0.033	1.13 - 1.17	0.78, 1.52
18:3 Linolenic Site IARL	1.24	1.20	3.35	0.014	1.22 - 1.26	0.78, 1.52
Grain Vitamin (mg/kg dw)						
Vitamin B2 Site ARNE	3.27	2.36	38.30	0.004	3.05 - 3.56	0, 4.47
Vitamin B2 Site IARL	1.41	1.93	-26.71	0.042	1.17 - 1.60	0, 4.47

Table VI-1 (continued). Summary of Differences (α=0.05) for the Comparison of Maize Component Levels for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean[3]	Control[4] Mean	Mean Difference (Test minus Control)		Test Range	Commercial Tolerance Interval[5]
			Mean Difference (% of Control)	Significance (p-Value)		
Statistical Differences Observed in One Individual Site						
Grain Proximate (% dw)						
Carbohydrates Site IARL	84.24	83.11	1.36	0.047	83.60 - 84.96	80.77, 89.46
Moisture (% fw) Site IARL	10.93	10.40	5.13	0.043	10.90 - 11.00	7.56, 14.80
Protein Site IARL	10.60	11.73	-9.64	0.019	9.91 - 11.35	5.79, 13.43
Grain Fiber (% dw)						
Acid Detergent Fiber Site ILWY	3.78	3.05	23.75	0.020	3.33 - 4.27	1.84, 4.39
Grain Amino Acid (% dw)						
Arginine Site IARL	0.48	0.53	-9.19	0.033	0.45 - 0.49	0.24, 0.68
Cystine Site IARL	0.24	0.26	-5.95	0.012	0.24 - 0.25	0.14, 0.30
Serine Site IARL	0.49	0.56	-11.21	0.037	0.46 - 0.51	0.24, 0.66

Table VI-1 (continued) . Summary of Differences (α=0.05) for the Comparison of Maize Component Levels for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean[3]	Control[4] Mean	Mean Difference (Test minus Control)		Test Range	Commercial Tolerance Interval[5]
			Mean Difference (% of Control)	Significance (p-Value)		
Statistical Differences Observed in One Individual Site						
Grain Amino Acid (% dw)						
Tryptophan Site ARNE	0.062	0.052	19.32	0.006	0.059 - 0.064	0.032, 0.069
Grain Fatty Acid (% total FA)						
18:0 Stearic Site ARNE	2.17	2.04	6.43	0.002	2.16 - 2.17	0.87, 2.88
20:0 Arachidic Site ARNE	0.48	0.46	4.63	0.002	0.47 - 0.48	0.15, 0.67
22:0 Behenic Site ARNE	0.21	0.19	11.00	0.007	0.21 - 0.23	0, 0.32
Grain Mineral						
Calcium (% dw) Site ARNE	0.0077	0.0067	14.03	0.024	0.0075 - 0.0079	0.0019, 0.0076
Zinc (mg/kg dw) Site IARL	23.54	26.51	-11.20	0.010	22.45 - 24.61	11.46, 30.37
Grain Vitamin (mg/kg dw)						
Folic Acid Site IARL	0.36	0.45	-19.59	0.020	0.31 - 0.40	0.11, 0.61

Table VI-1 (continued). Summary of Differences (α=0.05) for the Comparison of Maize Component Levels for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean[3]	Control[4] Mean	Mean Difference (Test minus Control)		Test Range	Commercial Tolerance Interval[5]
			Mean Difference (% of Control)	Significance (p-Value)		
Statistical Differences Observed in One Individual Site						
Grain Anti-nutrient (% dw)						
Raffinose Site ARNE	0.11	0.13	-18.51	0.031	0.11 - 0.11	0.024, 0.29
Forage Proximate (% dw)						
Carbohydrates Site IARL	86.46	84.12	2.78	0.029	86.21 - 86.75	80.13, 94.05
Moisture (% fw) Site IARL	69.90	74.71	-6.44	0.008	67.70 - 71.20	51.70, 86.22
Protein Site IARL	7.03	8.63	-18.59	0.037	6.75 - 7.40	1.34, 11.57

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2] MON 87427 treated with glyphosate.
[3]Mean = least-square mean.
[4]Control refers to the near isogenic, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial references. Negative limits were set to zero.

Table VI-2. Summary of Combined-Site Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	1.58 (0.036) (1.43 - 1.81)	1.56 (0.038) (1.48 - 1.67)	0.013 (0.042) (-0.14 - 0.14)	-0.074, 0.099	0.765	1.13, 1.97 (1.18 - 1.82)
Carbohydrates	84.88 (0.56) (83.60 - 86.33)	84.51 (0.57) (82.96 - 85.76)	0.37 (0.33) (-0.87 - 1.63)	-0.40, 1.14	0.305	80.77, 89.46 (82.26 - 87.17)
Moisture (% fw)	11.62 (0.46) (10.90 - 13.30)	11.41 (0.46) (10.20 - 12.40)	0.22 (0.21) (-0.30 - 1.10)	-0.27, 0.71	0.337	7.56, 14.80 (9.31 - 12.70)
Protein	10.05 (0.63) (8.46 - 11.35)	10.26 (0.63) (8.62 - 11.92)	-0.21 (0.38) (-1.50 - 1.20)	-1.08, 0.66	0.594	5.79, 13.43 (8.07 - 12.13)
Total Fat	3.50 (0.13) (3.13 - 3.83)	3.69 (0.13) (3.47 - 3.98)	-0.19 (0.075) (-0.52 - 0.11)	-0.36, -0.015	0.036	2.12, 5.35 (2.90 - 4.30)
Fiber (% dw)						
Acid Detergent Fiber	3.37 (0.23) (2.67 - 4.27)	3.19 (0.23) (2.80 - 3.54)	0.18 (0.27) (-0.27 - 1.09)	-0.43, 0.79	0.521	1.84, 4.39 (2.29 - 4.27)

Table VI-2 (continued). Summary of Combined-Site Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Neutral Detergent Fiber	10.00 (0.51) (9.17 - 10.97)	10.12 (0.51) (9.21 - 11.27)	-0.12 (0.24) (-0.90 - 0.98)	-0.68, 0.43	0.628	5.69, 11.81 (7.06 - 10.66)
Total Dietary Fiber	13.00 (0.37) (12.13 - 14.35)	13.05 (0.37) (12.64 - 13.75)	-0.044 (0.24) (-0.67 - 1.07)	-0.53, 0.44	0.854	8.67, 15.32 (10.25 - 14.30)
Amino Acid (% dw)						
Alanine	0.75 (0.061) (0.61 - 0.89)	0.76 (0.061) (0.55 - 0.90)	-0.0061 (0.033) (-0.15 - 0.080)	-0.082, 0.069	0.857	0.32, 1.12 (0.58 - 0.98)
Arginine	0.48 (0.024) (0.40 - 0.55)	0.49 (0.025) (0.39 - 0.56)	-0.010 (0.015) (-0.079 - 0.065)	-0.040, 0.020	0.501	0.24, 0.68 (0.34 - 0.57)
Aspartic Acid	0.64 (0.041) (0.54 - 0.71)	0.64 (0.042) (0.48 - 0.73)	-0.0025 (0.025) (-0.099 - 0.064)	-0.059, 0.054	0.920	0.34, 0.92 (0.52 - 0.78)
Cystine	0.24 (0.010) (0.21 - 0.27)	0.24 (0.010) (0.21 - 0.26)	-0.0022 (0.0068) (-0.015 - 0.020)	-0.018, 0.013	0.750	0.14, 0.30 (0.18 - 0.26)

Table VI-2 (continued). Summary of Combined-Site Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Glutamic Acid	1.87 (0.15) (1.53 - 2.24)	1.89 (0.15) (1.38 - 2.28)	-0.020 (0.077) (-0.35 - 0.20)	-0.20, 0.16	0.801	0.77, 2.84 (1.46 - 2.49)
Glycine	0.38 (0.018) (0.34 - 0.43)	0.38 (0.018) (0.31 - 0.42)	0.0012 (0.0098) (-0.038 - 0.033)	-0.021, 0.024	0.906	0.23, 0.52 (0.32 - 0.43)
Histidine	0.30 (0.013) (0.27 - 0.34)	0.30 (0.013) (0.23 - 0.34)	-0.0014 (0.0081) (-0.045 - 0.033)	-0.018, 0.015	0.867	0.16, 0.39 (0.22 - 0.33)
Isoleucine	0.35 (0.026) (0.29 - 0.42)	0.36 (0.027) (0.26 - 0.42)	-0.0018 (0.014) (-0.081 - 0.039)	-0.035, 0.032	0.901	0.16, 0.53 (0.27 - 0.46)
Leucine	1.23 (0.11) (0.97 - 1.52)	1.25 (0.11) (0.89 - 1.56)	-0.022 (0.060) (-0.29 - 0.13)	-0.16, 0.12	0.725	0.43, 1.95 (0.93 - 1.69)
Lysine	0.30 (0.012) (0.27 - 0.33)	0.30 (0.013) (0.25 - 0.33)	-0.0020 (0.0072) (-0.024 - 0.026)	-0.018, 0.014	0.782	0.19, 0.40 (0.26 - 0.34)

Table VI-2 (continued). Summary of Combined-Site Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Methionine	0.24 (0.019) (0.20 - 0.29)	0.24 (0.019) (0.20 - 0.27)	0.00043 (0.0094) (-0.015 - 0.024)	-0.021, 0.022	0.964	0.11, 0.29 (0.17 - 0.25)
Phenylalanine	0.51 (0.040) (0.40 - 0.60)	0.52 (0.040) (0.38 - 0.61)	-0.0088 (0.023) (-0.10 - 0.052)	-0.063, 0.045	0.714	0.23, 0.75 (0.39 - 0.66)
Proline	0.90 (0.067) (0.74 - 1.08)	0.90 (0.067) (0.65 - 1.06)	-0.0045 (0.032) (-0.15 - 0.12)	-0.078, 0.069	0.889	0.40, 1.24 (0.66 - 1.07)
Serine	0.47 (0.033) (0.38 - 0.52)	0.48 (0.033) (0.36 - 0.58)	-0.011 (0.022) (-0.063 - 0.052)	-0.062, 0.040	0.625	0.24, 0.66 (0.38 - 0.59)
Threonine	0.35 (0.020) (0.29 - 0.39)	0.35 (0.020) (0.28 - 0.39)	-0.0022 (0.013) (-0.042 - 0.033)	-0.032, 0.028	0.871	0.20, 0.46 (0.28 - 0.41)
Tryptophan	0.054 (0.0032) (0.045 - 0.064)	0.053 (0.0033) (0.042 - 0.065)	0.00070 (0.0032) (-0.015 - 0.013)	-0.0067, 0.0081	0.835	0.032, 0.069 (0.039 - 0.063)

Table VI-2 (continued). Summary of Combined-Site Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Tyrosine	0.29 (0.029) (0.18 - 0.38)	0.30 (0.029) (0.21 - 0.39)	-0.0041 (0.026) (-0.12 - 0.11)	-0.057, 0.048	0.874	0.077, 0.45 (0.11 - 0.43)
Valine	0.48 (0.029) (0.41 - 0.55)	0.49 (0.029) (0.37 - 0.56)	-0.0015 (0.017) (-0.089 - 0.049)	-0.040, 0.037	0.930	0.25, 0.67 (0.38 - 0.58)
Fatty Acid (% total FA)						
16:0 Palmitic	10.91 (0.26) (10.44 - 11.52)	10.54 (0.26) (10.15 - 11.08)	0.37 (0.065) (0.14 - 0.59)	0.22, 0.52	<0.001	6.42, 15.23 (9.13 - 12.33)
18:0 Stearic	1.97 (0.091) (1.81 - 2.17)	1.90 (0.091) (1.77 - 2.07)	0.070 (0.028) (-0.028 - 0.18)	0.0048, 0.13	0.038	0.87, 2.88 (1.54 - 2.38)
18:1 Oleic	24.28 (0.92) (22.84 - 26.62)	23.52 (0.92) (21.74 - 25.71)	0.76 (0.23) (0.13 - 1.20)	0.23, 1.28	0.010	11.30, 43.27 (21.39 - 34.71)
18:2 Linoleic	60.84 (1.28) (57.61 - 62.70)	62.06 (1.28) (59.18 - 64.09)	-1.22 (0.29) (-1.69 - -0.46)	-1.88, -0.55	0.002	41.35, 74.78 (49.38 - 63.16)

Table VI-2 (continued). Summary of Combined-Site Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% total FA)						
18:3 Linolenic	1.20 (0.014) (1.13 - 1.26)	1.20 (0.014) (1.18 - 1.22)	-0.0012 (0.015) (-0.088 - 0.043)	-0.035, 0.033	0.935	0.78, 1.52 (0.97 - 1.35)
20:0 Arachidic	0.42 (0.030) (0.37 - 0.48)	0.41 (0.030) (0.37 - 0.46)	0.016 (0.0043) (-0.0022 - 0.034)	0.0063, 0.026	0.005	0.15, 0.67 (0.32 - 0.53)
20:1 Eicosenoic	0.21 (0.0080) (0.19 - 0.23)	0.21 (0.0080) (0.20 - 0.23)	-0.00097 (0.0017) (-0.0049 - 0.0033)	-0.0049, 0.0029	0.583	0.12, 0.36 (0.21 - 0.31)
22:0 Behenic	0.17 (0.018) (0.14 - 0.23)	0.16 (0.018) (0.14 - 0.20)	0.0076 (0.0050) (-0.0099 - 0.031)	-0.0039, 0.019	0.167	0, 0.32 (0.057 - 0.23)
Mineral						
Calcium (% dw)	0.0060 (0.00063) (0.0048 - 0.0079)	0.0055 (0.00063) (0.0046 - 0.0076)	0.00049 (0.00033) (-0.00037 - 0.0017)	-0.00027, 0.0013	0.176	0.0019, 0.0076 (0.0038 - 0.0068)
Copper (mg/kg dw)	1.63 (0.11) (1.21 - 2.07)	1.71 (0.12) (1.49 - 1.99)	-0.085 (0.11) (-0.42 - 0.18)	-0.33, 0.16	0.458	0.17, 3.48 (1.10 - 2.62)

Table VI-2 (continued). Summary of Combined-Site Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Iron (mg/kg dw)	23.61 (0.78) (22.21 - 25.84)	23.03 (0.79) (20.66 - 25.57)	0.58 (0.61) (-2.12 - 2.11)	-0.82, 1.98	0.368	11.42, 28.01 (16.55 - 24.10)
Magnesium (% dw)	0.13 (0.0033) (0.13 - 0.14)	0.13 (0.0033) (0.12 - 0.14)	-0.00021 (0.0034) (-0.0062 - 0.010)	-0.0080, 0.0076	0.952	0.080, 0.16 (0.11 - 0.15)
Manganese (mg/kg dw)	7.91 (1.06) (5.52 - 9.40)	8.07 (1.06) (4.89 - 9.82)	-0.16 (0.27) (-0.83 - 0.83)	-0.71, 0.39	0.567	0, 12.67 (4.00 - 9.17)
Phosphorus (% dw)	0.34 (0.0034) (0.32 - 0.35)	0.34 (0.0036) (0.33 - 0.35)	-0.0071 (0.0050) (-0.020 - 0.0053)	-0.018, 0.0040	0.185	0.24, 0.42 (0.28 - 0.37)
Potassium (% dw)	0.40 (0.0074) (0.38 - 0.42)	0.40 (0.0077) (0.38 - 0.43)	-0.0045 (0.0073) (-0.029 - 0.021)	-0.019, 0.010	0.546	0.24, 0.54 (0.33 - 0.46)
Zinc (mg/kg dw)	22.67 (1.06) (20.99 - 25.42)	23.99 (1.07) (21.65 - 28.08)	-1.32 (1.00) (-5.63 - 3.29)	-3.62, 0.99	0.225	11.46, 30.37 (17.30 - 25.45)

Table VI-2 (continued). Summary of Combined-Site Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Vitamin (mg/kg dw)						
Folic Acid	0.36 (0.025) (0.28 - 0.43)	0.39 (0.025) (0.29 - 0.49)	-0.030 (0.030) (-0.097 - 0.078)	-0.099, 0.040	0.347	0.11, 0.61 (0.24 - 0.57)
Niacin	27.22 (2.15) (22.56 - 33.37)	27.71 (2.18) (22.61 - 33.26)	-0.48 (1.34) (-3.30 - 2.66)	-3.22, 2.26	0.722	7.89, 49.83 (20.63 - 43.08)
Vitamin A	1.01 (0.050) (0.88 - 1.21)	0.96 (0.051) (0.76 - 1.16)	0.057 (0.043) (-0.094 - 0.21)	-0.029, 0.14	0.186	0.38, 1.68 (0.58 - 1.50)
Vitamin B1	2.97 (0.19) (2.58 - 3.41)	2.88 (0.20) (2.48 - 3.41)	0.084 (0.16) (-0.44 - 0.45)	-0.28, 0.45	0.606	2.21, 3.65 (2.41 - 3.48)
Vitamin B2	2.09 (0.37) (1.17 - 3.56)	1.93 (0.37) (1.32 - 2.58)	0.16 (0.33) (-0.72 - 1.23)	-0.59, 0.92	0.630	0, 4.47 (1.28 - 3.29)
Vitamin B6	7.48 (0.60) (5.91 - 8.69)	7.71 (0.60) (5.67 - 9.61)	-0.23 (0.41) (-1.40 - 1.76)	-1.16, 0.70	0.589	2.57, 12.07 (5.24 - 10.29)

Table VI-2 (continued). Summary of Combined-Site Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Vitamin (mg/kg dw)						
Vitamin E	13.14 (2.09) (7.04 - 17.44)	13.46 (2.10) (10.13 - 18.10)	-0.31 (0.86) (-6.54 - 4.52)	-2.05, 1.43	0.718	0, 25.61 (6.67 - 17.34)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2] MON 87427 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the near isogenic, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial references. Negative limits were set to zero.

Table VI-3. Summary of Combined-Site Grain Anti-nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Anti-nutrient (% dw)						
Phytic Acid	0.96 (0.031) (0.87 - 1.04)	1.02 (0.031) (0.94 - 1.12)	-0.060 (0.022) (-0.12 - 0.032)	-0.10, -0.016	0.008	0.73, 1.23 (0.82 - 1.07)
Raffinose	0.14 (0.028) (0.098 - 0.21)	0.15 (0.029) (0.11 - 0.21)	-0.0054 (0.0082) (-0.028 - 0.025)	-0.024, 0.013	0.524	0.024, 0.29 (0.092 - 0.21)

[1]dw = dry weight.
[2]MON 87427 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the near isogenic, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial references. Negative limits were set to zero.

Table VI-4. Summary of Combined-Site Grain Secondary Metabolites for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Secondary Metabolite (µg/g dw)						
Ferulic Acid	2348.63 (58.17) (2188.55 - 2559.19)	2387.92 (60.24) (2236.10 - 2500.00)	-39.29 (81.45) (-171.29 - 209.93)	-221.69, 143.10	0.640	1070.41, 2955.86 (1588.35 - 2630.98)
p-Coumaric Acid	204.94 (17.45) (166.11 - 260.43)	205.00 (17.54) (162.58 - 252.26)	-0.060 (8.82) (-28.53 - 32.92)	-20.17, 20.05	0.994	58.74, 313.97 (124.16 - 250.30)

[1]dw = dry weight.
[2]MON 87427 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the near isogenic, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial references. Negative limits were set to zero.

Table VI-5. Summary of Combined-Site Forage Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.73 (0.23) (4.39 - 5.13)	4.86 (0.23) (3.99 - 5.84)	-0.13 (0.19) (-0.74 - 0.66)	-0.53, 0.27	0.508	2.66, 6.48 (3.70 - 5.95)
Carbohydrates	87.23 (0.90) (86.21 - 89.23)	86.69 (0.91) (83.80 - 88.92)	0.54 (0.49) (-1.59 - 2.61)	-0.46, 1.54	0.277	80.13, 94.05 (83.23 - 90.37)
Moisture (% fw)	68.71 (2.30) (62.70 - 73.10)	69.76 (2.32) (64.10 - 75.00)	-1.05 (1.06) (-5.90 - 5.70)	-3.50, 1.40	0.350	51.70, 86.22 (61.00 - 76.00)
Protein	6.44 (0.75) (4.48 - 7.40)	6.78 (0.76) (5.17 - 8.94)	-0.34 (0.39) (-2.00 - 1.26)	-1.25, 0.57	0.413	1.34, 11.57 (4.37 - 9.31)
Total Fat	1.60 (0.17) (1.09 - 1.85)	1.69 (0.18) (0.58 - 2.28)	-0.092 (0.25) (-1.11 - 1.18)	-0.65, 0.46	0.720	0.44, 3.33 (0.78 - 3.16)
Fiber (% dw)						
Acid Detergent Fiber	24.96 (0.97) (21.08 - 29.00)	26.74 (1.03) (20.27 - 32.16)	-1.78 (1.42) (-8.15 - 3.58)	-4.65, 1.09	0.216	14.84, 38.51 (21.33 - 35.92)

Table VI-5 (continued). Summary of Combined-Site Forage Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Neutral Detergent Fiber	39.79 (1.32) (36.14 - 43.70)	38.12 (1.38) (33.07 - 43.43)	1.67 (1.76) (-1.55 - 4.79)	-2.32, 5.65	0.368	25.12, 54.99 (29.68 - 60.16)
Mineral						
Calcium (% dw)	0.19 (0.010) (0.14 - 0.22)	0.19 (0.011) (0.15 - 0.25)	-0.0083 (0.011) (-0.063 - 0.036)	-0.031, 0.014	0.455	0.075, 0.29 (0.10 - 0.24)
Phosphorus (% dw)	0.24 (0.021) (0.20 - 0.31)	0.24 (0.021) (0.19 - 0.31)	-0.0050 (0.013) (-0.074 - 0.038)	-0.032, 0.022	0.708	0.063, 0.37 (0.16 - 0.31)

[1]dw = dry weight; fw = fresh weight.
[2]MON 87427 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the near isogenic, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial references. Negative limits were set to zero.

Table VI-6. Literature and ILSI Ranges for Components in Maize Forage and Grain

Grain Tissue Components[1]	Literature Range[2]	ILSI Range[3]
Grain Nutrients		
Proximates (% dw)		
Ash	1.17 – 2.01[a]; 1.14 – 1.63[b]	0.616 – 6.282
Carbohydrates by calculation	81.31 – 87.06[a]; 82.10 – 86.65[b]	77.4 – 89.5
Fat, total	2.95 – 4.40[a]; 3.16 – 4.23[b]	1.742 – 5.823
Moisture (% fw)	8.74 – 11.30[a]; 11.00 – 13.20[b]	6.1 – 40.5
Protein	8.27 – 13.33[a]; 8.55 – 12.19[b]	6.15 – 17.26
Fiber (% dw)		
Acid detergent fiber	1.82 – 4.48[a]; 1.14 – 4.41[b]	1.82 – 11.34
Neutral detergent fiber	6.51 –12.28[a]; 6.08 – 10.36[b]	5.59 – 22.64
Total dietary fiber	10.65 – 16.26[a]; 10.24 – 14.56[b]	8.82 – 35.31
Amino Acids (% dw)		
Alanine	0.60 – 1.04[a]; 0.63 – 0.96[b]	0.439 – 1.393
Arginine	0.34 – 0.52[a]; 0.32 – 0.50[b]	0.119 –0.639
Aspartic acid	0.52 – 0.78[a]; 0.56 – 0.77[b]	0.335 – 1.208
Cystine	0.19 – 0.26[a]; 0.20 – 0.26[b]	0.125 – 0.514
Glutamic acid	1.54 – 2.67[a]; 1.62 – 2.44[b]	0.965 – 3.536
Glycine	0.33 – 0.43[a]; 0.31 – 0.42[b]	0.184 – 0.539
Histidine	0.25 – 0.37[a]; 0.24 – 0.34[b]	0.137 – 0.434
Isoleucine	0.30 – 0.48[a]; 0.30 – 0.44[b]	0.179 – 0.692
Leucine	1.02 – 1.87[a]; 1.06 – 1.65[b]	0.642 – 2.492
Lysine	0.26 – 0.33[a]; 0.25 – 0.31[b]	0.172 – 0.668
Methionine	0.17 – 0.26[a]; 0.16 – 0.30[b]	0.124 – 0.468
Phenylalanine	0.43 – 0.72[a]; 0.43 – 0.63[b]	0.244 – 0.930
Proline	0.74 – 1.21[a]; 0.72 – 1.11[b]	0.462 – 1.632
Serine	0.39 – 0.67[a]; 0.40 – 0.60[b]	0.235 – 0.769
Threonine	0.29 – 0.45[a]; 0.29 – 0.39[b]	0.224 – 0.666
Tryptophan	0.047 – 0.085[a]; 0.040 – 0.070[b]	0.0271 – 0.215
Tyrosine	0.13 – 0.43[a]; 0.12 – 0.41[b]	0.103 – 0.642
Valine	0.42 – 0.62[a]; 0.41 – 0.58[b]	0.266 – 0.855
Fatty Acids (% Total FA)		
16:0 Palmitic	8.80 – 13.33[a]; 9.53 – 12.33[b]	7.94 – 20.71
16:1 Palmitoleic	0.059 – 0.23[a]	0.095 – 0.447
18:0 Stearic	1.36 – 2.14 [a]; 1.28 – 2.13[b]	1.02 – 3.40
18:1 Oleic	19.50 – 33.71[a]; 19.59 – 31.09[b]	17.4 – 40.2
18:2 Linoleic	49.31 – 64.70[a]; 55.17 – 65.65[b]	36.2 – 66.5
18:3 Linolenic	0.89 – 1.56[a]; 1.00 – 1.38[b]	0.57 – 2.25
20:0 Arachidic	0.30 – 0.49[a]; 0.29 – 0.42[b]	0.279 – 0.965
20:1 Eicosenoic	0.17 – 0.29[a]; 0.17 – 0.31[b]	0.170 – 1.917
22:0 Behenic	0.069 – 0.28[a]; 0.059 – 0.33[b]	0.110 – 0.349
Minerals		
Calcium (% dw)	0.0036 – 0.0068[a]; 0.0032 – 0.0070[b]	0.00127 – 0.02084
Copper (mg/kg dw)	1.14 – 3.43[a]; 1.29 – 4.16[b]	0.73 – 18.50
Iron (mg/kg dw)	14.17 – 23.40[a]; 14.37 – 24.66[b]	10.42 – 49.07
Magnesium (% dw)	0.091 – 0.14[a]; 0.095 – 0.14[b]	0.0594 – 0.194
Manganese (mg/kg dw)	4.83 – 8.34[a]; 4.55 – 9.35[b]	1.69 – 14.30
Phosphorous (% dw)	0.24 – 0.37[a]; 0.26 – 0.38[b]	0.147 – 0.533
Potassium (% dw)	0.29 – 0.39[a]; 0.32 – 0.45[b]	0.181 – 0.603
Zinc (mg/kg dw)	16.78 – 28.17[a]; 18.12 – 30.44[b]	6.5 – 37.2

Table VI-6 (continued). Literature and ILSI Ranges for Components in Maize Forage and Grain

Grain Tissue Components[1]	Literature Range[2]	ILSI Range[3]
Vitamins (mg/kg DW)		
Folic acid	0.19 – 0.35[a]; 0.22 – 0.42[b]	0.147 – 1.464
Vitamin A [β–Carotene]	Not Available	0.19 – 46.81
Vitamin B_1 [Thiamine]	2.33 – 4.17[a]; 2.71 – 4.78[b]	1.26 – 40.00
Vitamin B_2 [Riboflavin]	0.94 – 2.42[a]; 1.46 – 2.81[b]	0.50 – 2.36
Vitamin B_3 [Niacin]	15.07 – 32.38[a]; 13.64 – 42.60[b]	10.37 – 46.94
Vitamin B_6 [Pyridoxine]	4.93 – 7.53[a]; 4.01 – 8.27[b]	3.68 – 11.32
Vitamin E [α–Tocopherol]	5.96 – 18.44[a]; 2.83 – 15.53[b]	1.5 – 68.7
Grain Anti–Nutrients (%DW)		
Phytic acid	0.69 – 1.09[a]; 0.58 – 0.97[b]	0.111 – 1.570
Raffinose	0.079 – 0.22[a]; 0.028 – 0.15[b]	0.020 – 0.320
Grain Secondary Metabolites (µg/g DW)		
Ferulic acid	1205.75 – 2873.05[a]; 820.14 – 2539.86[b]	291.9 – 3885.8
p–Coumaric acid	94.77 – 327.39[a]; 64.03 – 259.68[b]	53.4 – 576.2

Forage Tissue Components[1]	Literature Range[2]	ILSI Range[3]
Forage Nutrients		
Proximates (% dw)		
Ash	2.67–8.01[a]; 3.88 – 6.90[b]	1.527 – 9.638
Carbohydrates by calculation	81.88 – 89.26[a]; 84.11 – 89.52[b]	76.4 – 92.1
Fat, total	1.28 – 3.62[a]; 0.20 – 2.33[b]	0.296 – 4.570
Moisture (% FW)	64.20 – 75.70[a] ; 71.40 – 78.00[b]	49.1 – 81.3
Protein	5.80 – 10.24[a]; 5.56 – 9.14[b]	3.14 – 11.57
Fiber (% dw)		
Acid detergent fiber	19.11 – 30.49[a]; 20.73 – 33.39[b]	16.13 – 47.39
Neutral detergent fiber	27.73 – 49.62[a]; 31.81 – 50.61[b]	20.29 – 63.71
Minerals (% dw)		
Calcium	0.12 – 0.33[a]; 0.17 – 0.41[b]	0.0714 – 0.5768
Phosphorous	0.090 – 0.26[a]; 0.13 – 0.21[b]	0.0936 – 0.3704

[1]dw=dry weight; fw=fresh weight, FA = fatty acids.
[2]Literature range references: a(Harrigan et al., 2009)[US 2006], b(Harrigan et al., 2009)[Chile 2006/2007].
[3]ILSI range is from ILSI Crop Composition Database (ILSI, 2009).

VI.B. Compositional Assessment of MON 87427 Summary and Conclusion

Analyses of nutrient, anti-nutrient, and secondary metabolite levels in MON 87427 and the conventional control were conducted to assess compositional equivalence. The tissues analyzed included forage and grain harvested from plants grown at three field sites in the U.S. during the 2008 field season. The composition analysis, conducted in accordance with OECD guidelines, also included measurement of nutrients, anti-nutrients, and secondary metabolites in commercial maize reference hybrids that have a history of safe consumption to establish the natural range of variability. MON 87427, the conventional control, and commercial references were treated with conventional weed control programs. In addition, MON 87427 plots were treated with glyphosate herbicide at a target rate of 1.0 lb ai/acre (1.13 kg ai/ha).

There were no significant differences identified for grain secondary metabolites or forage nutrients. The significant differences (α=0.05) in nutrient and anti-nutrient content were evaluated using considerations relevant to the safety and nutritional quality of MON 87427 when compared to the conventional control:

1) All nutrient and anti-nutrient component significant differences observed in the combined-site analysis, whether reflecting increased or decreased MON 87427 mean values with respect to the conventional control were small. Relative magnitude of differences ranged from 1.96% to 5.92%.

2) Mean values for these nutrient and anti-nutrient components from the combined-site analysis of MON 87427 fell within the 99% tolerance interval established from the commercial references grown concurrently and were, therefore, within the range of natural variability of that component in commercial maize hybrids with a history of safe consumption (Tables VI-1 - VI-3).

3) Assessment of the reproducibility of the combined-site differences at the three individual sites showed significant differences (α=0.05) for 18:0 stearic acid and 20:0 arachidic acid at one individual site and differences for 16:0 palmitic acid, 18:1 oleic acid, and 18:2 linoleic acid differed across all three sites. No difference was observed for total fat and phytic acid at any of the individual sites. Individual site mean values of MON 87427 for all components with significant differences were within the 99% tolerance interval established from the commercial references grown concurrently and were, therefore, within the range of natural variability of that component in commercial maize hybrids with a history of safe consumption.

4) All of the compositional components identified as significantly different from the conventional control were within the natural variability of these components in commercial commercial maize composition as published in the scientific literature and available in the ILSI Crop Composition Database.

This analysis provides a comprehensive comparative assessment of the levels of key nutrients, anti-nutrients, and secondary metabolites in grain and of key nutrients in forage of MON 87427 and the conventional control, discussed in the context of natural

variability of commercial maize. Results of the comparison indicate that the composition of the forage and grain of MON 87427 is equivalent to that of the conventional maize control and that neither the genetic modification in MON 87427, nor the glyphosate herbicide treatment have a meaningful impact on the composition and therefore on the food and feed safety or nutritional quality of this product compared to conventional maize.

VII. PHENOTYPIC, AGRONOMIC, AND ENVIRONMENTAL INTERACTIONS ASSESSMENT

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.

VII.A. Characteristics Measured for Assessment

In the phenotypic, agronomic, and environmental interactions assessment of MON 87427, data were collected to evaluate specific aspects of altered plant pest potential. A detailed description of the regulated article phenotype is requested to be included as part of the petition for determination of nonregulated status in 7 CFR § 340. 6 including differences from the unmodified recipient organism that would "substantiate that the regulated article is unlikely to pose a greater plant pest risk than unmodified organism from which it was derived…" As part of the characterization of MON 87427, data were collected to provide a detailed phenotypic, agronomic, and environmental interaction description of MON 87427 and included an evaluation of specific characteristics related to altered plant pest potential.

The MON 87427 plant characterization encompassed five general data categories: 1) germination, dormancy, and emergence; 2) vegetative growth; 3) reproductive growth (including pollen characteristics); 4) seed retention on the plant and lodging; and 5) environmental interactions (plant response to abiotic stress and interactions with diseases and arthropods). An overview of the characteristics assessed is presented in Table VII-1.

The phenotypic, agronomic, and environmental interactions data were evaluated from a basis of familiarity (Hokanson, et al., 1999; OECD, 1993) and were comprised of a combination of field and laboratory studies conducted by scientists who are familiar with the production and evaluation of maize. In each of these assessments, MON 87427 was compared to a conventional control that had a genetic background similar to MON 87427 but did not possess the *cp4 epsps* expression cassette. In addition, multiple commercial maize references (see Appendices F and G, and Tables F-1, and G-1) were included to

provide a range of comparative values that are representative of existing commercial maize hybrids for each measured phenotypic, agronomic, and environmental interaction characteristic. Commercial references are selected from commercially available hybrids that have a range of genetics and relative maturities and are appropriate to use at the field locations used in these studies. The commercial references provide a range of variation for characteristics and context for interpreting experimental results.

Table VII-1. Phenotypic, Agronomic and Environmental Interaction Characteristics Evaluated in U.S. Field Trials or Laboratory Studies

Data category	Characteristics measured	Evaluation timing[1]	Evaluation description
Germination, dormancy, and emergence	Normal germinated[2]	Day 4 and 7 (20/30°C)	Percentage of seed producing seedlings exhibiting normal developmental characteristics
	Abnormal germinated[2]	Day 7 (20/30°C)	Percentage of seed producing seedlings that could not be classified as normal germinated
	Germinated[2]	Day 4, 7, and 12 (5, 10, 20, 30, 10/20 and 10/30°C)	Percentage of seed that had germinated normally and abnormally
	Dead	Day 4 and 7 (5, 10, 20, 30, 10/20, 10/30, and 20/30°C); Day 12 (5, 10, 20, 30, 10/20 and 10/30°C)	Percentage of seed that had visibly deteriorated and become soft to the touch (also included non-viable hard and non-viable firm-swollen seed)
	Viable hard	Day 7 (20/30°C); Day 12 (5, 10, 20, 30, 10/20 and 10/30°C)	Percentage of seed that did not imbibe water and remained hard to the touch (viability determined by a tetrazolium test[3])
	Viable firm-swollen	Day 7 (20/30°C); Day 12 (5, 10, 20, 30, 10/20 and 10/30°C)	Percentage of seed that imbibed water and were firm to the touch but did not germinate (viability determined by a tetrazolium test[3])
	Early stand count	Stage V2 - V4	Number of emerged plants in two rows, standardized to 20 ft rows
	Final stand count	Pre-harvest	Number of plants in two rows, standardized to 20 ft rows
Vegetative growth	Seedling vigor	V2 - V4	Rated on a 1-9 scale, where 1 = good and 9 = poor; a rating of 3 – 6 is normal
	Stay green	Maturity	Rated as: 1 = 90-100% green tissue, 5 = 50-59% green tissue, 9 = 0-19% green tissue
	Ear height	Maturity	Distance from the soil surface at the base of the plant to the ear attachment node
	Plant height	Maturity	Distance from the soil surface to the uppermost node on the main stem of five representative plants per plot
Reproductive growth	Days to 50% pollen shed	Pollen shed	Days from planting until 50% of the plants have begun to shed pollen
	Days to 50% silking	Silking	Days from planting until 50% of the plants have silks exposed
	Pollen viability	Tasseling	Percentage of viable pollen based on pollen grain staining characteristics
	Pollen morphology	Tasseling	Diameter of viable pollen grains
	Grain moisture	Harvest	Percentage moisture of harvested shelled grain
	Test weight	Harvest	Test weight of harvested shelled grain
	Yield	Harvest	Bushels of harvested seed per acre, adjusted to 15.5% moisture

Table VII-1 (continued). Phenotypic, Agronomic and Environmental Interaction Characteristics Evaluated in U.S. Field Trials or Laboratory Studies

Data category	Characteristics measured	Evaluation timing[1]	Evaluation description
Seed retention and lodging	Stalk lodged plants	Pre-harvest	Number of plants per plot broken below the ear
	Root lodged plants	Pre-harvest	Number of plants per plot leaning at the soil surface at >30°from the vertical
	Dropped ears	Pre-harvest	Number of mature ears dropped from plants
Environmental interactions	Plant response to abiotic stress	Four times per growing season	Qualitative assessment of each plot, with rating on a 0-9 scale, where 0 = no symptoms and 9 = severe symptoms
	Disease damage	Four times per growing season	Qualitative assessment of each plot, with rating on a 0-9 scale, where 0 = no symptoms and 9 = severe symptoms
	Arthropod damage	Four times during growing season	Qualitative assessment of each plot, with rating on a 0-9 scale, where 0 = no symptoms and 9 = severe symptoms
	Stalk rot disease	Harvest	Qualitative assessment of each plot, with rating on a 0-9 scale, where 0 = no symptoms and 9 = severe symptoms
	Ear and kernel rot disease	Harvest	Qualitative assessment of each plot, with rating on a 0-9 scale, where 0 = no symptoms and 9 = severe symptoms
	Corn earworm damage	Harvest	Damage assessed on 10 representative plants per plot using a 0-9 scale adapted from Widstrom (1967)
	European corn borer damage	Harvest	Number of live larvae, number of entry and exit holes, number of feeding galleries, and total length of feeding galleries in each stalk of ten plants per plot
	Arthropod abundance	Five collection times during growing season	Identification and enumeration of pest and beneficial arthropods abundance in sticky trap samples

[1] Maize plant growth stages were determined using descriptions and guidelines outlined in Maize Growth and Development (Ritchie et al., 1997).
[2] For the 20/30 °C temperature regime both normal and abnormal germination measurements are taken. For all other temperature regimes germination only is noted.
[3] Viability of hard and firm-swollen seed were determined by a tetrazolium test (AOSA, 2000).

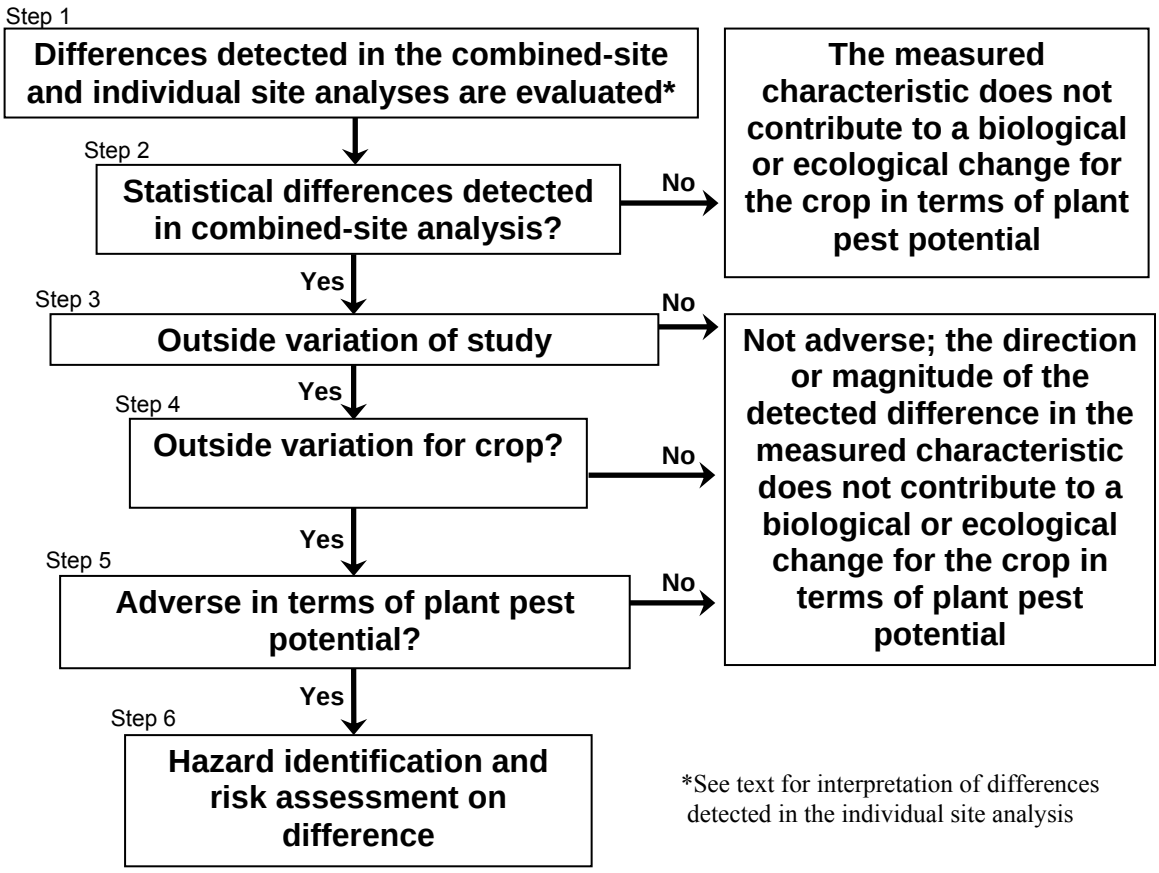
VII.B. Interpretation of Phenotypic and Environmental Interaction Data

Plant pest risk assessments for biotechnology-derived crops are comparative assessments. Familiarity provides a basis from which the potential environmental impact of a biotechnology-derived plant can be evaluated. The concept of familiarity is based on the fact that the biotechnology-derived plant is developed from a well-characterized conventional plant variety. Familiarity considers the biology of the crop, the introduced trait, the receiving environment and the interaction of these factors, and provides a basis for comparative environmental risk assessment between a biotechnology-derived plant and the conventional control.

Expert knowledge and experience with conventionally bred maize was the basis for selecting appropriate endpoints and estimating the range of responses that would be considered typical for maize. As such, assessment of phenotypic and agronomic characteristics and environmental interactions was essential to compare the biotechnology-derived plant to the conventional control. An overview of the characteristics assessed is presented in Table VII-1. A subset of the data relating to well-understood weediness criteria (e.g., seed dormancy, pre-harvest seed loss characteristics, lodging) was used to assess whether there was an increase in weediness potential, an element of APHIS's plant pest determination. Evaluation of environmental interaction characteristics (e.g., plant-abiotic stress, plant-disease, and plant-arthropod interactions) was also considered in the plant pest assessment. Based on all of the data collected, an assessment was made whether the biotechnology-derived plant is likely to pose an increased plant pest risk compared to the conventional control. Prior to statistical analysis, the overall dataset was evaluated for evidence of biologically relevant changes, and for possible evidence of an unexpected plant response. No unexpected observations or issues were identified.

VII.B.1. Criteria for Interpretation of Detected Differences Criteria

Comparative plant characterization data between a biotechnology-derived crop and the conventional control are interpreted in the context of contributions to increased plant pest potential as assessed by APHIS. Under the framework of familiarity, characteristics for which no differences are detected support a conclusion of no increased plant pest potential of the biotechnology-derived crop compared to the conventional crop. Characteristics for which differences are detected are considered in a step-wise method (Figure VII-1 or a similar method). All detected differences for a characteristic are considered in the context of whether or not the difference would increase the plant pest potential of the biotechnology-derived crop. Ultimately, a weight of evidence approach considering all characteristics and studies was used for the overall risk assessment of differences and their significance. In detail, Figure VII-1 illustrates the stepwise assessment process employed:



Note: A "no" answer at any step indicates that the characteristic does not contribute to a biological or environmental change for the crop in terms of plant pest potential and subsequent steps are not considered. If the answer is "yes" or "uncertain", the subsequent step is considered.

Figure VII-1. Schematic Diagram of Agronomic and Phenotypic Data Interpretation Methods

Steps 1 and 2 - Evaluate Detected Statistical Differences

Data on each measured characteristic are statistically analyzed, where appropriate, within each individual site and in a combined-site analysis, in which the data are pooled among sites. Differences detected in the individual-site analysis must be observed in the combined-site analysis to be considered further for plant pest potential. Any difference detected in the combined-site analysis is further assessed.

Step 3 - Evaluate Differences Relative to Reference Range

If a difference for a characteristic is detected in the combined-site analysis across multiple environments, then the mean value of the biotechnology-derived crop for that characteristic is assessed relative to the commercial reference varieties.

Step 4 - Evaluate Differences in the Context of the Crop

If the mean value of the biotechnology-derived crop is outside the variation of the reference substances (e.g., reference range), the mean value of the biotechnology-derived crop is considered in the context of known values common for the crop.

Step 5 - Plant Pest Potential

If the mean value of the biotechnology-derived crop is outside the range of values common for the crop, the detected difference for the characteristic is then assessed for whether or not it is adverse in terms of plant pest potential.

Step 6 - Conduct Risk Assessment on Identified Hazard

If an adverse effect (hazard) is identified, risk assessment on the difference is conducted. The risk assessment considers contributions to enhanced plant pest potential of the crop itself, the impact of differences detected in other measured characteristics, and potential for and effects of trait transfer to feral populations of the crop or to a sexually compatible species.

VII.C. Comparative Assessments of Phenotypic, Agronomic and Environmental Interactions Characteristics of MON 87427

This section provides the results of comparative assessments conducted in replicated laboratory and/or multi-site field experiments to provide a detailed phenotypic, agronomic, and environmental interaction description of MON 87427. The characteristics for MON 87427 evaluated in these assessments included: seed dormancy and germination characteristics (Section VII.C.1), plant phenotypic and environmental interaction observations under field conditions (Section VII.C.2), and pollen characteristics (Section VII.C.3). Additional details for each assessment are provided in Appendices F, G, and H.

VII.C.1. Seed Dormancy and Germination Characteristics

USDA-APHIS considers the potential for weediness to constitute a plant pest factor (7 CFR § 340.6). Seed germination and dormancy mechanisms vary with species and their genetic basis tends to be complex. Seed dormancy (e.g., hard seed) is an important characteristic that is often associated with plants that are considered weeds (Anderson, 1996; Lingenfelter and Hartwig, 2003). Standardized germination assays are available and routinely used to measure the germination characteristics of maize seed. The Association of Official Seed Analysts (AOSA), an internationally recognized seed testing organization, recommends a temperature range of 20/30 °C as optimal for testing the germination characteristics of maize seed (AOSA, 2007).

Comparative assessments of seed dormancy and germination characteristics were conducted on MON 87427 and the conventional control. In addition, seven reference hybrids were included to provide a range of comparative values that are representative of natural variability of commercial maize hybrids. The seed lots for MON 87427, the

conventional control, and the reference hybrids were produced in replicated field trials during 2008 in Iowa (IA) and Illinois (IL), geographic areas which represent environmentally relevant conditions for maize production for this product. These plots were not treated with glyphosate. In addition to the AOSA recommended temperature range of alternating 20/30 °C, seed was also tested at temperature regimes of 5, 10, 20, 30, alternating 10/20, and 10/30 °C to assess seed germination properties. The details of the materials, experimental methods, and germination data from all individual production sites are presented in Appendix F.

In the combined-site analysis, in which data were pooled for the two seed production sites, no statistically significant differences (5% level of significance) were detected between MON 87427 and the conventional control for any characteristic at the AOSA temperature regime (20/30 °C), or at the temperature regimes of 10, 30, 10/20, 10/30 °C (Table VII-2). In addition, no hard seed were observed at any temperature. MON 87427 had significantly lower percent dead seed than the conventional control (6.5 vs. 11.1%) and higher percent viable firm swollen seed than the conventional control (93.5 vs. 88.9%) at 5 °C. MON 87427 had statistically significant higher percent germinated seed (98.9 vs. 97.1%) and lower percent dead seed (1.1 vs. 2.9%) than the conventional control at 20 °C. These differences were small in magnitude and were not observed consistently across temperature regimes. Additionally, the mean values of percent dead and percent viable firm swollen seed at 5 °C and of percent germinated and dead seed at 20 °C for MON 87427 fell within the range of the natural variability of the commercial references. Therefore, the statistically significant differences in percent dead and viable firm swollen seed at 5 °C and percent germinated and dead seed at 20°C are unlikely to be biologically meaningful in terms of altered dormancy or germination characteristics (Figure VII-1, step 3).

The biological characteristics evaluated in this study were used to characterize MON 87427 in the context of plant pest risk assessment. Based on the dormancy and germination characteristics assessed, the results of this study, particularly the lack of increased hard seed, demonstrate there were no changes indicative of increased weediness or plant pest potential of MON 87427 compared to conventional maize.

Table VII-2. Germination Characteristics of MON 87427 and the Conventional Control

Temperature (°C)	Germination Characteristic	Mean % (S.E.)[1]		Reference Range[2]	
		MON 87427	Control	Min	Max
5	Germinated[†]	0.0 (0.0)	0.0 (0.0)	0.0	0.0
	Viable hard[†]	0.0 (0.0)	0.0 (0.0)	0.0	0.0
	Dead	6.5* (1.5)	11.1 (2.5)	3.8	6.8
	Viable firm swollen	93.5* (1.5)	88.9 (2.5)	93.3	96.3
10	Germinated	82.4 (2.4)	79.0 (1.8)	87.3	98.3
	Viable hard[†]	0.0 (0.0)	0.0 (0.0)	0.0	0.0
	Dead	3.9 (0.8)	4.4 (0.6)	1.0	5.3
	Viable firm swollen	13.8 (1.9)	16.6 (1.8)	0.0	11.3
20	Germinated	98.9* (0.3)	97.1 (0.6)	95.8	99.3
	Viable hard[†]	0.0 (0.0)	0.0 (0.0)	0.0	0.0
	Dead	1.1* (0.3)	2.9 (0.6)	0.8	4.3
	Viable firm swollen[†]	0.0 (0.0)	0.0 (0.0)	0.0	0.0
30	Germinated	97.4 (0.5)	98.4 (0.5)	97.0	99.8
	Viable hard[†]	0.0 (0.0)	0.0 (0.0)	0.0	0.0
	Dead	2.6 (0.5)	1.6 (0.5)	0.3	3.0
	Viable firm swollen[†]	0.0 (0.0)	0.0 (0.0)	0.0	0.0
10/20	Germinated	98.0 (0.5)	98.4 (0.6)	95.8	99.8
	Viable hard[†]	0.0 (0.0)	0.0 (0.0)	0.0	0.0
	Dead	2.0 (0.5)	1.6 (0.6)	0.3	4.3
	Viable firm swollen[†]	0.0 (0.0)	0.0 (0.0)	0.0	0.0
10/30	Germinated	98.5 (0.3)	97.9 (0.6)	97.0	99.5
	Viable hard[†]	0.0 (0.0)	0.0 (0.0)	0.0	0.0
	Dead	1.5 (0.3)	2.1 (0.6)	0.5	3.0
	Viable firm swollen[†]	0.0 (0.0)	0.0 (0.0)	0.0	0.0
20/30 (AOSA)	Normal germinated	94.4 (0.9)	94.9 (0.9)	93.8	98.8
	Abnormal germinated	3.8 (1.2)	3.6 (1.1)	0.8	3.8
	Viable hard[†]	0.0 (0.0)	0.0 (0.0)	0.0	0.0
	Dead	1.9 (0.5)	1.5 (0.3)	0.3	4.0
	Viable firm swollen[†]	0.0 (0.0)	0.0 (0.0)	0.0	0.0

Note: experimental design was a split-plot with four replications by two sites (n = 8).
*Indicates statistically significant difference (α=0.05) between MON 87427 and the conventional maize control using analysis of variance.
[†]No statistical comparison could be made due to lack of variability in the data.
[1]In some instances, the total percentage of both MON 84727 and the conventional control did not equal 100% due to numerical rounding of the means. S.E. = standard error
[2]Minimum and maximum mean values determined from among the commercial references.

VII.C.2. Field Phenotypic, Agronomic Characteristics, and Environmental Interactions

Plant growth, development, and yield characteristics were assessed under field conditions as part of the plant characterization assessment of MON 87427. These data were developed to provide USDA-APHIS with a detailed description of MON 87427 relative to the conventional control and commercial maize hybrids. According to 7 CFR § 340.6, as part of the petition to seek deregulation, a petitioner must submit "A detailed description of the phenotype of the regulated article." This information is being provided to assess whether there are phenotypic differences between MON 87427 and the conventional control that may impact its pest potential. Certain growth, reproduction, and pre-harvest seed loss characteristics (e.g., lodging, ear drop) were used to assess whether there is an increase in weediness of MON 87427, an element of APHIS's plant pest determination. Environmental interactions were also assessed as an indirect indicator of phenotypic changes to MON 87427 compared to the same comparators described above and are also considered in the plant pest assessment.

Data were collected at 16 field locations in the U.S. during 2008 to evaluate phenotypic, agronomic, and environmental interaction characteristics. These 16 locations provided a diverse range of environmental and agronomic conditions representative of commercial maize production areas in the U.S. (Table VII-3). The experiments were arranged as randomized complete block designs with three replications at each field location. The observations were taken on plants not treated with glyphosate. The categories and timings of phenotypic characteristics and environmental interactions evaluated are included in Table VII-1. The methods and detailed results of the individual-site data comparisons are presented and discussed in Appendix G, while the combined-site analyses are summarized in Table VII-4. The results of this assessment demonstrated that the tissue-selective glyphosate tolerance did not alter MON 87427 compared to conventional maize in terms of weediness or pest potential. The lack of biologically meaningful differences in plant response to abiotic stress, disease damage, arthropod damage, and pest and beneficial arthropod abundance further support the conclusion that the tissue-selective glyphosate tolerance in MON 87427 is not likely to result in increased plant pest potential or an altered environmental impact from MON 87427 compared to conventional maize.

VII.C.2.1. Field Phenotypic and Agronomic Characteristics

A total of 14 phenotypic and agronomic characteristics were evaluated. In a combined-site analysis in which the data were pooled among the sites, no statistically significant differences were detected (5% level of significance) between MON 87427 and the conventional control for early stand count, days to 50% pollen shed and silking, stay green, ear height, plant height, dropped ears, stalk and root lodging, final stand count, grain moisture, test weight, and yield (Table VII-4). One statistically significant difference was detected between MON 87427 and the conventional control. MON 87427 was less vigorous than the conventional control (2.7 vs. 2.4 rating on a 1-9 scale). However, the mean value for seedling vigor for MON 87427 fell within the natural variability of the commercial references. Therefore, the difference in seedling vigor is

unlikely to be biologically meaningful in terms of increased weed potential (Figure VII-1, step 3).

The phenotypic and agronomic characteristics evaluated in this study were used to provide a detailed description of MON 87427 compared to the conventional control. A subset of these characteristics was used to assess the weediness of MON 87427. Based on the assessed phenotypic and agronomic characteristics, the results support a determination that MON 87427 is not fundamentally different than conventional maize and is no weedier and no more likely to pose a plant pest risk or have a significant environmental impact than conventional maize.

Table VII-3. Field Phenotypic Evaluation Sites for MON 87427 during 2008

Location	Location Code	USDA-APHIS Notification Number
Jackson County, AR	ARNE	08-058-103n
Jefferson County, IA	IARL	08-058-103n
Stark County, IL	ILWY	08-058-103n
Parke County, IN	INRC	08-058-103n
Wapello County, IA	IA1	08-050-101n
Benton County, IA	IA2	08-050-101n
Stark County, IL	IL1	08-066-102n
Clinton County, IL	IL2	08-050-101n
Montgomery County, IN	IN1	08-066-102n
Boone County, IN	IN2	08-066-102n
Ottawa County, MI	MI	08-050-101n
Butler County, MO	MO	08-050-101n
Caddo County, OK	OK	08-066-102n
Berks County, PA	PA	08-050-101n
Armstrong County, TX	TX	08-066-102n
Walworth County, WI	WI	08-050-101n

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 (α=0.05).

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

[2]Early stand count was dropped for all reps of MON 87427 and the conventional control from the IA1, IL1, OK, and WI sites due to heavy overplanting. After early stand count, plots were thinned to a uniform density, and no other assessments were affected. N = 36 for MON 87427 and the conventional control.

[3]Stay green ratings from the ARNE, IARL, ILWY, and INRC sites were included in the individual site analysis but excluded from combined site analysis due to a reversal of the rating scale at these sites. Stay green was dropped from all reps at the IN1 site because it was rated incorrectly. Stay green was excluded from a single rep of MON 87427 at the OK site because it was identified as an outlier. N = 32 for MON 87427; N = 33 for the conventional control.

[4]Plant height data were excluded from a single rep of MON 87427 at the IN2 site due to broken stalk tops that prevented accurate plant height measurement. N = 47 for MON 87427; N = 48 for the conventional control.

[5]Dropped ears and stalk lodging were not recorded from a single rep of the conventional control at the OK site. N = 48 for MON 87427; N = 47 for the conventional control.

[6]Yield assessment data were dropped from all reps at the MO site due to wind damage, at the OK site due to insect damage, and at the TX site due to frost damage. Yield assessment data were dropped from two reps of MON 87427 at the IA2 site and one rep of MON 87427 at the INRC site, and one rep of the conventional control at the IARL site because the final stand count in these reps was more than 20% below the target population. N = 36 for MON 87427; N = 38 for the conventional control.

VII.C.2.2. Environmental Interaction Analyses

USDA-APHIS considers the environmental interaction of the biotechnology-derived crop compared to its conventional control to determine the potential for increased plant pest characteristics. Qualitative and quantitative environmental interactions assessments were conducted as part of the plant characterization for MON 87427. In the 2008 U.S. field trials conducted to evaluate the phenotypic and agronomic characteristics of MON 87427, data were also collected on plant response to abiotic stress (drought, wind, nutrient deficiency, etc.), disease damage, arthropod damage, and arthropod abundance (Tables VII-5 and VII-6; Appendix G; Tables G-4, G-5, G-6, G-7, G-8, and G-9, respectively). These data are used as part of the environmental risk assessment to assess plant pest potential and provide an indication of potential adverse effects of MON 87427 on NTO compared to the conventional control (see Section IX and Section X for additional discussion). In addition, multiple commercial maize hybrids were included as references in the analysis to establish a range of natural variability for each assessed characteristic. The results of the field evaluations of non-glyphosate treated plants showed that the tissue-selective glyphosate tolerance in MON 87427 did not alter the assessed environmental interactions of MON 87427 compared to conventional maize. The lack of biologically meaningful differences in plant response to abiotic stress, disease damage, arthropod damage, and pest and beneficial arthropod abundance support the conclusion that the tissue-selective glyphosate tolerance in MON 87427 is unlikely to result in increased plant pest potential or an altered environmental impact from MON 87427 compared to conventional maize.

Corn earworm damage and European corn borer damage were evaluated quantitatively at four of the 16 sites at harvest (i.e., IL1, IN2, MO, and PA). In a combined-site analysis in which the data were pooled among the four sites, no statistically significant differences (5% level of significance) were detected between MON 87427 and the conventional control for corn earworm damage or for European corn borer damage (Table VII-5).

In a qualitative assessment of plant response to abiotic stressors and disease damage, no differences were observed between MON 87427 and the conventional control for 172 comparisons involving any of 12 assessed abiotic stressors or for 210 comparisons involving any of the 24 assessed diseases (Table VII-6; Appendix G; Tables G-4, G-5). In a qualitative assessment of arthropod damage, no differences were observed between MON 87427 and the conventional control for any of the 167 comparisons for the 22 assessed arthropods among all observations at the sites (Table VII-6; Appendix G; Table G-6).

In a quantitative assessment of pest and beneficial arthropod abundance, no statistically significant differences (5% level of significance) were detected between MON 87427 and the conventional control for 191 out of 203 comparisons, including 98 pest arthropod comparisons and 105 beneficial arthropod comparisons, among the observations at the four sites (Table VII-6; Appendix G; Tables G-8, G-9). In addition, p-values were not calculated for four pest arthropod comparisons because there was no variability in the data between MON 87460 and the conventional control.

The four differences detected out of 98 comparisons for pest arthropod abundance included observations for corn flea beetles, grasshoppers, and leafhoppers (Table G-8). MON 87427 had higher abundance than the conventional control of corn flea beetles at the first and fifth observations from the PA site (91.7 vs. 59.0 per plot and 12.0 vs. 5.3 per plot, respectively), higher abundance of grasshoppers from the fourth observation at the MO site (1.3 vs. 0.0 per plot), and higher abundance of leafhoppers from the second observation at the PA site (7.7 vs. 2.0 per plot). The mean abundance values for MON 87427 were within the reference range for all detected differences in arthropod abundance with the exception of the difference detected for corn flea beetles from the fifth observation at the PA site and grasshopper abundance at the MO site; however, no differences were detected between MON 87427 and the conventional control for corn flea beetle abundance from three of five observations from the PA site or in observations from any other site or for grasshopper abundance from any other site or observation times. Furthermore, the differences detected in corn flea beetle, grasshopper, and leaf hopper abundance were small in magnitude and unlikely to be biologically meaningful in terms of increased pest potential. These results support a conclusion that the detected differences in pest arthropod abundance were not indicative of a consistent response associated with the tissue-selective glyphosate tolerance in MON 87427 and are not considered biologically meaningful in terms of increased plant pest potential or an altered environmental impact from MON 87427 compared to conventional maize.

The eight detected differences out of 105 comparisons for beneficial arthropod abundance included observations for Aranae, ladybird beetles, macro-parasitic Hymenoptera, *Nabis* spp., and *Orius* spp. (Table G-9). MON 87427 had higher abundance than the conventional control of Aranae from the fourth observation at the PA site (2.3 vs. 0.7 per plot), lower abundance of ladybird beetles from the third observation at the IL1 site (0.3 vs. 1.3 per plot), and higher ladybird beetle abundance from the third observation of the IN2 site (10.7 vs. 3.0 per plot). MON 87427 had higher abundance compared to the conventional control of macro-parasitic Hymenoptera from the fourth and fifth observations at the IN2 site (26.7 vs. 9.7 per plot and 47.7 vs. 27.7 per plot, respectively), lower abundance of *Nabis* spp. from the first observation at the PA site (0.7 vs. 2.7 per plot), lower abundance of *Orius* spp. from the second observation at the IN2 site (0.3 vs. 3.7 per plot) and higher abundance of *Orius* spp. from the fifth observation at the PA site (14.0 vs. 7.3 per plot). The mean abundance values for MON 87427 were within the reference range for all differences detected with the exception of the difference detected for ladybird beetles at the IN2 site, *Nabis* spp. from the PA site, and macro-parasitic Hymenoptera from the IN2 site at the fourth and fifth observation times; however, the differences observed for ladybird beetles and *Nabis* spp. were not consistent across observation times or across sites, and the differences observed for macro-parasitic Hymenoptera were not observed at other sites. These results support the conclusion that the detected differences in arthropod abundance were not indicative of a consistent response associated with the tissue-selective glyphosate tolerance in MON 87427 and, therefore, are not considered biologically meaningful in terms of plant pest potential or an adverse environmental impact of MON 87427 compared to the conventional control. Thus, there was not a consistently observed response associated with MON 87427, and the detected differences in beneficial arthropod abundance are not considered biologically

meaningful in terms of plant pest potential or an adverse environmental impact of MON 87427 compared to conventional maize.

The results of the field evaluations demonstrated that the tissue-selective glyphosate tolerance in MON 87427 did not alter the assessed environmental interactions of MON 87427 compared to conventional maize. The lack of significant biological differences in plant responses to abiotic stress, disease damage, arthropod damage, and pest and beneficial arthropod abundance support the conclusion that the tissue-selective glyphosate tolerance in MON 87427 is unlikely to result in increased plant pest potential or an altered environmental impact from MON 87427 compared to conventional maize.

Table VII-5. Quantitative Assessment of Corn Earworm and European Corn Borer Damage across Four[1] Locations during 2008

Pest	Damage Assessment	Mean (S.E.)		Reference Range[2]
		MON 87427	Control	
Corn earworm	Mean of 10 ears (0 – 9 rating scale)	0.76 (0.31)	0.98 (0.33)	0.00 - 3.03
European corn borer	Number of larva/10 plants	0.07 (0.03)	0.08 (0.03)	0.00 - 0.27
	Number of stalk entry/ exit holes of 10 plants	0.73 (0.19)	0.58 (0.14)	0.13 - 1.47
	Number of stalk galleries per plant of 10 plants	0.60 (0.12)	0.59 (0.14)	0.17 - 1.67
	Stalk gallery length (in.) per plant of plants with at least one gallery	1.63 (0.31)	1.67 (0.28)	0.65 - 3.83

No statistical differences were detected between MON 87427 and the conventional control ($p > 0.05$).
Note: The experimental design was a randomized complete block with three replications at each site; S.E. = standard error; N = 12.
[1] Combined site analysis included only the four sites at which quantitative arthropod damage were collected. Sites included Stark Co., IL (IL1); Boone Co., IN (IN2); Butler Co., MO (MO); and Berks Co., PA (PA).
[2]Reference range was calculated from the minimum and maximum mean values from among the 14 unique reference hybrids.

Table VII-6. Summary of Qualitative and Quantitative Environmental Interactions Assessments during 2008

Assessments	Number of sites	Number of observations across sites	Number of observations where no differences were detected	Detected Differences[1]				
				Variable Name	Site	Observation Number	Within reference range?	Consistently detected across observations or sites?
Qualitative								
Plant response to abiotic stress	16	172	172	-	-	-	-	-
Disease damage	16	210	210	-	-	-	-	-
Arthropod damage	16	167	167	-	-	-	-	-
Quantitative								
Pest arthropod abundance	4	98	94[2]	Corn flea beetle	PA	1	Yes	No
				Corn flea beetle	PA	5	No	No
				Grasshopper	MO	4	No	No
				Leafhopper	PA	2	Yes	No
Beneficial arthropod abundance	4	105	97	Araneae	PA	4	Yes	No
				Ladybird beetle	IL1	3	Yes	No
				Ladybird beetle	IN2	3	No	No
				Macro-parasitic Hymenoptera	IN2	4	No	No
				Macro-parasitic Hymenoptera	IN2	5	No	No
				Nabis spp.	PA	1	No	No
				Orius spp.	IN2	2	Yes	No
				Orius spp.	PA	5	Yes	No

[1] For qualitative assessments, MON 87427 was considered different from the conventional control if the severity of injury to MON 87427 did not overlap with the severity of injury to the conventional control across all three replications. Quantitative assessments were statistically analyzed at the 5% level of significance.
[2] Four additional pest arthropods were observed, but statistical comparisons were not made between MON 87427 and the conventional control due to lack of variability in the data.

VII.C.3. Pollen Characteristics

APHIS considers the potential for gene flow and introgression of the tissue-selective glyphosate tolerance in MON 87427 into other maize plants and wild relatives to determine the potential for increased weedy or invasive characteristics of the receiving species. Pollen morphology and viability information are pertinent to this assessment and therefore were assessed on MON 87427. In addition, morphological characterization of pollen produced by MON 87427 and the conventional control is relevant to the plant pest risk assessment because it adds to the detailed description of the phenotype of MON 87427 compared to the conventional control.

The purpose of this evaluation was to assess the morphology and viability of pollen collected from non-glyphosate treated plants of MON 87427 compared to the conventional control. Pollen was collected from MON 87427, the conventional control, and four commercial references grown under similar agronomic conditions in a field trial in Missouri. The trial was arranged in a randomized complete block design with three replications. Pollen was collected from three non-systematically selected plants per plot and stained with Alexander's stain (Alexander, 1980). Pollen viability was evaluated from at least 100 pollen grains of each sample and pollen grain diameter was measured for ten representative viable pollen grains per replication. General shape and form (morphology) of the pollen was observed for one replication of MON 87427, the conventional control, and the reference hybrids (see Appendix H).

No statistically significant differences were detected between MON 87427 and the conventional control for pollen diameter (Table VII-7). Based on visual observations made during the experiment there were no differences observed in general pollen morphology between MON 87427 and the conventional control. MON 87427 had a statistically significant higher percent viability than the conventional control (99.7 vs. 98.9%). Although the percent viability of MON 87427 pollen was slightly greater than the reference range, both MON 87427 and the conventional control demonstrated a high level of pollen viability, and the difference was small in magnitude (0.8%). The small difference in pollen viability is unlikely to be biologically meaningful, and therefore, does not represent altered pollen viability in MON 87427 compared to the conventional control.

These results demonstrate that the tissue-selective glyphosate tolerance in MON 87427 did not alter the overall morphology or viability of MON 87427 pollen compared to the conventional control. The pollen characterization data contribute to the detailed phenotypic description of MON 87427 compared to the conventional control. The results support an overall conclusion that MON 87427 is not fundamentally different than conventional maize and no more weedy or likely to pose a plant pest risk or have a significant environmental impact than conventional maize.

Table VII-7. Pollen Characteristics

	Mean (S.E.)		Reference Range[1]	
Characteristic	**MON 87427**	**Control**	**Min**	**Max**
Viability (%)	99.7* (0.0)	98.9 (0.3)	99.2	99.6
Diameter (μm)	89.9 (1.2)	90.8 (1.6)	87.2	91.5

Note: The experimental design was a randomized complete block with three replications at each site; S.E. = standard error; N = 12.

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

[1] Reference range is the minimum and maximum mean value observed among the four reference maize hybrids.

VII.D. Conclusions for Phenotypic, Agronomic, and Environmental Interactions Evaluation

An extensive and robust set of information and data were assessed to determine whether the tissue-selective glyphosate tolerance in MON 87427 altered the plant pest potential of MON 87427 compared to conventional maize. Phenotypic, agronomic, and environmental interaction characteristics of MON 87427 were evaluated and compared to those of the conventional control and considered within the variation among commercial references. These assessments included 14 plant growth and development characteristics; five seed dormancy and germination characteristics evaluated under seven different temperature regimes; two pollen characteristics; observations of abiotic stress response, disease damage, arthropod damage and arthropod abundance. Results from the phenotypic, agronomic, and environmental interactions assessment indicate that MON 87427 does not possess weedy characteristics, increased susceptibility or tolerance to specific abiotic stress, diseases, or arthropods, or characteristics that would confer a plant pest risk or significant environmental impact compared to conventional maize.

VIII. U.S. AGRONOMIC PRACTICES

VIII.A. Introduction

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 seed[2] 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.

VIII.B. Overview of U.S. Maize Production

VIII.B.1. Maize Grain Production

The U.S., China, European Union, Brazil, and Mexico are the top five producers of maize (FAOSTAT, 2009). Globally in 2008, approximately 5.8 million metric tonnes (MMT) of maize seed were used to produce more than 820 MMT of grain harvested from more than 160 million hectares (FAOSTAT, 2009). For this same year, approximately 0.59 MMT of hybrid maize seed were used in the U.S. to produce approximately 37% (307 MMT) of the world's maize crop (FAOSTAT, 2009). Maize is grown in the U.S. almost totally from hybrid seed, and is the largest crop based on acreage planted and net crop value, accounting for >90% of total value and production of feed grains (USDA-ERS, 2009b). The U.S. is a major player in the world maize trade market, with approximately 20 percent of the maize crop exported to other countries (USDA-ERS, 2009b).

The U.S. acreage for cultivating maize has varied. Since 1900, maize acreage ranged from a high of 113 million acres in 1932 to a low of 60.2 million acres in 1983. In the past 10 years (2000-2009), total annual maize acreage planted varied from approximately

[2] F1 hybrid maize seed is produced by a homozygous inbred (the female parent) through pollination by a different homozygous inbred (the male parent). The divergence between the parent lines promotes improved growth and yield characteristics through the phenomenon of heterosis ("hybrid vigor"), whilst the homozygosity of the parent lines ensures a phenotypically uniform F1 generation.

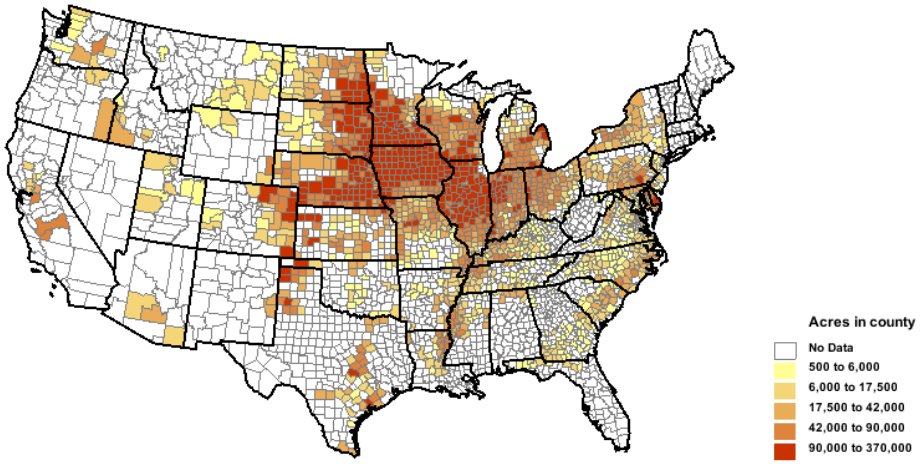
75 to 93 million acres. Total annual production during this period ranged from about 9 to 13 billion bushels, and total annual value fluctuated from about 18 to 54 billion dollars depending on production output and commodity prices (Table VIII-1) (USDA-NASS, 2010).

Hybrid maize was planted in almost every state in the continental U.S. in 2008 (Figure VIII-1), with the two largest maize producing regions being the Midwest and the Great Plains (USDA-ERS, 2009a). The Midwest region is comprised of eight states: Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Ohio and Wisconsin. The Great Plains includes portions of ten states: Colorado, Kansas, Montana, Nebraska, New Mexico, North Dakota, Oklahoma, South Dakota, Texas and Wyoming (Riebsame, 1990). The Midwest and Great Plains regions contributed 64% and 27%, respectively, of the national maize production total for 2009 (Table VIII-2) (USDA-NASS, 2010).

Table VIII-1. Maize Production in the U.S., 2000-2009

Year	Acres Planted (×1000)	Acres Harvested (×1000)	Average Yield (bushels/acre)	Total Production (×1000 bushels)	Value (billions $)
2009	86,482	79,590	164.7	13,110,062	48.59
2008	85,982	78,570	153.9	12,091,648	49.31
2007	93,527	86,520	150.7	13,037,875	54.67
2006	78,327	70,638	149.1	10,531,123	32.08
2005	81,779	75,117	147.9	11,112,187	22.19
2004	80,929	73,631	160.3	11,805,581	24.38
2003	78,603	70,944	142.2	10,087,292	24.48
2002	78,894	69,330	129.3	8,966,787	20.88
2001	75,702	68,768	138.2	9,502,580	18.88
2000	79,551	72,440	136.9	9,915,051	18.50

Source: (USDA-NASS, 2010)



Source: (USDA-ERS, 2009a)
Figure VIII-1. U.S. Maize Production by County in 2008

Table VIII-2. U.S. Maize Production by Region and State in 2009

Region/State	Acres Planted[1] (×1000)	Acres Harvested[1] (×1000)	Average Yield[1] (bu/acre)	Total Production[1] (×1000 bu)	Value[1] (billions $)
Midwest Region					
Illinois	12,000	11,800	174	2,053,200	7.54
Indiana	5,600	5,460	171	933,660	3.50
Iowa	13,700	13,400	182	2,438,800	9.15
Michigan	2,350	2,090	148	309,320	1.12
Minnesota	7,600	7,150	174	1,244,100	4.63
Missouri	3,000	2,920	153	446,760	1.63
Ohio	3,350	3,140	174	546,360	2.02
Wisconsin	3,850	2,930	153	448,290	1.66
Region Totals	**51,450**	**48,890**	**166**	**8,420,490**	**31.25**
Northeast Region					
Connecticut	26				
Maine	28				
Massachusetts	17				
New Hampshire	15				
New York	1,070	595	134	79,730	0.31
Pennsylvania	1,350	920	143	131,560	0.51
Rhode Island	2				
Vermont	91				
Region Totals	**2,599**	**1,515**	**139**	**211,290**	**0.82**
Mid-Atlantic Region					
Delaware	170	163	145	23,635	0.09
Maryland	470	425	145	61,625	0.25
New Jersey	80	70	143	10,010	0.03
Virginia	480	330	131	43,230	0.16
West Virginia	47	30	126	3,780	0.01
Region Totals	**1,247**	**1,018**	**138**	**142,280**	**0.54**

Table VIII-2 (continued). U.S. Maize Production by Region and State in 2009

Region/State	Acres Planted[1] (×1000)	Acres Harvested[1] (×1000)	Average Yield[1] (bu/acre)	Total Production[1] (×1000 bu)	Value[1] (billions $)
Southeast Region					
Alabama	280	250	108	27,000	0.11
Arkansas	430	410	148	60,680	0.23
Florida	70	37	100	3,700	0.01
Georgia	420	370	140	51,800	0.19
Kentucky	1,220	1,150	165	189,750	0.71
Louisiana	630	610	132	80,520	0.29
Mississippi	730	695	126	87,570	0.32
North Carolina	870	800	117	93,600	0.36
South Carolina	335	320	111	35,520	0.14
Tennessee	670	590	148	87,320	0.32
Region Totals	**5,655**	**5,232**	**130**	**717,460**	**2.68**
Great Plains Region					
Colorado	1,100	990	153	151,470	0.58
Kansas	4,100	3,860	155	598,300	2.15
Montana	72	26	152	3,952	0.02
Nebraska	9,150	8,850	178	1,575,300	5.83
New Mexico	130	50	185	9,250	0.04
North Dakota	1,950	1,740	115	200,100	0.71
South Dakota	5,000	4,680	151	706,680	2.44
Oklahoma	390	320	105	33,600	0.13
Texas	2,350	1,960	130	254,800	1.03
Wyoming	90	45	140	6,300	0.03
Region Totals	**24,332**	**22,521**	**146**	**3,539,752**	**12.96**
Northwest Region					
Idaho	300	80	180	14,400	0.06
Oregon	60	32	215	6,880	0.03
Washington	170	105	215	22,575	0.10
Region Totals	**530**	**217**	**203**	**43,855**	**0.19**

[1]Source: (USDA-NASS, 2010)

VIII.B.2. Maize Seed Production

Introduction – Use of hybrid maize seed dates back to the early 1920s when there were approximately 1000 open pollinated inbred lines available in the U.S. (Troyer, 1999). As described in Wych (1988), "Copper Cross" was the first hybrid developed for the U.S. maize belt, and its launch in 1924 was limited to a total of 15 bushels of hybrid maize seed produced on a one-acre production plot. It was under the drought conditions of 1934 and 1936 that farmers noticed the improved performance of hybrid maize seed over the open pollinated inbred varieties, and began to accept and eventually demand access to new hybrids developed for their growing regions (Wych, 1988).

Early maize inbreds were weak and low yielding, which made it difficult and expensive to produce large quantities of hybrid maize seed. The cost-effective production of hybrid maize seed with these early inbreds was achieved through double cross hybrids. Pair wise crossing of four inbreds were made by separately making two single crosses: A × B and C × D. These higher yielding single cross hybrids were then crossed to produce a double cross hybrid: (A × B) × (C × D). The double cross hybrid, although perhaps lower yielding than the best single cross hybrids, yielded much better than the best open pollinated maize varieties. The production of double cross hybrid maize seed resulted in lower seed prices and better farmer affordability (Duvick, 2001).

Initially, the adoption of hybrid maize seed was slow, and in 1933 only 1% of the maize grown in the U.S. was produced from hybrid seed. However, by the 1960s the newest inbreds were so high-yielding that it became practical to use them as seed parents for the cost-effective production of single cross hybrid maize seed, and virtually all maize plantings in the U.S. now use single cross hybrid seed (Duvick, 2001). Although nearly all of the maize planted today in the U.S. is hybrid maize, growers in some other regions of the world have not adopted the use of hybrids as widely. Recently, 29%, 56% and 57% of the maize planted in Brazil, India and Romania, respectively, was open pollinated inbred varieties (Edgerton, 2009).

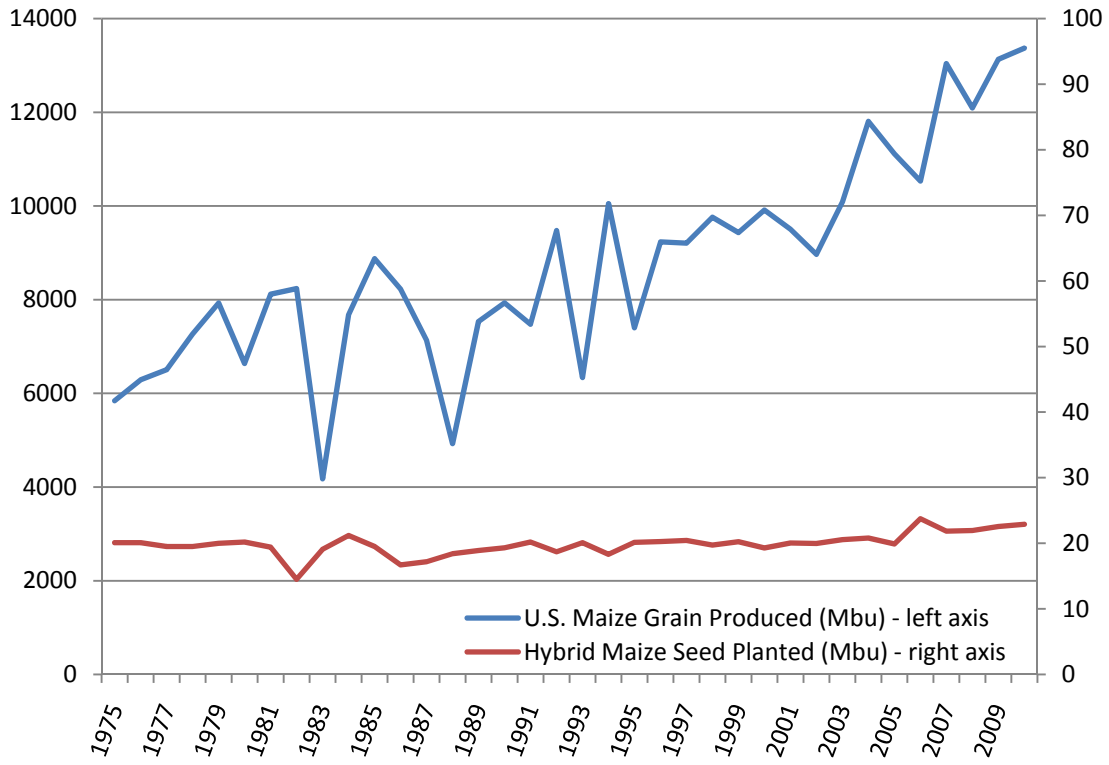
Hybrids are not entirely responsible for the advances in maize yields. Starting around the 1950s, the widespread use of synthetic nitrogen fertilizers, herbicides, and more efficient planting and harvesting machinery also contributed to higher yields. Surprisingly, improvements in hybrid vigor have not contributed to higher yields (Duvick, 2001). Experiments have shown that hybrid vigor, calculated as the difference in yield between a single cross hybrid and the mean of its two inbred parents, is unchanged over the years (Duvick, 2001). The yields of the inbred lines have increased at almost the same rate as hybrid yield (Duvick, 2001). Yield gains appear to have come primarily from genetic improvements in tolerance to stresses that include disease and insects, dense planting, drought or low soil fertility (Duvick, 2001).

Commercial hybrid maize seed production is a labor intensive process. Single cross hybrid maize seed production involves planting male and female parent inbreds in separate rows or blocks in an isolated field. The female parent inbred is prevented from shedding pollen to ensure only pollination by the male parent inbred that is usually destroyed by mechanical means following pollination to prevent seed mixing during

harvest. Ears that are produced from the cross-pollinated female parent inbred are harvested, processed, and the seed sold to farmers for planting as hybrid maize seed.

Maintaining an adequate supply of the parental inbred lines is vital to producing an adequate supply of hybrid maize seed. Often referred to as foundation seed, parental inbred lines are produced and maintained under strict isolation in the production field to preserve the identity and integrity of the genetics within each inbred. Quality control checks performed during the production of inbreds include visual inspections of the plants grown in isolation, and the use of molecular tools to verify the genetics of each inbred line (Hoisington, et al., 1998).

Hybrid maize seed is produced in the U.S. on approximately 0.5 million acres (Jugenheimer, 1976), an area that is not expected to change with the introduction of MON 87427. Over the last 35 years, the volume of hybrid maize seed planted in the U.S. has changed very little, with 20.10 million bushels (MBu) planted in 1975 and 22.55 MBu planted in 2009 (USDA-ERS, 2010). Grain yields have increased significantly over this same period (Figure VIII-2).



Source: (USDA-ERS, 2010)

Figure VIII-2. Hybrid maize seed Planted and Grain Produced in the U.S. from 1975-2009.

A number of factors must be considered in hybrid maize seed production, including: 1) forecasting the quantity of specific hybrid maize seed that will be needed at least a year in advance; 2) selection of a production area that mitigates risks and maximizes the yield of hybrid maize seed; and 3) agronomic practices. Key considerations and practices for producing hybrid maize seed are described in the following sections, which may differ from the cultivation of commercial maize.

Production Area and Input Considerations – Seed production begins with the selection of a suitable growing area. Factors such as temperature, rainfall, day length, and soil nutrient status are important because seed yields may be sensitive to unfavorable conditions during particular periods. For example, extremely high temperatures and dry conditions can affect the timing of silk emergence and growth, pollen shed and pollen viability resulting in poor seed formation and yield. To reduce costs of transportation to distribution points, the production area should be reasonably accessible to the final market for the seed, except in those areas that are unsuitable for producing high-quality seed. Distant regions may also be chosen for growing conditions that allow seed production during the off-season.

Maize is a warm-season crop. Like commercial maize for grain production, the inbred lines used in seed production grow and yield best with moderate temperatures (70-90°F) and a plentiful supply of water during the growing season (McDonald and Copeland, 1997). The ideal daytime temperature is about 80-86°F, or higher provided there is adequate water (Hoeft, et al., 2000a). Climatic conditions in the U.S. maize belt are well suited for maize seed production and include the major maize belt states of Nebraska, Iowa, Illinois, and Indiana (McDonald and Copeland, 1997). Only limited quantities of maize seed are produced in the southern states due to high temperatures during pollination, inadequate rainfall during the growing season, and a higher incidence of insects and diseases (Chad Peters, Monsanto Global Operations, personal communication, 2010). Maize seed is also not produced in the most northern portions of the maize belt due to colder temperatures where the mean number of growing degree days accumulated during the season may not be sufficient for maize to reach maturity prior to frost (Hoeft et al., 2000a). Hybrid maize seed is typically harvested prior to damaging frost that can reduce seed viability (Wych, 1988).

Planting conditions for hybrid maize seed production are generally the same as for the cultivation of commercial maize. A minimum soil temperature of 50°F is recommended for planting maize to achieve good germination and stands. Delayed emergence from colder soil conditions can result in damage from microorganisms and insects. Foundation seed will generally not be among the first maize planted mainly because colder soil temperatures may result in non-uniform emergence of inbred lines and a risk of frost damage. Medium-textured, well-drained soils with high water-holding capacities are ideal for commercial maize and maize seed production (Hoeft et al., 2000a; Hoeft, et al., 2000b; a). Sandy soils are less desirable because of their low water-holding capacities, but are suitable if adequate rainfall or irrigation is available during the growing season. Fields with non-uniform soil conditions may result in variable growth and variable timing of pollination and silk emergence (Chad Peters, Monsanto Global Operations, personal

communication, 2010). Minimum tillage and, in some locations, conventional tillage are used for seed production (McDonald and Copeland, 1997).

Nutrient requirements for hybrid maize seed production are generally the same as for the cultivation of commercial maize. Nutrient management programs include the addition of nitrogen, phosphorus and potassium fertilizer to optimize maize yields and profitability. Soil tests are used to measure pH and the levels of phosphorus and potassium. Soil pH affects nutrient availability and should be maintained at or above 6.0 for maximum maize yields (Hoeft et al., 2000b). Supplemental nitrogen requirements for the crop year may be based on soil tests or calculated from target yields (Hoeft et al., 2000b). Deficiencies in secondary nutrients (calcium, magnesium, and sulfur) or micronutrients (boron, chloride, copper, iron, manganese, molybdenum, and zinc) are uncommon but can result in yield reduction unless corrected with supplemental nutrient applications (Hoeft et al., 2000b).

Control of Weeds, Diseases, and Insects – Control of weeds, insects, and diseases within the hybrid maize seed production field is an integral and necessary part of seed production (Wych, 1988). Seed growers rely heavily on herbicides for effective weed control, since inbred maize lines do not compete effectively with weeds. In addition, insecticides are used to control above and below ground pests, and protect against insect damage to stands, the growing plants, and the female parent inbred ears. Seed companies practice integrated pest management principles and evaluate seed fields to determine if and when insecticide application is justified. Fungicides are also an important component of hybrid maize seed production and are used to protect susceptible parent lines from damaging fungal diseases. Chemical protection is often needed when disease resistance is not adequate in the parent line. Spray applications can effectively reduce damage from foliar disease in susceptible inbred lines, and seed treatments are widely used to prevent seed and seedling diseases.

Plant Density – The optimum seeding rate and subsequent plant population in commercial maize is specific to each hybrid. The same is true for inbred lines in hybrid seed production (Chad Peters, Monsanto Global Operations, personal communication, 2010). Seeding rates for male and female parent inbreds planted in 30-inch row spacing are generally the same and are specified by seed companies (Chad Peters, Monsanto Global Operations, personal communication, 2010). Male and female parent inbreds may be planted at different populations, though, with the female parent inbred population typically being higher than the male parent inbred population. There has been considerable interest in recent years in narrower row spacing in maize seed production (Chad Peters, Monsanto Global Operations, personal communication, 2010). Narrowing the row spacing from 30 inches can result in better distribution and spacing of maize plants for greater light penetration and less evaporation of water from the soil, to provide higher plant populations with no yield loss (Abendroth and Elmore, 2006).

Planting Patterns of Production Plots – In maize hybrid seed production, the male and female parent inbreds are physically separated to control pollination within the field. The male inbred parent can be double planted to extend the pollen shedding period, so that the timing of peak pollen shedding coincides with the timing of peak silk exposure in the

female parent inbred. Planting patterns in seed production fields include 4:1 (four rows of female parent inbred to one row of male parent inbred), 4:2, 4:1:2:1, 6:2, and solid female parent inbred with interplanted male parent inbred. The female parent inbred is never more than two rows from the male parent inbred in the first three patterns. One-half of the female parent inbred rows are adjacent to a male parent inbred in the 4:1 and 4:2 patterns, and two-thirds of the female parent inbred rows are adjacent to a male parent inbred in the 4:1:2:1 pattern. The 6:2 pattern has been used for production of double-cross hybrids, and for the production of single cross hybrids with male parent inbreds that shed an abundant supply of pollen. A planting pattern where every other or every fourth between-row space of a solid planted female parent inbred is interplanted with the male parent inbred fully utilizes the land area for female parent inbred production and achieves closer placement of the male and female parent inbreds (Craig, 1977; Wych, 1988).

Isolation of Production Plots – Hybrid maize seed production plots are isolated from neighboring maize fields to avoid inadvertent cross-pollination during the flowering stage by wind-borne pollen. Physical isolation is used because temporal isolation is difficult to manage and would require the flowering time of the male and female parent inbreds to occur in synchrony, yet independently from the flowering times of other nearby maize. The isolation distance from other maize is regulated by seed certification standards, and is typically at least 660 feet from other maize (AOSCA, 2009). Planting additional male parent inbred border rows around the perimeter of the seed production plots increases desirable pollen shed from the male parent inbred during silking of the female parent inbred, and reduces the potential for contamination from external pollen sources. Official seed certification regulations often allow isolation distances between seed production fields to be reduced as the number of male parent inbred border rows increases (Agrawal, et al., 1998).

Parent Delay Techniques – Various parent delay techniques can be used to synchronize the flowering of male and female parent inbreds that would otherwise occur at different times. Split-date planting is the most common of these techniques, where the male and female parent inbreds are planted at different times, based on a combination of the number of days, growth stages, and heat units accumulated from the date when the first parent was planted (Wych, 1988). In addition, the pollen-shedding period may be extended by planting the male parent inbred at two or more dates. Plantings are timed so that peak pollen shed coincides with maximum female parent inbred silk exposure.

Special techniques are also available to manipulate the flowering dates of one or both of the seed parents (Wych, 1988). These techniques can delay flowering or extend the duration of flowering by days. The timing of flowering of the second-planted seed parent may be advanced by varying planting depth or fertilization rate. The flowering date of the first-planted parent can be delayed using techniques that involve burning off the above-ground leaves and stalk of young plants, or cutting off the tops of the plants. These techniques are rarely used to delay the female parent inbred because they typically result in reduced seed yield.

Methods of Pollen Control – Pollen control refers to practices that ensure complete pollination of female parent inbreds by male parent inbreds to produce hybrid maize seed. Pollen control in hybrid maize seed production is critical for producing hybrid maize seed with high purity of the background genetics. A number of methods have been described, including the two most common production methods of detasseling and cytoplasmic male sterility (Craig, 1977; Wych, 1988). Chemical hybridization agents (male gametocides) have also been developed for pollen control, but their use is severely limited due to off-target effects (Loussaert, 2004).

1. Detasseling

Detasseling is the most widely used method of pollen control in the production of hybrid maize seed. Tassels are physically removed from female parent inbred maize before undergoing pollen shed or silk emergence. Removal of all of the tassels from the female parent inbred avoids self-fertilization of silks by the pollen that could have been produced from this genetic line. Instead, fertilization of the detasseled female parent inbred is achieved by pollination from a male parent inbred with different background genetics that is grown in close proximity. The window for detasseling averages 3-4 days, and occurs between tassel emergence from the leaf sheath and the initiation of pollen shedding (Hoeft et al., 2000a).

Pollen shed usually occurs in maize over a 5-8 day period with the peak production on about the third day (Hoeft et al., 2000a). Pollen shed is not always a continuous process, and can stop and restart depending on climatic conditions or when additional pollen has matured (Hoeft et al., 2000a). As a result, the window for detasseling that averages 3-4 days prior to the initiation of pollen shed is a critical step in maize seed production that, once begun, must be performed on a regular basis, regardless of weather. Removal of the tassel from the female parent inbred in seed production fields is accomplished by a combination of mechanical and manual detasseling methods (Wych, 1988). Mechanical detasseling methods came into widespread use in the 1970s as a way to better control rising production costs that resulted from increasing labor costs and a declining labor supply (Craig, 1977).

Mechanical detasseling machines either cut or pull the tassels from the maize in all the female parent inbred rows. Mechanical cutters use a rotating blade or knife to remove the top of the maize plant and tassel. Mechanical pullers are complementary to cutters, and use two counter-rotating wheels or rollers to grasp and remove the tassel and upper leaves. Mechanical detasseling is delayed as long as possible before silk emergence, to permit maximum exsertion of tassels and enable their removal with minimum leaf damage. Best results are achieved in a uniform seed field in which the tassels are well exserted ahead of pollen shedding. As conditions become less favorable, the percentage of tassels removed per pass will decrease and leaf damage will increase. Removal of the entire tassel can result in the removal of too much leaf tissue, and reduce maize seed yields by as much as 10% (McDonald and Copeland, 1997). In addition, the tassels that have been removed can become lodged in the leaf canopy and shed pollen, resulting in unwanted self-pollination. This complication is resolved by hand detasseling crews.

Crews also detassel the maize that was not completely detasseled with the mechanical methods.

Although detasseling is relatively straightforward to accomplish, the production of hybrid maize seed is expensive and labor-intensive. It employs tens of thousands of teenage, migrant, and other agricultural workers each year to hand detassel maize in the U.S. The large manual labor force is needed for only a relatively short period of time that may last from less than a week to many weeks depending upon the volume of production and the range in female parent inbred maturity dates planted within a seed production area. A detasseling operation is at risk from weather such as heavy rain or windstorms that can lodge or tangle the female parent inbreds just as the tassels begin to emerge, making it difficult to walk or drive through the field. Extreme heat or drought during the onset of flowering can delay the emergence of tassels and silks. Seed fields need to be monitored and inspected closely during the detasseling period, as even a slight mistake can have considerable economic consequences. The labor force must be well trained, closely supervised, and effectively managed. This is complicated because of the reliance on temporary seasonal workers. Increasing wage rates and changing population demographics (labor supply and its distribution) are two factors that pose challenges to the industry. Liability and worker safety issues associated with employing temporary manual labor are also important considerations.

Field inspections are conducted throughout the pollination and detasseling period to measure the progress of the male pollination and female silk emergence, to ensure that female parent inbreds are not shedding pollen or self-pollinating, and to evaluate the effectiveness of the detasseling operations. Genetic purity of intended crosses is dependent on compliance with quality standards that certifying agencies have established when the female parent inbred has 5% receptive silks (silks emerged and turgid), which includes a limit of 1% shedding tassels in the female parent inbred at any one inspection and a total of 2% shedding tassels for three inspections at different dates, plus a limit of 0.1% male off-types at any inspection (AOSCA, 2009)). Tassels are counted as shedding when more than 2 inches of the central spike and/or side branches have emerged and have shedding anthers (AOSCA, 2009).

2. Cytoplasmic Male Sterility

Cytoplasmic male sterility (CMS) is a genetic method that was widely adopted in the U.S. in the 1950s and 1960s as a means to eliminate pollen from the female parent inbred without the need of manual or mechanical detasseling (Craig, 1977; Ullstrup, 1972). The genetics by which CMS functions is based on the presence of mitochondrial DNA genes that produce pollen sterility when dominant fertility restoration genes are absent in the nuclear DNA (Schnable and Wise, 1998). Pollen fertility is restored in the F1 hybrid maize seed produced from crossing this female parent inbred with a male parent inbred that possesses the dominant fertility restoration genes in its nuclear DNA.

A number of CMS systems have been identified to facilitate the crossing of two inbreds, and include S-*cms*, C-*cms* and T-*cms*. With the T-*cms* system, detasseling is eliminated through the use of a female parent inbred that is completely male sterile. Unfortunately,

this genotype also carries a hyper-susceptibility to *Helminthosporium maydis* race T that resulted in a virulent epidemic from southern maize leaf blight in U.S. maize in 1969-1970 (Pring and Lonsdale, 1989; Ullstrup, 1972). Continued use of this genotype was problematic because the male sterility trait was inseparable from *H. maydis* disease susceptibility (Levings and Siedow, 1992).

The C and S cytoplasms are not linked to disease susceptibility (Craig, 1977), and became important in the late 1970s as a cost-competitive and satisfactory technique for producing hybrid maize seed (Wych, 1988). Both the production of hybrid seed using CMS, and the cultivation of field maize from this hybrid maize seed are complicated. For example, C and S cytoplasms in certain genetic backgrounds result in only partial male sterility and still require some detasseling during the production of hybrid maize seed. Furthermore, the hybrid seed produced using CMS is typically blended with hybrid seed of the same genetic background that was produced without CMS, to ensure adequate pollination of the commercial maize grown from this hybrid seed.

Harvesting and Conditioning of Hybrid Maize Seed – Maize harvested for grain is almost entirely harvested and shelled with combines in the field. In contrast, hybrid maize seed is almost entirely harvested, and then dried, on the ear to minimize the amount of mechanical damage to the seed. The black layer that forms at the base of the seed at physiological maturity is an indication that maximum dry weight has been reached, and generally occurs when the seed has 30-38% moisture content (McDonald and Copeland, 1997). Freezing is a major concern to seed viability, and can be minimized by harvesting early when seed moisture content is high. This necessitates the need for artificial drying methods (McDonald and Copeland, 1997). The drying systems for maize seed are typically fan systems that force heated air through bins filled with maize seed on the ear. High-moisture seed is more sensitive to germination damage by heat than low-moisture seed, so the temperature is generally held below 95°F until 20% seed moisture content is achieved, and then the temperature can be increased to a maximum of 115°F. Seed is typically dried to a moisture content of 12-13% which is suitable for subsequent shelling and conditioning operations (McDonald and Copeland, 1997).

Conditioning seed consists of three steps: 1) cleaning the seed to remove cob and kernel pieces, husks, silks, and other debris; 2) separating the seed into sizes and shapes based on width, thickness and length; and 3) treating the seed with an insecticide and/or fungicide (McDonald and Copeland, 1997).

Labeling and Certification Requirements – Standardized seed production practices are responsible for maintaining high quality seed stocks, an essential basis for U.S. agriculture. By the early 20th century, agronomists learned how to develop specific plant varieties with desirable traits. In the U.S., state agricultural experiment stations developed many seed varieties which were distributed to farmers for use. As seeds were saved by farmers and later sold to neighbors, the desirable traits of the varieties often were lost through random genetic changes and contamination with other crop and weed seeds. The value of seed quality (including genetic purity, vigor, weed seed presence,

seed borne diseases and inert materials such as dirt) was quickly identified as a major factor in crop yields. States developed seed laws and certification agencies to ensure that purchasers who received certified seed could be assured that the seed met established seed quality standards (Bradford, 2006). The federal government passed the U.S. Federal Seed Act of 1939 to recognize seed certification and official certifying agencies. Regulations first adopted in 1969 under the Federal Seed Act recognize land history, field isolation, and varietal purity standards for foundation, registered, and certified seed. Under international agreements such as the OECD scheme, the U.S. and other countries mutually recognize minimum seed quality standards (Bradford, 2006). The Association of Official Seed Certifying Agencies (AOSCA) represents state and private seed certification in the U.S., and includes international member countries in North and South America, as well as Australia and New Zealand.

Seed certification is based on varietal lineage, as well as quality production and processing standards. Seeds produced for sale to a crop grower (certified seeds) are a limited number of generations from a verified seed stock of the specified variety (Bradford, 2006). Breeder seed is generally produced under the strictest standards and under the supervision of the breeder. Breeder seed is used to produce foundation seed, which is used to produce registered seed, which is then used to produce certified seed that is sold for commercial planting (Bradford, 2006). In addition to documenting the pedigree of the seed, certification programs also monitor crop rotations, previous crops and weeds in the field, as well as isolation of the field from other plants of the same genus or species (Bradford, 2006). Inspectors walk the fields to note the occurrence of off-type plants, other crop plants, weeds, or disease. After seed harvesting and cleaning, the seed is later tested for germination capacity, and analyzed for the presence of seed of other varieties or other crops, weed seeds and inert matter to assure high quality before the seed bags are tagged as "certified" (Bradford, 2006). Within a seed crop, the main sources of off-types, or seed from another plant, result from "volunteers," or seed from crops grown in the field at an earlier date, pollen transfer and mixing that occurs during harvesting and handling (Bradford, 2006). Seed producers take steps, such as cleaning equipment, appropriate crop rotation and other stewardship measures, to minimize these factors.

Hybrid maize seed must meet state and federal seed standards and labeling requirements. AOSCA is dedicated to assisting companies in the production, identification, distribution and promotion of certified classes of seed and establishes minimum standards for quality and identity. Its goal is to standardize certification regulations and procedures internationally so companies compete with one set of standards. The association cooperates with the OECD and other international organizations to develop standards, regulations, procedures, and policies to expedite movement of seed and encourage international commerce in improved varieties. The AOSCA standards for maize seed are as follows: 98% pure seed (minimum), 2% inert matter (maximum), no weed seed, 0.5% other hybrids, 90% germination (minimum), and 14% moisture (maximum) (AOSCA, 2009). State seed certification standards vary slightly from state to state and can be more restrictive than the seed standards of AOSCA. Certification by the OECD is applied to hybrids that meet established conditions of identity, uniformity, and stability. OECD

certified hybrids have an added economic value and are published in official OECD lists. The OECD helps ensure the varietal identity and quality of seed by setting appropriate requirements and controls throughout production, processing and labeling. Certified seeds are produced and officially controlled according to common harmonized procedures. OECD certification provides official worldwide recognition of "quality-guaranteed" seed, facilitating international trade and contributing to removal of technical trade barriers.

Seed containing MON 87427 will be produced and marketed in accordance with OECD and AOSCA standards and the U.S. Federal Seed Act, and will have no adverse impact on current hybrid seed production practices.

VIII.C. Production Management Considerations

Tissue-selective expression of CP4 EPSPS protein in MON 87427 enables the formation of a male sterile phenotype for hybrid maize seed production, through tissue-selective tolerance to glyphosate. MON 87427 utilizes a specific promoter and intron combination to drive CP4 EPSPS protein expression in vegetative and female reproductive tissues, conferring tolerance to glyphosate in the leaves, stalk, and root tissues and tissues that develop into seed or grain and silks. This specific promoter and intron combination also results in limited or no production of CP4 EPSPS protein in two key male reproductive tissues: pollen microspores which develop into pollen grains, and tapetum cells that supply nutrients to the pollen. Thus, in MON 87427, male reproductive tissues critical for male gametophyte development are not tolerant to glyphosate. Application of glyphosate at approximate vegetative growth stages ranging from V8 through V13 when these tissues are rapidly developing, results in a male sterile phenotype through tissue-selective glyphosate tolerance, and eliminates or greatly reduces the need for manual and/or mechanical detasseling or the use of genetic mechanisms such as cytoplasmic male sterility for producing hybrid maize seed.

Only specifically timed applications of glyphosate will produce the male sterile phenotype through tissue-selective glyphosate tolerance, and enable specific cross pollinations to be made in maize. Glyphosate is a systemic herbicide that is readily translocated in plants to areas of high meristematic activity, following a typical source to sink distribution (Franz, et al., 1997a). Early tassel growth stages start at the approximate maize vegetative growth stage V9, therefore glyphosate applications made at approximately this time allow maximum translocation of glyphosate to the male reproductive tissues, and selectively cause cell death in only those cells that are not tolerant to glyphosate (i.e. tapetum and pollen cells). Glyphosate applications made during early vegetative stages, consistent with the application timing specified in the current Roundup agricultural product label for weed control purposes, do not affect pollen production of MON 87427 because the sensitive male reproductive tissues are not actively developing at that time.

Upon deregulation, MON 87427 will be commercialized as a stack with other commercial products. Hybrid maize seed carrying the introduced trait in MON 87427 will be produced from pollination of a MON 87427 female parent inbred with a male

parent inbred that has an approved trait with vegetative and reproductive tolerance to glyphosate. These two inbred lines are planted in patterns as previously described in hybrid maize seed production (VIII.B.2). Post-emergent weed control can be achieved in the entire production field through glyphosate treatment as directed on the Roundup agricultural product labels.

During the production of the hybrid maize seed for stacked products, the entire field will be sprayed with glyphosate at early tassel growth, which induces the male sterile phenotype through tissue-selective glyphosate tolerance in only the MON 87427 inbred. Since maize can be at different growth stages in a field due to environmental factors, glyphosate applications at the labeled rates are generally made twice during the approximate V8 to V13 growth stages to ensure complete suppression of pollen development. There is an approximate two-week window for suppressing pollen development with MON 87427 that is significantly longer that the average 3-4 day window for manual detasseling, and provides an advantage for producing high-quality, high-purity hybrid maize seed. Subsequent to these glyphosate treatments, pollination of the MON 87427 female parent inbred occurs through cross-pollination by the male parent inbred. The F1 hybrid maize seed is viable and carries both MON 87427 inherited from the female parent inbred, and another glyphosate-tolerance trait inherited from the male parent inbred. The resulting F1 hybrid is fully tolerant to glyphosate.

The production practices for producing hybrid maize seed using MON 87427 are generally similar to those using detasseling or CMS, except for the use of glyphosate at early tassel development timings to cause the male sterile phenotype through tissue-selective glyphosate tolerance. Further, the amount of glyphosate recommended for use with MON 87427 will not exceed the total amount of glyphosate already approved for in crop use with Roundup Ready corn 2 products. Cultivation practices for hybrid maize containing MON 87427 will not differ from other maize with glyphosate-tolerance that is already commercially available.

VIII.D. Weed Management

Weeds compete with maize for light, nutrients, and moisture resources, and can lead to reductions in yield (Knake, et al., 1990). Numerous studies have shown that weed control early in the growing season is necessary to reduce yield losses in maize. Some weeds can tolerate cold, wet conditions better than maize, and can gain an advantage prior to planting. Fields infested with perennial weeds present special problems for maize growers. Like annual weeds, most perennials can reproduce by seeds, but they also re-grow and spread vegetatively. Vegetative structures such as rhizomes propagate new shoots, usually soon after maize is planted. Unless effectively controlled, perennial weeds can quickly gain a season-long advantage over the maize crop.

Annual weed species such as giant foxtail (*Setaria* spp.), barnyardgrass (*Echinochloa crus-galli* (L.) *Beauv*.) and pigweed (*Amaranthus* spp.) can reduce maize yields by up to 13, 35 and 50%, respectively (Bosnic and Swanton, 1997; Fausey, et al., 1997; Gianessi, et al., 2002; Knake and Slife, 1965). In a study of mixed weed populations competing with maize, maize yields were reduced by up to 20% when the weed plants reached a

height of eight inches (Carey and Kells, 1995; Gianessi et al., 2002). A survey of Extension Service weed scientists solicited estimates of the percent of maize acreage infested with individual weed species by state or region, as well as the potential impact on maize yields if the species were left uncontrolled. In this survey, twelve annual broadleaf, nine annual grass, and seven perennial species were identified as troublesome weeds (Table VIII-3) (Gianessi et al., 2002). Estimates of yield loss ranged from a low of 15% due to wirestem muhly and sandburs to a high of 48% from burcucumber.

Until the early 1950s, tillage and cultivation practices were primarily used for weed control in maize, but they have been largely replaced by the use of herbicides. Herbicide use in maize became widespread by the end of the 1970s, and in 2005, herbicides were applied to 97% of the planted maize acreage (USDA-NASS, 2006). Atrazine continues to be the most widely applied herbicide with 66% of the planted acreage being treated at an average rate of 1.133 pounds per acre (USDA-NASS, 2006). Glyphosate isopropylamine salt was applied to 31% of planted acres, up from 19% in 2003, at an average rate of 0.963 pounds per acre (USDA-NASS, 2006). In terms of area applied, that was followed closely by S-metolachlor and acetochlor, at 23% of the planted maize acreage treated (USDA-NASS, 2006).

The introduction of biotechnology-derived herbicide-tolerant maize has offered the growers an alternative and effective solution for the control of weeds in maize and in 2009 approximately 68% of the total maize acreage in the U.S. was planted with hybrids possessing herbicide-tolerance traits (USDA-NASS, 2009).

Weed management practices in the production of hybrid maize seed using a system with MON 87427 are anticipated to be substantially the same as current hybrid maize seed production practices, with the added option of using glyphosate for early post-emergent weed control. Weed management practices in the cultivation of commercial maize with MON 87427 stacked with other already approved glyphosate-tolerant maize traits will also remain unchanged. Producers of hybrid maize seed and growers of commercial maize will be able to achieve the same high level of weed control as other biotechnology-derived herbicide tolerant maize hybrids. Additionally, because MON 87427 is agronomically and phenotypically equivalent to conventional maize as described in Section VII, it is not anticipated that MON 87427 will respond differently to commonly used herbicides, except glyphosate.

Table VIII-3. Common Weeds in Maize Production

Weed Species ***Latin name***	**Area Infested [1]**	**Acreage Infested (%)**	**Potential Yield Loss (%)**
Annuals			
Broadleaves			
Bur Cucumber *Sicyos angulatus*	PA/OH/TN/SE	5-10	48
Cocklebur *Xanthiums strumarium*	MW/NP/SE	20-60	33
Jimsonweed *Datura stramonium*	MW/CO	5-20	17
Kochia *Kochia scopari*	NP/NW	10-70	33
Lambsquarters *Chenopodium album*	MW/SE/NE/CA	15-80	33
Morningglory *Ipomoea purpurea*	MW/SE/SP	20-75	33
Nightshade *Solanum nigrum*	MW/NP/CA	25-50	26
Pigweeds/Waterhemp *Amaranthus* spp.	US	30-90	36
Ragweed, Common *Ambrosia artemisiifolia* L.	MW/SE/NE	20-70	30
Ragweed, Giant *Ambrosia trifida*	MW/NP	10-45	28
Smartweeds *Polygonum* spp.	MW/SD/NE/SE	30-70	22
Velvetleaf *Abutilon theophrasti*	MW/NE/NP	25-70	28
Grasses			
Barnyardgrass *Echinochloa crus-galli* (L.) *Beauv*.	SP/NW/CA	80-90	23
Bermudagrass *Cynodon dactylon*	MD/SE/UT/CA	10-20	47
Crabgrass spp. *Digitaria* spp.	MW/SE/NE	20-80	29
Cupgrass, Woolly *Eriochloa villosa*	IA/WI	15-20	29
Foxtail spp. *Setaria* spp.	MW/NE/NP	50-90	31
Millet, Wild-Proso *Panicum miliaceum*	UT/WY/CO/ID	15-40	31

Weed Species ***Latin name***	**Area Infested [1]**	**Acreage Infested (%)**	**Potential Yield Loss (%)**
Panicum, Fall *Panicum dichotomiflorum*	MW/SE/NE/NP	15-80	30
Sandburs *Cenchrus* spp.	NP/UT/WY	5-30	15
Shattercane *Sorghum bicolor*	MW/SP	5-40	33
Perennials			
Bindweed, Field *Convolvulus arvensis*	ND/SW/CA	40-80	18
Dogbane, Hemp *Apocynum cannabinum* L.	IL/MO	2-20	21
Johnsongrass *Sorghum halepense*	MW/SE/SW/CA	20-60	45
Muhly, Wirestem *Muhlenbergia frondosa*	PA	2	15
Nutsedge, Yellow *Cyperus esculentus*	MW/SE/NE/NP/CA	10-70	21
Quackgrass *Elytrigia repens*	MW/NE/UT	10-70	27
Thistle, Canada *Cirsium arvense*	NE/MW/NP/CO	5-25	26

Source: (Gianessi et al., 2002)

[1] **Regions**

US:	United States	**States**			
MW:	Midwest	CA:	California	OH:	Ohio
NE:	Northeast	CO:	Colorado	PA:	Pennsylvania
NP:	Northern Plains	ID:	Idaho	SD:	South Dakota
NW:	Northwest	IA:	Iowa	TN:	Tennessee
SE:	Southeast	MD:	Maryland	UT:	Utah
SW:	Southwest	MO:	Missouri	WI:	Wisconsin
SP:	Southern Plains	ND:	North Dakota	WY:	Wyoming

VIII.E. Management of Insects

Maize is subject to attack by a complex of insects from the time it is planted until it is harvested and used as food and feed. The economically important insect pests in North America include wireworms, black cutworm, European corn borer, Southwestern corn borer, corn rootworms, grasshoppers, fall armyworm, and corn earworm. Table VIII-4 lists the insect pests in U.S. maize. Approximately 27 active pesticide ingredients are registered for use in maize for the control of insect pests. In its annual survey of agricultural chemical usage, USDA determined that 23% of the maize acreage was treated with chemical insecticides in 2005 (USDA-NASS, 2006). Tefluthrin, cyfluthrin, and tebupirimphos were the most widely applied insecticides to maize, at 7%, 7%, and 6% of total acreage, respectively (USDA-NASS, 2006). Chlorpyrifos was only applied

to 2% of total maize acreage, but total quantity applied is more than three times greater than the next highest insecticide at 2.0 million pounds (USDA-NASS, 2006).

The introduction of biotechnology-derived insect protected maize has offered growers an alternative and effective solution for the control of major insect pests in maize and in 2009 approximately 63% of the total maize acreage in the U.S. was planted with hybrids possessing insect protection traits (USDA-NASS, 2009).

MON 87427 has no insecticidal activity. Environmental observations in field studies have demonstrated no apparent impact on arthropods of maize (Section VII). Therefore, no changes to current insect management practices are anticipated from the introduction of MON 87427, including pesticide use, conventional breeding selection for resistance, or when used in conjunction with biotechnology-derived traits.

Table VIII-4. Insect Pests in Maize

Common Name	Latin name
Soil Insects	
Northern corn rootworm	*Diabrotica barberi*
Western corn rootworm	*Diabrotica virgifera virgifera*
Southern corn rootworm	*Diabrotica undecimpunctata*
Black cutworm	*Agrotis ipsilon*
Wireworms	*A. mancus, Horistonotus uhlerii, Melanotus cribulosus,* others
Billbugs	*Sphenophorus* spp.
White grubs	*Phyllophaga* spp.
Corn root aphid	*Anuraphis maidiradicis*
Seedcorn maggot	*Delia platura*
Grape colaspis	*Colaspis brunnea*
Seedcorn beetle	*Stenolophus lecontei*
Insects attacking the leaf, stalk, and ear	
Corn earworm	*Helicoverpa zea*
European corn borer	*Ostrinia nubilalis*
Corn leaf aphid	*Rhopalosiphum maidis*
Fall armyworm	*Spodoptera frugiperda*
Stalk borers	*Diatraea spp.*
Armyworm	*Pseudaletia unipuncta*
Lesser cornstalk borer	*Elasmopalpus lignosellus*
Chinch bug	*Blissus leucopterus leucopterus*
Grasshoppers	*Melanoplus differentialis*
Corn flea beetle	*Chaetocnema pulicaria*
Japanese beetle	*Popillia japonica*
Other insects	
Thrips	*Anaphothrips* spp., *Frankliniella* spp.
Leafhoppers	*Trigonotylus brevipes*, others
Western bean cutworm	*Striacosta albicosta*
Corn blotch leaf miner	*Agromyza parvimaizeis*
Spider mites	*Oligonychus* spp., *Tetranychus* spp.
Pink scavenger caterpillar	*Pyroderces rileyi*
Garden symphlan	*Scuttigerella immaculata*
Hop-vine borer	*Hydraecia immanis*
Potato stem borer	*Hydraecia micacea*
Sod webworms	Subfamily Cramdinae
Leaf rollers	
Stink bugs	
Insect disease vectors	Several

Sources: (Dicke and Guthrie, 1988; O'Day, et al., 1998)

VIII.F. Management of Diseases and Other Pests

Management of diseases during maize growth and development is essential for protecting the yield of the harvested grain. Estimates for annual yield losses because of diseases have ranged from 7 to 17% (Shurtleff, 1980). Incidence of disease infestation is highly variable and depends on many factors such as location, climate, and other environmental factors. Most maize hybrids on the market today have acceptable levels of resistance to common diseases. The diseases found to occur in maize grown in the U.S. are summarized in Table VIII-5. In addition, several nematode species have been known to cause diseases in maize (Smith and White, 1988). The use of fungicides in maize is limited because the incidence and severity of most diseases tends to be low and quite variable. Fungicides used on maize plants in the U.S. during 2004 include azoxystrobin, trifloxystrobin, chlorothalonil, propiconazole, and sulfur (USDA-NASS, 2004)).

Environmental observations in field studies have demonstrated no apparent impact of MON 87427 or the glyphosate resistance trait on diseases of maize (Section VII). Therefore, no changes in current disease management practices are anticipated from the introduction of MON 87427, including pesticide use or conventional breeding selection for disease resistance.

Table VIII-5. Diseases of Maize

Common Name	Causative Agent [transmittal agent]
Seed rots and seedling blights	*Fusarium moniliform, Pythium* spp.
Foliar Diseases	
Bacterial leaf blight and stalk rot	*Pseudomonas avenae*
Bacterial stripe	*Pseudomonas andropogonis*
Stewart's wilt	*Erwinia stewartii*
Chocolate spot	*Pseudomonas coronafaciens*
Goss's wilt	*Clavibacter michiganense*
Holcus spot	*Pseudomonas syringe*
Anthracnose	*Colletotrichum graminicola*
Eyespot	*Kabatiella zeae*
Gray leaf spot	*Cercospora zeae-maydis*
Northern leaf spot	*Bipolaris zeicola*
Northern corn leaf blight	*Exserohilum turcicum*
Physoderma brown spot	*Physoderma maydis*
Southern corn leaf blight	*Bipolaris maydis*
Yellow leaf blight	*Phyllosticta maydis*
Common corn rust	*Puccinia sorghi*
Southern corn rust	*Puccinia polysora*
Common corn smut	*Ustilago maydis*
Systemic Diseases	
Head smut	*Sphacelotheca reiliana*
Crazy top	*Sclerophthora macrospora*
Sorghum downy mildew	*Peronosclerospora sorghi*
Maize dwarf mosaic virus	[aphids]
Maize chlorotic dwarf virus	[leafhoppers]
Corn lethal necrosis	[chrysomelid beetles]
Maize white line mosaic virus	[not identified]
Corn stunt	[leafhoppers]
Maize bushy stunt	[leafhoppers]
Stalk and root rots	
Gibberella stalk rot	*Gibberella zeae*
Diplodia stalk rot	*Stenocarpella maydis*
Anthracnose stalk rot	*Colletotrichum graminicola*
Charcoal rot	*Macrophomina phaseolina*
Fusarium stalk rot	*Fusarium moniliforme*
Pythium stalk rot	*Pythium aphanidermatum*
Bacterial stalk rot diseases	*Erwinia chrysanthemi*
Root rots	*Pythium* spp.
Ear rots and storage molds	
Fusarium ear rot	*Fusarium moniliforme*
Gibberella ear rot	*Gibberella zeae*
Diplodia ear rot	*Diplodia maydis*
Aspergillus ear and kernel rot	*Aspergillus flavus*
Storage molds	*Penicillium* spp., *Aspergillus* spp.

Source: (Smith and White, 1988)

VIII.G. Crop Rotation Practices in Maize

Crop rotation is a well-established farming practice and a key management tool for maize seed and grain growers. The purpose of rotating maize with other crops is to improve the yield and profitability of one or more crops over time, decrease the need for nitrogen fertilizer on the crop following soybean, increase residue cover, mitigate or break disease, insect and weed cycles, reduce soil erosion, increase soil organic matter, improve soil tilth and soil physical properties, and reduce runoff of nutrients, herbicides, and insecticides (Al-Kaisi, et al., 2003). According to USDA Economic Research Service, approximately 75% of the maize acreage in the 10 major producing states used a maize-soybean rotation system in 2001 (USDA-ERS, 2006). Although the benefits of crop rotations can be substantial, the grower must make cropping decisions by evaluating both the agronomic and economic returns on various cropping systems. Crop rotations also afford growers the opportunity to diversify farm production in order to minimize market risks.

Since most hybrid seed is produced in the maize belt, a two-year rotation of maize-soybean is the most widely used crop rotation. Wheat could be added to the rotation or replace soybean in the cropping sequence, particularly in the western maize belt. Although seed producers prefer not to plant maize following maize, it is necessary in some areas (NE and MI) or situations because of the limited soybean acres or other crops available on the farm for rotation (Chad Peters, Monsanto Global Operations, personal communication, 2010). Planting maize following maize can result in a higher incidence of diseases, increase nutrient requirements and make management of volunteer maize plants more difficult and expensive.

No changes are anticipated from the introduction of MON 87427 on current rotational practices for the production of hybrid maize seed or for the cultivation of commercial maize.

VIII.H. Volunteer Management

Volunteer maize commonly occurs in rotational crops in the season following cultivation of conventional or biotechnology-derived maize. Viable grain is not produced on the approximately 9% of U.S. maize acres that is cultivated for the production of silage, and volunteer maize plants typically do not occur in the rotational crops that follow maize harvested as silage. In the warmer climates of the Southeast and Southwest, the occurrence of volunteer maize is rare because maize grain remaining after harvest is likely to germinate in the fall and the resulting plants can usually be controlled by tillage or by freezing temperatures in the winter. In the Northern maize-growing regions, volunteer maize does not always occur in the rotational crop because of seed decomposition over the winter, efficient harvest procedures, and tillage prior to planting rotational crops.

Management of volunteer maize in rotational crops involves minimizing or reducing the potential for volunteers through practices that include: 1) adjusting harvest equipment to minimize the amount of maize grain lost in the field; 2) planting maize hybrids that

reduce the extent of ear drop; 3) choosing maize hybrids with superior stalk strength and reduced lodging; and 4) practicing no-till production to significantly reduce the potential for volunteer growth in the rotational crop. If volunteer maize does occur in subsequent crops, pre-plant tillage or in-crop cultivation are very effective management tools. Selective herbicides labeled for the effective post-emergent control of volunteer maize in specific crops include Assure II® (quizalofop), Fusilade® DX (fluazifop), Fusion® (fluazifop + fenoxaprop), Poast® (sethoxydim), and Select® 2EC (clethodim). These herbicides are labeled for use in 11 vegetable rotation crops and eight field crops that include soybean, cotton, sugar beet and alfalfa.

In hybrid maize seed production where maize follows maize, volunteer maize must be controlled to avoid inadvertent cross pollination of the female parent inbred, and to ensure high genetic purity of the hybrid maize seed. Effective management is typically achieved by planting maize seed following soybean or another crop in the crop rotation (Chad Peters, Monsanto Global Operations, personal communication, 2010). Under situations of continuous maize production, the volunteer maize is controlled by cultivation and hand weeding. Off-type plants or rogue plants may also be present in the maize inbreds. They differ phenotypically from the inbred maize and are removed from seed fields by hand weeding prior to pollination.

No changes are anticipated from the introduction of MON 87427 on current volunteer management practices in the production of hybrid maize seed or in the cultivation of commercial maize.

VIII.I. Stewardship of MON 87427

Under the Coordinated Framework for Regulation of Biotechnology, the responsibility for regulatory oversight of biotechnology-derived crops falls on three lead 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 both FDA and USDA have completed their reviews and assessments under their respective jurisdictions.

Food and feed from biotechnology-derived crops are subject to regulatory review by FDA under the Federal Food, Drug, and Cosmetic Act [21 U.S.C. 346 a(d)]. Since 1992, FDA has used a voluntary consultation process to work together with the developers of biotechnology-derived products to identify and resolve any issues regarding the safety and nutritional content of food and feed derived from these crops. In compliance with this policy, Monsanto has initiated a consultation with the FDA on the food and feed safety and nutritional assessment of MON 87427. Monsanto will be submitting a safety and nutritional assessment summary document to FDA in the near future.

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The Animal and Plant Health Inspection Service (APHIS) of the U.S. Department of Agriculture (USDA) has responsibility under the Plant Protection Act (7 U.S.C. § 7701-7772) to prevent the introduction and dissemination of plant pests into the U.S. The APHIS regulation at 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.

An EPA submission has been made to change the glyphosate label for the production of hybrid maize seed, and will be obtained prior to commercial use/marketing of MON 87427.

Monsanto Company develops effective products and technologies and is committed to assuring that its products and technologies are safe and environmentally responsible. Monsanto demonstrates this commitment by implementing product stewardship processes throughout the lifecycle of a product and by participation in the Excellence Through Stewardship[SM] (ETS) Program[3]. These policies and practices include rigorous field compliance and quality management systems and verification through auditing. Monsanto's Stewardship Principles are also articulated in Technology Use Guides[4] that are distributed annually to growers who utilize Monsanto branded traits.

As an integral action of fulfilling this commitment, Monsanto will seek biotechnology regulatory approvals for MON 87427 in all key maize import countries with a functioning regulatory system to assure global compliance and support the flow of international trade. These actions will be consistent with the Biotechnology Industry Organization (BIO) Policy on Product Launch[5] Monsanto continues to monitor other countries that are key importers of maize from the U.S., for the development of formal biotechnology approval processes. If new functioning regulatory processes are developed, Monsanto will make appropriate and timely regulatory submissions.

Monsanto also commits to best industry practices on seed quality assurance and control to prevent adventitious presence of unapproved traits. As with all of Monsanto's products, before commercializing MON 87427 in any country, a detection method will be made available to grain producers, processors, and buyers.

VIII.J. Impact of the Introduction of MON 87427 on Agricultural Practices Summary and Conclusions

MON 87427 offers an alternative to detasseling methods and CMS technology for consistently producing high-quality, high-purity hybrid maize seed. As a result of introducing MON 87427, there are no changes in the production of hybrid maize seed or

[3] Excellence Through Stewardship Program can be found at: http://www.excellencethroughstewardship.org/.
[4] Monsanto Technology Use Guides can be found at: http://www.monsanto.com/monsanto/ag_products/pdf/stewardship/technology_use_guide.pdf.
[5] BIO's Product Launch guidelines can be found at: http://www.excellencethroughstewardship.org/facts/documents/Guide%20for%20Product%20Launch%20Stewardship.pdf.

in the cultivation of commercial maize that are anticipated in current management practices for the control of insects, diseases and other pests, or in crop rotation and volunteer management. With the exception of the intended impact of glyphosate applications during reproductive development, other agricultural management practices for the production of hybrid maize seed and for the cultivation of commercial maize would also be no different for MON 87427 than for the management practices used in conventional maize hybrids.

IX. ENVIRONMENTAL CONSEQUENCES

IX.A. Introduction

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.

The following lines of evidence form the basis for the plant pest risk assessment in this petition: (1) insertion of a single functional copy of the *cp4 epsps* expression cassette, (2) characterization of the CP4 EPSPS protein expressed in MON 87427, (3) safety and mode of action of the CP4 EPSPS protein, (4) compositional equivalence of MON 87427 forage and grain as compared to conventional control, (5) phenotypic and agronomic characteristics demonstrating no increased plant pest potential including disease and pest susceptibilities, (6) negligible risk to non target organisms (NTO) and threatened or endangered species (TES), (7) modern maize has inherently low plant pest potential including the potential for gene flow to other species with which it can interbreed, and (8) no greater likelihood to impact agronomic practices, including land use, cultivation practices, or the management of weeds, diseases, and insects than commercial maize.

Using the assessment above, the data and analysis presented in this petition lead to a conclusion that MON 87708 is unlikely to be a plant pest, and therefore should no longer be subject to regulation under 7 CFR § 340.

In 2008, APHIS proposed amendments to 7 CFR § 340 that included provisions to utilize its noxious weed authority in regulating genetically engineered plants (73 FR 600008). Because the data presented in this petition demonstrate that MON 84727 has no potential to cause injury or damage to protected interests under the noxious weed authority, MON 84727 would not be considered a "noxious weed" as defined by the Plant Protection Act.

IX.B. Plant Pest Assessment of MON 87427 Insert and Expressed Substances

IX.B.1. Characteristics of the Genetic Insert and Expressed Protein

IX.B.1.1. Genetic Insert

MON 87427 was produced by *Agrobacterium*-mediated transformation of maize tissue using the PV-ZMAP1043. This plasmid vector contains one transfer DNA (T-DNA) that is delineated by Right and Left Border sequences. The T-DNA contains an expression cassette consisting of the *cp4 epsps* coding sequence regulated by the *e35S* promoter, the *hsp70* intron, and the *nos* 3′ untranslated region (Figure III-2; Table III-1). Southern blot analyses were used to confirm the copy number of the integrated T-DNA sequences in the genome and the presence or absence of backbone from PV-ZMAP1043 in the genome of MON 87427 (Figures IV-2 to 4). The data demonstrate that MON 87427 contains one copy of the insert at a single integration locus and that all expression elements are present in the T-DNA. The data also demonstrate that MON 87427 does not contain detectable backbone from PV-ZMAP1043. The complete DNA sequence of the insert and adjacent genomic DNA sequence in MON 87427 confirmed the integrity of the inserted *cp4 epsps* expression cassette and identified the 5' and 3' insert-to-genomic DNA junctions.

Additional characterization of the insertion site in conventional maize confirmed that 140 bases of genomic DNA were deleted at the insertion site in MON 87427. Additionally, there is 41 bp insertion at the 5' insertion junction and 24 bp insertion at the 3' insertion junction that occurred during the *Agrobacterium*-mediated transformation in MON 87427 (Section IV.D). Such changes are quite common during plant transformation; these changes presumably resulted from double-stranded break repair mechanisms in the plant during *Agrobacterium*-mediated transformation process (Salomon and Puchta, 1998). Finally, Southern blot analysis demonstrated that the DNA fingerprint of the T-DNA insert in MON 87427 has been maintained through multiple generations of breeding, thereby confirming the stability of the insert in multiple generations.

These data demonstrates that there are no unintended changes in the MON 87427 genome as a result of the insertion of the *cp4 epsps* expression cassette, and supports the overall conclusion that MON 87427 is unlikely to be a plant pest.

IX.B.1.2. Mode of Action

Monsanto Company has developed 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 which confer tolerance to glyphosate. In most plants, the endogenous EPSPS protein, an enzyme involved in the shikimate pathway for the biosynthesis of aromatic amino acids, is inhibited by the herbicide glyphosate resulting in cell death (Franz et al., 1997b). MON 87427 utilizes a specific promoter and intron combination to drive CP4 EPSPS protein expression in vegetative and female reproductive tissues, conferring tolerance to glyphosate in the leaves, stalk, and root tissues and tissues that develop into seed or grain and silks. This

specific promoter and intron combination also results in limited or no production of CP4 EPSPS protein in two key male reproductive tissues: pollen microspores which develop into pollen grains, and tapetum cells that supply nutrients to the pollen. Thus, in MON 87427, male reproductive tissues critical for male gametophyte development are not tolerant to glyphosate. Two glyphosate applications beginning just prior and/or during tassel development stages (approximate maize vegetative growth stages ranging from V8 to V13) will produce a male sterile phenotype through tissue-selective glyphosate tolerance, and will eliminate or greatly reduce the need for detasseling which is currently used in the production of hybrid corn seed. (Section I.). However, glyphosate applications at the early vegetative stages, as directed on Roundup herbicide agricultural product labels, should not affect pollen viability of MON 87427 because the tapetum and pollen microspore cells are not actively developing at these stages. In addition, the use of glyphosate in the hybrid seed production of MON 87427 results in viable hybrid maize seed carrying the tissue-selective glyphosate tolerance trait.

IX.B.1.3. Expressed Protein Safety

Multiple Roundup Ready crops that produce the CP4 EPSPS protein have been reviewed by USDA, determined to not pose a plant pest risk, and cleared for environmental release. The CP4 EPSPS protein expressed in MON 87427 is identical to the CP4 EPSPS proteins in other Roundup Ready crops including Roundup Ready soybeans (Figure V-1), Roundup Ready corn 2, Roundup Ready canola, Roundup Ready sugar beet, Roundup Ready cotton and Roundup Ready Flex cotton. Results from the protein characterization studies included in this petition confirmed the identity of the MON 87427-produced CP4 EPSPS protein and established the equivalence of MON 87427-produced protein to the *E. coli*-produced CP4 EPSPS reference protein standard (Section V.B.). The safety of CP4 EPSPS proteins present in biotechnology-derived crops has been extensively assessed (Harrison et al., 1996). The Environmental Protection Agency (EPA) also reviewed the safety of the CP4 EPSPS protein and has established a tolerance exemption for the protein and the genetic material necessary for its production either in or on all raw agricultural commodities (40 CFR § 174.523). This exemption was based on a safety assessment that included rapid digestion in simulated gastric fluids, lack of homology to known toxins and allergens, and lack of toxicity in an acute oral mouse gavage study. A history of safe use is supported by the lack of any documented reports of adverse effects since the introduction of Roundup Ready crops in 1996. Therefore, it is concluded that the CP4 EPSPS protein poses no risk to human or animal health.

IX.B.1.4. CP4 EPSPS Protein Expression Levels

As discussed in Section I, MON 87427 utilizes a specific promoter and intron combination (*e35S-hsp70*) to drive CP4 EPSPS protein expression in vegetative and female reproductive tissues, conferring tolerance to glyphosate in these tissues. Use of this promoter and intron combination drives little to no CP4 EPSPS protein production in the tapetum and pollen microspore cells of MON 87427, thus these tissues and pollen from MON 87427 are not tolerant to glyphosate. The CP4 EPSPS protein expression data from MON 87427 is consistent with the MON 87427 product concept. Protein expression analyses determined that the mean CP4 EPSPS protein levels in MON 87427

from different maize tissues and across all sites ranged from 290 – 680 µg/g dwt in leaf, 73 – 140 µg/g dwt in root, and 240 – 500 µg/g dwt in whole plant (Section V.C.). Little to no CP4 EPSPS is expected to be produced in MON 87427 pollen, and CP4 EPSPS protein levels in MON 87427 pollen across the sites were either < LOD µg/g dwt or were very low (Section V.C.). The small amount of CP4 EPSPS protein found in these pollen samples might be attributed to the presence of anther tissue collected with the pollen from MON 87427. Alternatively, a low amount of CP4 EPSPS protein in MON 87427 pollen samples may be inherent to this product due to the use of the *e35S* promoter (CaJacob et al., 2004). The levels of CP4 EPSPS protein in MON 87427 from all maize tissues tested, and across all sites are comparable (except in the pollen) to other commercialized CP4 EPSPS protein-containing maize products that have been cleared for environmental release by regulatory agencies around the world.

IX.B.2. Compositional Characteristics

Compositional analyses of MON 87427 and a conventional control were carried out to confirm that MON 87427 was compositionally equivalent to the conventional control maize. The conventional control had background genetics similar to MON 87427 but did not produce CP4 EPSPS.

Nutrients assessed in this analysis included proximates (ash, carbohydrates by calculation, moisture, protein, and fat), acid detergent fiber (ADF), neutral detergent fiber (NDF), total dietary fiber, amino acids, fatty acids (C8-C22), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), and vitamins [folic acid, niacin, A (β-carotene), B_1, B_2, B_6, and E] in the grain, and proximates, ADF, NDF, calcium and phosphorus in forage. The anti-nutrients assessed in grain included phytic acid and raffinose. Secondary metabolites assessed in grain included furfural, ferulic acid, and p-coumaric acid. All components analyzed in MON 87427 forage and grain were either not statistically significantly different (α=0.05) compared to the conventional control, or, if significantly different, were within the natural variability of maize composition as expressed in the 99% tolerance interval. Collectively, the compositional analyses data support the conclusion that MON 87427 is compositionally equivalent to conventional maize. Therefore there is no known risk related to change in composition of maize products as a result of introducing MON 87427.

IX.B 3. Phenotypic and Agronomic and Environmental Interaction Characteristics

The plant phenotypic and environmental interaction parameters evaluated in this study were used to characterize the plant and its interactions with the environment and to assess the plant pest or weed potential of MON 87427 compared to the conventional control. Evaluations were taken on plants not treated with glyphosate. These assessments included seed dormancy and germination parameters, plant growth and development characteristics, as well as pollen morphology and viability (Section VII). Results from the phenotypic and agronomic assessments demonstrate that MON 87427 does not possess characteristics that would confer a plant pest risk compared to conventional maize (Section VII.C.2). Data on environmental interactions also indicate that MON 87427 does not confer any biologically meaningful increased susceptibility or

tolerance to specific disease, insect, or abiotic stressors, or changes in agronomic and phenotypic characteristics (Section VII.C.2.2). Based on the assessed characteristics, the results of this study demonstrate that there were no unexpected changes in the phenotype or ecological interactions indicative of increased plant pest or weed potential of MON 87427 compared to conventional maize. Taken together, these data support the conclusion that MON 87427 is not likely to pose increased plant pest risk compared to conventional maize.

IX.B.3.1. Seed Dormancy and Germination

Seed dormancy and germination are both considered indicators of weediness potential. Seed dormancy (e.g. hard seed) is often associated with plants that are considered as weeds (Anderson, 1996; Lingenfelter and Hartwig, 2003). Seed dormancy and germination characterization indicated that MON 87427 seed had germination characteristics similar to seed of conventional maize. In particular, the lack of hard seed in MON 87427, supports a conclusion of no increased weediness of MON 87427 compared to conventional maize from germination and dormancy characteristics.

IX.B.3.2. Plant Growth and Development

Evaluations of plant growth and development characteristics in the field are useful for assessing potential weediness characteristics such as stalk and root lodging. Phenotypic characteristics such as early stand count, days to 50% pollen shed and silking, stay green, ear height, plant height, dropped ears, final stand count, grain moisture, test weight, and yield were assessed. In the combined site analyses, one statistically significant difference was detected between MON 87427 and the conventional control for seedling vigor (Table VII-4). However, the mean value for seedling vigor for MON 87427 was within the range of the references. Therefore, this difference in seedling vigor is unlikely to be biologically significant in terms of increased weed potential (Section VII.C). Taken together, these comparative assessments of plant growth and development characteristics indicate that MON 87427 is not likely to have increased weed or plant pest potential compared to conventional maize.

IX.B.3.3. Pollen Morphology and Viability

Evaluations of pollen morphology and viability from non glyphosate treated field-grown plants provide useful information in a plant pest assessment as it relates to the potential for gene flow to, and introgression of the biotechnology-derived trait into other maize plants and wild relatives. For pollen characteristic assessments, there were no statistically significant differences (5% level of significance) detected between MON 87427 and the conventional control for pollen diameter. MON 87427 had a statistically significant higher percent viability than the conventional control (99.7 vs. 98.9%) and was slightly outside the reference range. However, the difference between MON 87427 and the conventional control for pollen viability was less than one percentage point and is not deemed biologically meaningful.

IX.B.3.4. Interactions with Non-target Organisms Including Threatened and Endangered Species

Evaluation of MON 87427 for potential adverse impacts on non target organisms (NTO) is a component of the plant pest risk assessment. Roundup Ready corn 2 has been studied for impacts on NTO and found to exhibit no significant difference when compared to conventional maize (Croon, 2000). MON 87427 produces the same CP4 EPSPS protein as in commercial Roundup Ready corn 2 (Section IX.B.1.2), therefore it is expected that MON 87427 will have no impact on NTO, consistent with Roundup Ready corn 2. The CP4 EPSPS protein is a member of the larger family of EPSPS proteins that are ubiquitous in plants and microbes in the environment (CaJacob et al., 2004) and the mode of action of this family of proteins is well known (Alibhai and Stallings, 2001). The information for this evaluation included the CP4 EPSPS protein safety assessments (Section IX.B.1.3) and information from the environmental interaction assessment (Section VII.C.2.2).

The safety of CP4 EPSPS proteins present in biotechnology-derived crops has been extensively assessed (Harrison et al., 1996). A mouse gavage study demonstrated no acute oral toxicity and consequently the low potential for impact to terrestrial vertebrate NTO including threatened and endangered vertebrate species. Additionally, lack of impact on water quality is discussed in section I.5.1.1. Data from the 2008 U.S. phenotypic and agronomic study, observational data on environmental interactions such as plant-disease interaction, arthropod damage and arthropod abundance, were collected at select sites for MON 87427 and conventional control (Section VII; Appendix G.6; Section VI C.2.2). These results support the conclusion of no adverse environmental impact from cultivation of MON 87427 to non-target arthropod populations.

Taken together, these data support the conclusion that MON 87427 has no reasonable mechanism for harm to NTO, or impact threatened and endangered species compared to the cultivation of conventional maize.

IX.C. Weediness Potential of MON 87427

Maize is not listed as a weed in the major weed references (Crockett, 1997; Muenscher, 1980), nor is it present on the lists of noxious weed species distributed by the federal government (7 CFR § 360). In addition, maize has been grown throughout the world without any report that it is a serious weed. Modern maize does not survive as a weed because of past selection in the development of maize. During domestication of maize, traits often associated with weediness, such as, seed dormancy, a dispersal mechanism, or the ability to form reproducing populations outside of cultivation, have not been selected. For example, the maize ear is enclosed with a husk; therefore seed dispersal of individual kernels is limited. Even if individual kernels of maize were distributed within a field or along transportation routes from the fields to storage or processing facilities, sustainable volunteer maize populations are not found growing in fence rows, ditches, and road sides. Maize is poorly suited to survive without human assistance and is not capable of surviving as a weed (Galinat, 1988; Keeler, 1989). Although maize seed can overwinter

into a crop rotation with soybeans, mechanical and chemical measures can be used to control volunteers.

In comparative studies between MON 87427 and a conventional control, phenotypic, agronomic and environmental interaction data were evaluated (Section VII) for changes that would impact the plant pest potential, in particular, plant weediness potential. Results of these evaluations show that there is no fundamental difference between MON 87427 and the conventional control for traits potentially associated with weediness. Furthermore, comparative field observations between MON 87427 and its conventional control and their response to abiotic stressors, such as cold, drought, heat, mineral toxicity, soil compaction, wet soil, and wind, indicated no differences and, therefore, no increased weediness potential. Collectively, these findings support the conclusion that MON 87427 has no increased weed potential compared to conventional maize and it is no more likely to become a weed than conventional maize.

IX.D. Potential for Pollen Mediated Gene Flow

Pollen mediated gene flow (often referred to as "cross pollination) is a process whereby one or more genes successfully integrate into the genome of a recipient plant. Introgression is affected by both biotic and abiotic factors such as plant biology, pollen biology/volume, plant phenology, overlap of flowering times, proximity of the pollen source and sink, ambient conditions such as temperature and humidity, and field architecture. Because gene introgression is a natural biological process, it does not constitute an environmental risk in and of itself. Gene introgression must be considered in the context of the transgenes inserted into the biotechnology derived plant, and the likelihood that the presence of the transgenes and their subsequent transfer to recipient plants will result in increased plant pest potential. The potential for gene introgression from MON 87427 is discussed below.

IX.D.1. Hybridization with Cultivated Maize *Zea mays* L.

Maize morphology fosters cross pollination; therefore, high levels of pollen mediated gene flow can occur in this species. In addition, researchers recognize that (1) the amount of gene flow that occurs can be high because of open pollination; (2) the percent gene flow will vary by population, hybrid or inbred; (3) the level of gene flow decreases with greater distance between the source and recipient plants; (4) environmental factors affect the level of gene flow; (5) maize pollen is viable for a short period of time under field conditions; (6) maize produces ample pollen over an extended period of time; and, (7) maize is wind pollinated; pollinating insects, especially bees, are occasional visitors to the tassels but rarely visit silks of maize.

Based on several studies conducted on the extent of pollen mediated gene flow between maize fields, results were found to vary depending on the experimental design, environmental conditions, and detection method, as expected. In general, the percent of gene flow diminished with increasing distance from the source field, generally falling below 1% at distances >200 m (~660 feet) (Table IX-1). This information is useful for managing gene flow during maize breeding, seed production, identity preservation or

other applications; in addition, it forms the basis for the USDA-APHIS performance standards for maize. All testing and production of regulated MON 87427 seed or grain have been conducted under USDA notification according to these standards. Gene flow from fields planted with MON 87427 to other maize would not be of concern because of the lack of potential to cause harm to humans and to the environment.

IX.D.2. Hybridization with Wild Annual Species of Subgenus *Zea mays* subsp. *mexicana*

For gene flow to occur by normal sexual transmission, the following conditions must exist: (1) the two parents must be sexually compatible; (2) there must be overlapping phenology; and (3) a suitable factor (such as wind or insects) must be present and capable of transferring pollen between the two parents.

Maize and annual teosinte (*Zea mays* subsp. *mexicana*), are genetically compatible, wind-pollinated and hybridize when in close proximity to each other e.g., in areas of Mexico and Guatemala (Wilkes, 1972). Maize crosses with teosinte; however, teosinte is not present in the U.S. other than as an occasional botanical garden specimen or small feral populations of *Zea mays* subsp. *mexicana* in Florida, Alabama and Maryland. In an experimental field study where maize and teosinte species were planted together (Ellstrand, et al., 2007), very low hybridization rates were observed for maize and *Zea mays* subsp. *mexicana.* Differences in factors such as flowering time, geographical separation, and development factors make natural crosses in the U.S. highly unlikely.

IX.D.3. Hybridization with the Wild Perennial Species of Subgenus *Tripsacum*

In contrast with maize and teosinte, which hybridizes under certain conditions, it is only with extreme difficulty and special techniques that maize and the closely related perennial species, *Tripsacum* (gamma grass) hybridize. Furthermore, the offspring of the cross show varying levels of sterility and are genetically unstable (Galinat, 1988; Russell and Hallauer, 1980).

A single species, *Tripsacum floridanum* (Florida gamma grass), found in the extreme southern Florida counties of Miami-Dade, Collier and Monroe has been categorized as a threatened species by the state of Florida and listed on the USDA Natural Resources Conservation Service (USDA-NRCS) database. Another species, *Tripsacum dactyloides* (Eastern gamma grass), found primarily throughout the eastern U.S., has been categorized as endangered in Massachusetts and Pennsylvania, and as threatened in New York (USDA-NRCS, 2010). However, given the level of difficulty for natural hybridization between species of *Tripsacum* and *Zea* as mentioned above, the occurrence of *T. floridanum* primarily in both highly urbanized and non-agricultural, swampy areas of the state where commercial maize is not typically grown, as well as the preference of *T. dactyloides* for wet habitats where hybrid maize production would not occur, it is very unlikely there would be any impact on this species due to the introduction of MON 87427.

IX.D.4. Transfer of Genetic Information to Species with which Maize Cannot Interbreed (Horizontal Gene Flow)

Monsanto is aware of no reports confirming the transfer of genetic material from maize to other species with which maize cannot sexually interbreed. The probability for horizontal gene flow to occur is judged to be exceedingly small. Even if it were to occur, the consequences would be negligible since the CP4 EPSPS protein produced in MON 87427 is the same CP4 EPSPS protein as in commercial Roundup Ready maize products and shown to have no meaningful toxicity to humans and other NTO under the conditions of use.

IX.E. Summary of Plant Pest Assessments

Plant pests, as defined in the Plant Protection Act, are living organisms that can directly or indirectly injure, cause damage to, or cause disease to any plant or plant product (7 U.S.C. § 7702[14]). Data presented in Sections IV through VII of this petition confirm that MON 87427, with the exception of glyphosate tolerance, is not significantly different from conventional maize, in terms of pest potential. Monsanto is not aware of any study results or observations associated with MON 87427 that would suggest that an increased plant pest risk would result from its introduction.

The plant pest assessment was based on multiple lines of evidence developed from a detailed characterization of MON 87427 compared to conventional maize, followed by a risk assessment on detected differences. The plant pest risk assessment in this petition was based on the following lines of evidence: (1) insertion of a single functional copy of the *cp4 epsps* expression cassette; (2) characterization of the CP4 EPSPS protein expressed in MON 87427; (3) safety of the CP4 EPSPS protein; (4) compositional equivalence of MON 87427 forage and grain as compared to a conventional control; (5) phenotypic and agronomic characteristics demonstrating no increased plant pest potential; (6) negligible risk to NTO and threatened or endangered species; (7) modern maize has inherently low plant pest potential, and (8) no greater likelihood to impact agronomic practices, including land use, cultivation practices, or the management of weeds, diseases, and insects, than conventional maize.

Based on the data and information presented in this petition, it is concluded that, similar to currently deregulated maize products, MON 87427 is highly unlikely to be a plant pest. Thus, the results support a conclusion of no increased weediness potential of MON 87427 compared to conventional maize. In addition, APHIS has proposed to amend 7 CFR § 340 to include its noxious weed authority. MON 87427 would not be considered a "noxious weed" as defined by the Plant Protection Act because the data in this petition show that it has no potential to cause direct injury or damage (physical harm) to any protected interest. Therefore, Monsanto Company requests a determination from APHIS that MON 87427 and any progeny derived from crosses between MON 87427 and other commercial maize be granted non-regulated status under 7 CFR § 340.

Table IX-1. Summary of published literature on maize outcrossing

Pollinator Distance (m)	Reported Outcrossing (%)	Comments	Country	Reference
12-15	1	Frequencies by distance investigated. One year experiment. Single yellow male and white female used to measure outcrossing rates.	UK	(Bateman, 1947)
0	28.6	Frequencies by distance investigated. Three year study. Single male and female. Pollen source was a yellow dent and the female was a white sweet corn..	USA	(Jones and Brooks, 1950)
25	14.2			
75	5.8			
125	2.3			
200	1.2			
300	0.5			
400	0.8			
500	0.2			
1	-	Frequencies by distance investigated. Single yellow sweet corn hybrid was used as a pollen source and as pollen recipient. To measure levels of outcrossing, pollen recipient plants were detasseled.	UK	(Haskell and Dow, 1951)
3.6	95			
4.8	-			
6	-			
7.3	-			
8.5	-			
9.8	-			
11	-			
12	-			
13.4	-			
18	10			

Table IX-1 (continued). Summary of published literature on maize outcrossing

Pollinator Distance (m)	Reported Outcrossing (%)	Comments	Country	Reference
N/A	N/A	Dispersion and deposition investigated. Two year experiment. Two males and two females. Pollen deposition per unit area at 60 m = 0.2%. Pollen concentrations at 60m equal 1%.	USA	(Raynor, et al., 1972)
1 10 20 30 34	2.25 0.02 0.008 0.005 0.003	Dispersal of maize pollen investigated. Single hybrid. Gene flow decreased with greater distance from the source. Closer correlation of number of plants with gene flow than physical distance. Data reported in this table represent means from two of four fields	Brazil	(Paterniani and Stort, 1974)
2-4	0.01	Gene flow in isolated and crossing blocks was evaluated. Two year study. Single male and female. Bt female hybrid was detasseled.	Mexico	(Garcia, et al., 1998)
30 40 350	1.04 0.03 0	Frequencies by distance investigated. Two year study. Single RR male and non-RR female. Data reported in this table represent one of two years.	USA	(Jemison and Vayda, 2001)

Table IX-1 (continued). Summary of published literature on maize outcrossing

Pollinator Distance (m)	Reported Outcrossing (%)	Comments	Country	Reference
100 150 200 300 400	0.01 - 0.01 - -	Frequencies by distance and pollen viability investigated. Two year study. Single male and female. A purple gene marker was utilized to measure pollen mobility. Pollen viability lasted one hour in the driest-hottest year and two hours in the most humid, less hot year.	Mexico	(Luna, et al., 2001)
1 3 8 16 24 32	30-40 18-22 9-12 3-5 0-2 2-4	Frequencies by distance investigated. Two sites/one year. Six hybrid pairs. Six Bt and six near isogenic non-Bt hybrids. Hybridization was assessed by measuring the expression of Bt gene in kernels collected from neighboring plants. Alternatively, sampled kernels were grown and seedlings tested for expression of Bt gene. Data reported in this table represent estimates from a graph.	USA	(Chilcutt and Tabashnik, 2004)
1 5 10 14 19 24 28 33 36	9.7-19.0 1.3-2.6 0.7-2.0 0.3-0.6 0.4 0-0.3 0.1-0.5 0-0.3 0-0.1	Frequency by distance investigated. Three year, three sites. Single male and female/location.	Canada	(Ma, et al., 2004)

Table IX-1 (continued). Summary of published literature on maize outcrossing

Pollinator Distance (m)	Reported Outcrossing (%)	Comments	Country	Reference
200 300	0.03 0.02	Detasseling efficiency on pollen containment investigated. Four inbreds were used as a source of pollen; yellow inbred, two GM inbreds (Bt and RR) and an IT (imidazolinone tolerant) inbred. The recipient pollen traps were two white inbreds and a male-sterile hybrid. Two year/three locations study.	USA	(Stevens, et al., 2004)
24-32 60-62 123-125 244-254 486-500 743-745	0.01-0.7 0.01-0.2 0.001-0.08 0-0.02 0-0.005 0-0.002	Frequencies by distance investigated. Single male and 7 females with different RM used. The male parent source of pollen contains the genetic markers P1-rr and R1-nj. When male pollen pollinated female yellow plants a purple coloration occurred in the fertilized yellow kernels. Two year/two site study. Data reported in this table represent results from one site.	USA	(Halsey, et al., 2005)
1.8 9.4 20.6 35.8 200	1.0-2.5 1.2-2.5 1.0-2.2 0.5-2.3 0.6-1.4	Isolation distance investigated. The objectives were (i) to evaluate current industry isolation practices to produce hybrid seed that meets higher levels of genetic purity and (ii) to identify practices that will improve reproductive isolation in hybrid seed fields. Three year/315 fields. Multiple hybrids from 24 seed companies tested. Data reported in this table represent estimates from a graph.	USA	(Ireland, et al., 2006)

Table IX-1 (continued). Summary of published literature on maize outcrossing

Pollinator Distance (m)	Reported Outcrossing (%)	Comments	Country	Reference
1 10 35 100 150 200 250	17.0-29.9 1.5-2.5 0.4 0.03-0.05 0.01-0.03 0.007-0.03 0.002-0.03	Frequencies of cross-pollination by distance investigated. Pollination was quantified by measuring out-crossing from a transgenic hybrid plot into a conventional grain production field. A combination of three marker genes was utilized to detect outcrosses: y1 (seed color gene), Bt and RR. Two years/two sites. Single male and female.	USA	(Goggi, et al., 2006)
0 4.6 18.3	< 0.9% at distances ≤ 20 m	Efficiency of border rows and isolation distance on cross-pollination investigated. Available datasets were utilized to make predictions for reducing out-crossing to levels below 0.9%.	USA	(Gustafson, et al., 2006)
0 2 5 10 20 40 80	3-13 0.2-10 0.1-2.3 0.2-3.7 0.1-0.8 0-0.7 0.1-0.2	Frequencies of cross-pollination with a PCR based method investigated. The main objective of the study was to compare a PCR based method to real cross-fertilization rates as determined by phenotypic analysis. Four Bt hybrids and a single non-Bt hybrid were used as a male and female respectively. One year/one site.	Spain	(Pla, et al., 2006)

Table IX-1 (continued). Summary of published literature on maize outcrossing

Pollinator Distance (m)	Reported Outcrossing (%)	Comments	Country	Reference
0	10.5	Frequency of cross pollination (expressed as %GM DNA) by distance investigated. The study was conducted in large farm scale evaluation (FSE) across the UK. Data reported here are maximum raw values	UK	(Weekes, et al., 2007)
2	34.9			
5	9.9			
10	12.2			
15	0.5			
20	8.2			
25	4			
40	3.7			
50	5.9			
70	0.13			
75	0.28			
80	0.12			
100	2.3			
120	0.16			
142	0.06			
147	0			
150	5.4			
160	0			
200	0.24			

Table IX-1 (continued). Summary of published literature on maize outcrossing

Pollinator Distance (m)	Reported Outcrossing (%)	Comments	Country	Reference
52 85 105 125 149 150 200 287 371 402 458 4125 4440	0.009 0.015 0.003 0.01 0.016 0.007 0.009 0.005 0.008 0.005 0.0002 0.006 0.0005	Cross-pollination investigated using occurrence of yellow kernels in 13 white maize fields. In no case, the cross-pollination of the whole field was > than 0.02%. In every field some cross-pollination with a low rate, on an average of 1.8% of the sampled ears, could be found. These pollinations were mostly single cross-pollinations on the ear.	Switzerland	(Bannert and Stamp, 2007)
1 10 35 100	42.2 6.3 1.3 0.1	Frequencies of cross-pollination by distance investigated. The pollen source was a stacked RR/Bt yellow hybrid. The recipient was a nontransgenic white hybrid. Higher outcrossing detected when white hybrid used detasseled.	USA	(Goggi, et al., 2007)
12 12 12	4.2 11.7 3.8	Frequency of cross pollination and coexistence by distance investigated. Two crops were used as barriers to determine their usefulness as buffer crops in maize. Three genetic markers to measure outcrossing were used: GM Bt maize, a kernel color maize and a molecular marker test	EU	(Langhof, et al., 2008)

X. ADVERSE CONSEQUENCES OF INTRODUCTION

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. The characterization evaluations included molecular analyses, which confirmed the insertion of a single functional copy of the *cp4 epsps* expression cassette at a single locus within the maize genome. Additionally, protein expression analyses demonstrate the CP4 EPSPS protein is expressed in vegetative and female reproductive tissues. The CP4 EPSPS protein produced by MON 87427 is similar and functionally identical to endogenous plant EPSPS enzymes, but has a much reduced affinity for glyphosate relative to endogenous plant EPSPS. The amino acid sequence of the CP4 EPSPS protein expressed in MON 87427 is identical to the amino acid sequence of the recombinant *E. coli*-produced CP4 EPSPS protein standard previously utilized in the published safety assessment (Harrison et al., 1996). Analysis of key nutrients, anti-nutrients, and secondary metabolites of MON 87427 grain and of key nutrients in MON 87427forage demonstrate that MON 87427 is compositionally equivalent to conventional maize. The phenotypic evaluations of MON 87427, including an assessment of seed germination and dormancy characteristics, plant growth and development characteristics, pollen characteristics, ecological interaction characteristics, and environmental interactions also indicated MON 87427 is unchanged compared to conventional maize. There is no indication that MON 87427 would have an adverse impact on beneficial or non-target organisms, including threatened or endangered organisms. Therefore, based on the lack of increased pest potential or adverse environmental consequences compared to conventional maize, the risks for humans, animals, and other NTO from MON 87427 are negligible under the conditions of use.

The introduction of MON 87427 will not adversely impact cultivation practices or the management of weeds, diseases, and insects in maize production systems. Farmers familiar with the Roundup Ready maize system would continue to employ the same crop

rotational practices, weed control practices and/or volunteer control measures currently in place for Roundup Ready Corn 2 products.

REFERENCES

Abendroth, L. and R. Elmore. 2006. What row spacing is best? Iowa State University Extension, Ames, Iowa. http://www.agronext.iastate.edu/corn/production/management/planting/row.html. [Accessed June 1, 2010].

Adams, T.B., J. Doull, J.I. Goodman, I.C. Munro, P. Newberne, P.S. Portoghese, R.L. Smith, B.M. Wagner, C.S. Weil, L.A. Woods and R.A. Ford. 1997. The FEMA GRAS assessment of furfural used as a flavour ingredient. Food and Chemical Toxicology 35: 739-751.

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. http://www.ipm.iastate.edu/ipm/icm/2003/12-15-2003/croprotation.html [Accessed June 19, 2006].

Alexander, M.P. 1980. A versatile stain for pollen, fungi, yeast, and bacteria. Stain Technology 55: 13-18.

Alibhai, M.F. and W.C. Stallings. 2001. Closing down on glyphosate inhibition - with a new structure for drug discovery. Proceedings of the National Academy of Sciences of the United States of America 98: 2944-2946.

Anderson, K.S. and K.A. Johnson. 1990. Kinetic and structural analysis of enzyme intermediates: Lessons from EPSP Synthase. Chemical Reviews 90: 1131-1149.

Anderson, W.P. 1996. Weed ecology. Pages 27-38 in Weed Science: Principles and Applications. Third Edition. West Publishing Company, St. Paul, Minnesota.

AOSA. 2000. Tetrazolium testing handbook. Contribution no. 29. The Handbook on Seed Testing, Association of Official Seed Analysts, Lincoln, Nebraska.

AOSA. 2007. Rules for testing seeds. Association of Official Seed Analysts, Lincoln, Nebraska.

AOSCA. 2009. Seed certification handbook: including genetic and crop standards, procedures, and AOSCA service programs. Association of Official Seed Certifying Agencies, Moline, Illinois.

Armstrong, C.L., C.E. Green and R.L. Phillips. 1991. Development and availability of germplasm with High Type II culture formation response. Maize Genetics Cooperation Newsletter 65: 92-93.

Bannert, M. and P. Stamp. 2007. Cross-pollination of maize at long distance. European Journal of Agronomy 27: 44-51.

Barker, R.F., K.B. Idler, D.V. Thompson and J.D. Kemp. 1983. Nucleotide sequence of the T-DNA region from the *Agrobacterium-tumefaciens* octopine Ti plasmid pTil15955. Plant Molecular Biology 2: 335-350.

Barry, G.F., G.M. Kishore, S.R. Padgette and W.C. Stallings. 2001. Glyphosate-tolerant 5-enolpyruvylshikimate-3-phosphate synthases. Patent 6,248,876, U.S. Patent Office, Washington, D.C.

Bateman, A.J. 1947. Contamination in seed crops. III. Relation with isolation distance. Heredity 1: 303-336.

Bedinger, P. and S.D. Russell. 1994. Gametogenesis in maize. Pages 48-61 in The Maize Handbook. M. Freeling and V. Walbot (eds.). Springer-Verlag, New York, New York.

Bevan, M., W. Barnes and M.-D. Chilton. 1983. Structure and transcription of the nopaline synthase gene region of T- DNA. Nucleic Acid Research 11: 369-385.

Bosnic, A.C. and C.J. Swanton. 1997. Influence of barnyardgrass (*Echinochloa crus-galli*) time of emergence and density on corn (*Zea mays*). Weed Science 45: 276-282.

Bradford, K.J. 2006. Methods to maintain genetic purity of seed stocks. Publication 8189. University of California, Oakland, California.

Brown, S.M. and C.G. Santino. 1997. Enhanced expression in plants. Patent 5,593,874, U.S. Patent Office, Washington, D.C.

Buchanan, B.G., W. Gruissem and R. Jones. 2000. Biochemistry and molecular biology of plants. Pages 1286-1289 in Biochemistry and Molecular Biology of Plants. American Society of Plant Biologists, Rockville, Maryland.

CaJacob, C.A., P. Feng, G.R. Heck, M.F. Alibhai, D. Sammons and S.R. Padgette. 2004. Enigeering resistance to herbicides. Pages 353-372 in Handbook of Plant Biotechnology. P. Christou and H. Klee (eds.). John Wiely & Sons, Ltd., Hoboken, New Jersey.

Carey, J.B. and J.J. Kells. 1995. Timing of total postemergence herbicide applications to maximize weed control and corn (*Zea mays*) yield. Weed Technology 9: 356-361.

Chilcutt, C.F. and B.E. Tabashnik. 2004. Contamination of refuges by *Bacillus thuringiensis* toxin genes from transgenic maize. Proceedings of the National Academy of Sciences of the United States of America 101: 7526-7529.

Codex Alimentarius. 2003. Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. CAC/GL 45-2003. Codex Alimentarius Comission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organisation, Rome, Italy. ftp://ftp.fao.org/codex/Publications/Booklets/Biotech/Biotech_2003e.pdf [Accessed May 12, 2010].

Craig, W.F. 1977. Production of hybrid corn seed. Pages 671-719 in Corn and Corn Improvement. Second Edition. G.F. Sprange (ed.). American Society of Agronomy, Madison, Wisconsin.

Crockett, L. 1997. Wildly successful plants: A handbook of North American weeds. Macmillan Publishing Co., Inc., New York, New York and Collier Macmillan Publishers, London, United Kingdom.

Croon, K.A. 2000. Request for extension of determination of non regulated status to the additional regulated article: Roundup Ready Corn line NK603. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Washington, D.C. http://www.aphis.usda.gov/brs/not_reg.html [Accessed September 12, 2010].

Depicker, A., S. Stachel, P. Dhaese, P. Zambryski and H.M. Goodman. 1982. Nopaline synthase: Transcript mapping and DNA sequence. Journal of Molecular and Applied Genetics 1: 561-574.

Devine, M., S.O. Duke and C. Fedtke. 1993. Herbicide translocation. Pages 67-90 in Physiology of Herbicide Action. PTR Prentice Hall, Englewood Cliffs, New Jersey.

Dicke, F.F. and W.D. Guthrie. 1988. The most important corn insects. Pages 767-867 in Corn and Corn Improvement. Third Edition. G.F. Sprague and J.W. Dudley (eds.). American Society of Agronomy, Inc, Crop Science Society of America, Inc, Soil Science Society of America, Inc, New York, New York.

Duvick, D.N. 2001. Biotechnology in the 1930s: The development of hybrid maize. Nature Reviews in Genetics 2: 69-74.

Edgerton, M.D. 2009. Increasing crop productivity to meet global needs for feed, food, and fuel. Plant Physiology 149: 7-13.

EFSA. 2009. Applications (references EFSA-GMO-NL-2005-22, EFSA-GMO-RX-NK603) for the placing on the market of the genetically modified glyphosate tolerant maize NK603 for cultivation, food and feed uses, import and processing and for renewal of the authorisation of maize NK603 as existing products, both under Regulation (EC) No

1829/2003 from Monsanto. European Food Safety Authority, Parma, Italy. http://www.efsa.europa.eu/en/scdocs/doc/1137.pdf [Accessed October 4, 2010].

Ellstrand, N.C., L.C. Garner, S. Hegde, R. Guadagnuolo and L. Blancas. 2007. Spontaneous hybridization between maize and teosinte. Journal of Heredity 98: 183-187.

FAO/WHO. 1991. Strategies for assessing the safety of foods produced by biotechnology, report of joint FAO/WHO consultation. World Health Organization, Geneva, Switzerland.

FAOSTAT. 2009. Food and Agricultural Organization statistical database. Food and Agricultural Organization of the United Nations, New York, New York. http://faostat.fao.org/site/567/default.aspx#ancor [Accessed August 16, 2010].

Fausey, J.C., J.J. Kells, S.M. Swinton and K.A. Renner. 1997. Giant foxtail (*Setaria faberi*) interference in nonirrigated corn (*Zea mays*). Weed Science 45: 256-260.

Fling, M.E., J. Kopf and C. Richards. 1985. Nucleotide sequence of the transposon Tn7 gene encoding an aminoglycoside-modifying enzyme, 3"(9)-o-nucleotidyltransferase. Nucleic Acid Research 13: 7095-7106.

Franz, J.E., M.K. Mao and J.A. Sikorski. 1997a. Uptake, transport, and metabolism of glyphosate in plants. Pages 143-186 in Glyphosate: A Unique Global Herbicide. American Chemical Society, Washington, D.C.

Franz, J.E., M.K. Mao and J.A. Sikorski. 1997b. Glyphosate's molecular mode of action. Glyphosate: A Unique Global Herbicide. American Chemical Society, Washington, D.C.

Galinat, W.C. 1988. The origin of corn. Pages 1-31 in Corn and Corn Improvement. Volume 18. G.F. Sprague and J.W. Dudley (eds.). American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, Wisconsin.

Garcia, C.M., M.J. Figueroa, L.R. Gomez, R. Townsend and J. Schoper. 1998. Pollen control during transgenic hybrid maize development in Mexico. Crop Science 38: 1597-1602.

Gianessi, L.P., C.S. Silvers, S. Sankula and J.E. Carpenter. 2002. Plant biotechnology: Current and potential impact for improving pest management in U.S. agriculture. An analysis of 40 case studies - herbicide tolerant field corn. National Center for Food & Agricultural Policy, Washington, D.C.

Giza, P.E. and R.C.C. Huang. 1989. A self-inducing runaway-replication plasmid expression system utilizing the Rop protein. Gene 78: 73-84.

Goggi, A.S., P. Caragea, H. Lopez-Sanchez, M. Westgate, R. Arritt and C.A. Clark. 2006. Statistical analysis of outcrossing between adjacent maize grain production fields. Field Crops Research 99: 147-157.

Goggi, A.S., H. Lopez-Sanchez, P. Caragea, M. Westgate, R. Arritt and C.A. Clark. 2007. Gene flow in maize fields with different local pollen densities. International Journal of Biometerology 51: 493-503.

Goldberg, R.B., T.P. Beals and P.M. Sanders. 1993. Anther development: Basic principles and practical applications. Plant Cell 5: 1217-1229.

Goodman, M.M. and W.C. Galinat. 1988. The history and evolution of maize. Critical Reviews in Plant Sciences 7: 197-220.

Gustafson, D.I., I.O. Brants, M.J. Horak, K.M. Remund, E.W. Rosenbaum and J.K. Soteres. 2006. Empirical modeling of genetically modified maize grain production practices to achieve European Union labeling thresholds. Crop Science 46: 2133-2140.

Halsey, M.E., K.M. Remund, C.A. Davis, M. Qualls, P.J. Eppard and S.A. Berberich. 2005. Isolation of maize from pollen-mediated gene flow by time and distance. Crop Science 45: 2172-2185.

Hamilton, D.A., M. Roy, J. Rueda, R.K. Sindhu, J. Stanford and J.P. Mascarenhas. 1992. Dissection of a pollen-specific promoter from maize by transient transformation assay. Plant Molecular Biology 18: 211-218.

Harrigan, G.G., W.P. Ridley, K.D. Miller, R. Sorbet, S.G. Riordan, M.A. Nemeth, W. Reeves and T.A. Pester. 2009. The forage and grain of MON 87460, a drought-tolerant corn hybrid, are compositionally equivalent to that of conventional corn. Journal of Agricultural and Food Chemistry 57: 9754-9763.

Harrigan, G.G., D. Lundry, S. Drury, K. Berman, S.G. Riordan, M.A. Nemeth, W.P. Ridley and K.C. Glenn. 2010. Natural variation in crop composition and the impact of transgenesis. Nature Biotechnology 28: 402-404.

Harrigan, G.G., L.G. Stork, S.G. Riordan, T.L. Reynolds, W.P. Ridley, J.D. Masucci, S. MacIsaac, S.C. Halls, R. Orth, R.G. Smith, L. Wen, W.E. Brown, M. Welsch, R. Riley, D. McFarland, A. Pandravada and K.C. Glenn. 2007. Impact of genetics and environment on nutritional and metabolite components of maize grain. Journal of Agricultural and Food Chemistry 55: 6177-6185.

Harrison, L.A., M.R. Bailey, M.W. Naylor, J.E. Ream, B.G. Hammond, D.L. Nida, B.L. Burnette, T.E. Nickson, T.A. Mitsky, M.L. Taylor, R.L. Fuchs and S.R. Padgette. 1996. The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. Journal of Nutrition 126: 728-740.

Haskell, G. and P. Dow. 1951. Studies with sweet corn. V. Seed-settings with distances from pollen source. Empire Journal of Experimental Agriculture 19: 45-50

Haslam, E. 1993. Introduction, commentary and overview. Pages 1-50 in Shikimic Acid: Metabolism and Metabolites. John Wiley and Sons, Inc., Chichester, England.

Herrmann, K.M. 1995. The shikimate pathway: Early steps in the biosynthesis of aromatic compounds. Plant Cell 7: 907-919.

Herrmann, K.M. and L.M. Weaver. 1999. The shikimate pathway. Annual Reviews of Plant Physiology and Plant Molecular Biology 50: 473-503.

Hileman, R.E., A. Silvanovich, R.E. Goodman, E.A. Rice, G. Holleschak, J.D. Astwood and S.L. Hefle. 2002. Bioinformatic methods for allergenicity assessment using a comprehensive allergen database. International Archives of Allergy and Immunology 128: 280-291.

Hoeft, R.G., E.D. Nafziger, R.R. Johnson and S.R. Aldrich. 2000a. Corn as a crop. Pages 1-29 in Modern Corn and Soybean Production. MCSP Publications, Champaign, Illinois.

Hoeft, R.G., E.D. Nafziger, R.R. Johnson and S.R. Aldrich. 2000b. Nutrient management for top profit. Pages 107-171 in Modern Corn and Soybean Production. MCSP Publications, Champaign, Illinois.

Hoeft, R.G., E.D. Nafziger, R.R. Johnson and S.R. Aldrich. 2000a. Planting decisions and operations. Pages 81-105 in Modern Corn and Soybean Production. MCSP Publications, Champaign, Illinois.

Hoisington, D., G.M. Listman and M.L. Morris. 1998. Varietal development: Applied biotechnology. Pages 77-102 in Maize Seed Industries in Developing Countries. M.L. Morris (ed.). Lynne Rienner Publishers, Boulder, Colorado.

Hokanson, K., D. Heron, S. Gupta, S. Koehler, C. Roseland, S. Shantharam, J. Turner, J. White, M. Schechtman, S. McCammon and R. Bech. 1999. The concept of familiarity and pest resistant plants. Workshop on Ecological Effects of Pest Resistance Genes in Managed Ecosystems, Bethesda, Maryland.

Huang, M.-D., F.-J. Wei, C.-C. Wu, Y. Hsing and A.H.C. Huang. 2009. Analyses of advanced rice anther transcriptomes reveal global tapetum secretory functions and potential proteins for lipid exine formation [1[W][OA]]. Plant Physiology 149: 694-707.

ILSI. 2009. Crop Composition Database Version 3.0. International Life Science Institute. http://www.cropcomposition.org/.

Ireland, D.S., D.O. Wilson, M.E. Westgate, J.S. Burris and M.J. Lauer. 2006. Managing reproductive isolation in hybrid seed corn production. Crop Science 46: 1445-1455.

Jemison, J.M. and M.E. Vayda. 2001. Cross pollination from genetically engineered corn: Wind transport and seed source. AgBioForum 4: 87-92.

Jones, J.M. and J.S. Brooks. 1950. Effectiveness and distance of border rows in preventing outcrossing in corn. Oklahoma Agricultural Experimental Station Technicial Bulletin No. T-38. Oklahoma State Univeristy, Stillwater, Oklahoma.

Jugenheimer, R.W. 1976. Production of hybrid seed. Pages 441-455 in Corn: Improvement, Seed Production, and Uses. John Wiley & Sons, New York, New York.

Kay, R., A. Chan, M. Daly and J. McPherson. 1987. Duplication of CaMV 35S Promoter sequences creates a strong enhancer for plant genes. Science 236: 1299-1302.

Keeler, K.H. 1989. Can genetically engineered crop become weeds? Bio-Technology 7: 1134-1139.

Kishore, G., D. Shah, S. Padgette, G. Della-Cioppa, C. Gasser, D. Re, C. Hironaka, M. Taylor, J. Wibbenmeyer, D. Eichholtz, M. Hayford, N. Hoffmann, X. Delannay, R. Horsch, H. Klee, S. Rogers, D. Rochester, L. Brundage, P. Sanders and R.T. Fraley. 1988. 5-enolpyruvylskikimate 3-Phosphate synthase from biochemistry to genetic engineering of glyphosate tolerance. Pages 37-48 in Biotechnology for Crop Protection. American Chemical Society, Washington, D.C.

Klee, H.J., Y.M. Muskopf and C.S. Gasser. 1987. Cloning of an *Arabidopsis thaliana* gene encoding 5-enolpyruvylshikimate-3-phosphate synthase: sequence analysis and manipulation to obtain glyphosate-tolerant plants. Molecular and General Genetics 210: 437-442.

Knake, E.L. and F.W. Slife. 1965. Giant foxtail seeded at various times in corn and soybeans. Weeds 13: 331-334.

Knake, E.L., J.F. Miller, O.E. Strand and J.D. Doll. 1990. Annual grass weeds in corn. National Corn Handbook. Michigan State University, East Lansing, Michigan.

Langhof, M., B. Hommel, A. Husken, J. Schiemann, P. Wehling, R. Wilhelm and G. Ruhl. 2008. Coexistence in maize: Do nonmaize buffer zones reduce gene flow between maize fields? Crop Science 48: 305-316.

Laughnan, J.R. and S. Gabay-Laughnan. 1983. Cytoplasmic male sterility in maize. Annual Review of Genetics 17: 27-48.

Levin, J.G. and D.B. Sprinson. 1964. The enzymatic formation and isolation of 3-enolpyruvylshikimate-5-phosphate. Journal of Biological Chemistry 239: 1142-1150.

Levings, C.S., III and J.N. Siedow. 1992. Molecular basis of disease susceptibility in Texas cytoplasm of maize. Plant Molecular Biology 19: 135-147.

Liener, E.I. 2000. Non-nutritive factors and bioactive componets in soy. Soy in Animal Nutrition. J.K. Drackley (ed.). Federation of Animal Science Society, Savoy, Illinois.

Lingenfelter, D.D. and N.L. Hartwig. 2003. Introduction to weeds and herbicides. Pennsylvania State University Agricultural Research and Cooperative Extension, University Park, Pennsylvania.

Loussaert, D. 2004. Trihalogenated methylsulfonamides as specific male gametocides. Sexual Plant Reproduction 16: 299-307.

Luna, S.V., J.M. Figueroa, B.M. Baltazar, R.L. Gomez, R. Townsend and J.B. Schoper. 2001. Maize pollen longevity and distance isolation requirements for effective pollen control. Crop Science 41: 1551-1557.

Ma, B.L., K.D. Subedi and L.M. Reid. 2004. Crop ecology, management and quality. Crop Science 44: 1273-1282.

Ma, J., D.S. Skibbe, J. Fernandes and V. Walbot. 2008. Male reproductive development: Gene expression profiling of maize anther and pollen ontogeny. Genome Biology 9: R1811-R1817.

MaizeGDB. 2010. Stock data in MaizeGDB. Maize Genetics and Genomics Database. http://www.maizegdb.org/stock.php [Accessed October 4, 2010].

McDonald, M.B. and L.O. Copeland. 1997. Cereal seeds. Corn (*Zea mays* L.). Pages 193-205 in Seed Production: Principles and Practices. Chapman & Hall, New York, New York.

Metcalfe, D.D., J.D. Astwood, R. Townsend, H.A. Sampson, S.L. Taylor and R.L. Fuchs. 1996. Assessment of the allergenic potential of foods derived from genetically engineered crop plants. Critical Reviews in Food Science and Nutrition 36: S165-S186.

Morris, M.L. 1998. Overview of the world maize economy. Pages 13-34 in Maize Seed Industries in Devoloping Countries. Lynne Rienner Publishers Inc., Boulder, Colorado.

Muenscher, W.C. 1980. Mallow family. Pages 310-314 in Weeds. Second Edition. Comstock Publishing Associates, Ithaca,New York.

O'Day, M., A. Becker, A. Keaster, L. Kabrick and K. Steffey. 1998. Corn insect pests: a diagnostic guide. University of Missouri Extension, Columbia, Missouri.

Odell, J.T., F. Nagy and N.-H. Chua. 1985. Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. Nature 313: 810-812.

OECD. 1993. Safety considerations for biotechnology: scale-up of crop plants. Organization for Economic Co-operation and Development, Paris, France. www.biosafety.be/CU/BSL_Ressources/PDF/M00034525.pdf [Accessed January 9, 2009].

OECD. 2002. Consensus document on compositional considerations for new varieties of maize (*Zea mays*): key food and feed nutrients, anti-nutrients and secondary plant metabolites. OECD ENV/JM/MONO (2002)4. Organization of Economic Co-Operation and Development, Paris, France.

OECD. 2003. Consensus document on the biology of *Zea mays* subsp. *mays* (maize). ENV/JM/MONO(2003)11. Organization for Economic Co-Operation and Development, Paris, France.

OGTR. 2008. The biology of *Gossypium hirsutum* L. and *Gossypium barbadense* L. (cotton). Australian Government, Department of Health and Ageing, Office of the Gene Technology Regulator, Canberra, Australia.

Padgette, S.R., D.B. Re, G.F. Barry, D.E. Eichholtz, X. Delannay, R.L. Fuchs, G.M. Kishore and R.T. Fraley. 1996. New weed control opportunities: Development of soybeans with a Roundup Ready® gene. Pages 53-84 in Herbicide-Resistant Crops: Agriculture, Environmental, Economic, Regulatory and Technical Aspects. S. Duke (ed.). CRC Press, Boca Raton, Florida.

Paterniani, E. and A.C. Stort. 1974. Effective maize pollen dispersal in the field. Euphytica 23: 129-134.

Pla, M., J.L. La Paz, G. Penas, N. Garcia, M. Palaudelmas, T. Esteve, J. Messeguer and E. Mele. 2006. Assessment of real-time PCR based methods for quantification of pollen-mediated gene flow from GM to conventional maize in a field study. Transgenic Research 15: 219-228.

Pring, D.R. and D.M. Lonsdale. 1989. Cytoplasmic male sterility and maternal inheritance of disease susceptibility in maize
Annual Review of Phytopathology 27: 483-502.

Raynor, G.S., E.C. Ogden and J.V. Hayes. 1972. Dispersion and deposition of corn pollen from experimental sources. Agronomy Journal 64: 420-427.

Reynolds, T.L., M.A. Nemeth, K.C. Glenn, W.P. Ridley and J.D. Astwood. 2005. Natural variability of metabolites in maize grain: Differences due to genetic background. Journal of Agricultural and Food Chemistry 53: 10061-10067.

Ridley, W.P., R.D. Shillito, I. Coats, H.-Y. Steiner, M. Shawgo, A. Phillips, P. Dussold and L. Kurtyka. 2004. Development of the international life sciences institute crop composition database. Journal of Food Composition and Analysis 17: 423-438.

Riebsame, W.E. 1990. The United States great plains. Pages 561-575 in The Earth as Transformed by Human Action. B.L. Turner (ed.). Cambridge University Press, New York, New York.

Ritchie, S.W., J.J. Hanway and G.O. Benson. 1997. How a corn plant develops. Special Report No. 48. Iowa State University of Science and Technology Cooperative Extension Service, Ames, Iowa.

Russell, W.A. and A.R. Hallauer. 1980. Corn. Pages 299-312 in Hybridization of Crop Plants. W.R. Fehr and H.H. Hadley (eds.). American Society of Agronomy, Inc, and Crop Science Society of America, Inc, Madison, Wisconsin.

Salomon, S. and H. Puchta. 1998. Capture of genomic and T-DNA sequences during double-strand break repair in somatic plant cells. EMBO Journal 17: 6086-6095.

Schnable, P.S. and R.P. Wise. 1998. The molecular basis of cytoplasmic male sterility and fertility restoration. Trends in Plant Science 3: 175-180.

Schonbrunn, E., S. Eschenburg, W.A. Shuttleworth, J.V. Schloss, N. Amrhein, J.N.S. Evans and W. Kabsch. 2001. Interaction of the herbicide glyphosate with its target enzyme 5-enolpyvuvylshikimate 3-phosphate synthase in atomic detail. Proceedings of the National Academy of Sciences of the United States of America 98: 1376-1380.

Shurtleff, M.C. 1980. Introduction. Page 1 in Compendium of Corn Diseases Second Edition. The American Phytopathological Society, Urbana, Illinois.

Sidorov, V. and D. Duncan. 2009. *Agrobacterium*-mediated maize transformation: Immature embryos versus callus. Pages 47-58 in Methods in Molecular Biology: Transgenic Maize. M.P. Scott (ed.). Humana Press Inc, Totowa, New Jersey.

Silvanovich, A., M.A. Nemeth, P. Song, R. Herman, L.Tagliani and G.A. Bannon. 2006. The value of short amino acid sequence matches for prediction of protein allergenicity. Toxicological Sciences 90: 252-258.

Smith, D.R. and D.G. White. 1988. Diseases of corn. Pages 687-766 in Corn and corn improvement. G.G. Sprague and J.W. Dudley (eds.). American Society of Agronomy, Inc, Crop Science Society of America, Inc, and Soil Science Society of America, Inc, Madison, Wisconsin.

Stalker, D.M., C.M. Thomas and D.R. Helinski. 1981. Nucleotide sequence of the region of the origin of replication of the broad host range plasmid RK-2. Molecular and General Genetics 181: 8-12.

Stallings, W.C., S.S. Abdel-Meguid, L.W. Lim, H.S. Shieh, H.E. Dayringer, N.K. Leimgruber, R.A. Stegeman, K.S. Anderson and J.A. Sikorski. 1991. Structure and topological symmetry of the glyphosate target 5-enolpyruvylshikimate-3-phosphate synthase a distinctive protein fold. Proceedings of the National Academy of Sciences of the United States of America 88: 5046-5050.

Steinrucken, H.C. and N. Amrhein. 1980. The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimic-acid-3-phosphate synthase. Biochemical and Biophysical Research Communications 94: 1207-1212.

Steinrucken, H.C. and N. Amrhein. 1984. 5 enolpyruvylshikimate-3-phosphate synthase ec-2.5.1.19 of *Klebsiella-pneumoniae* 2. Inhibition by glyphosate [N-(phosphonomethyl)*glycine*]. European Journal of Biochemistry 143: 351-358.

Stevens, W.E., S.A. Berberich, P.A. Sheckell, C.C. Wiltse, M.E. Halsey, M.J. Horak and D.J. Dunn. 2004. Optimizing pollen confinement in maize grown for regulated products. Crop Science 44: 2146-2153.

Sutcliffe, J.G. 1979. Complete nucleotide sequence of the *Escherichia coli* plasmid pBR322. Pages 77-90, Cold Spring Harbor Symposia on Quantative Biology, Cold Spring Harbor, New York.

Thomas, K., G. Bannon, S. Hefle, C. Herouet, M. Holsapple, G. Ladics, S. MacIntosh and L. Privalle. 2005. In silico methods for evaluating human allergenicity to novel proteins: International Bioinformatics Workshop Meeting Report, 23-24 February 2005. Toxicological Sciences 88: 307-310.

Troyer, A.F. 1999. Review and interpretation: Background of US hybrid corn. Crop Science 39: 601-626.

U.S. EPA. 1996. Residue Chemistry Test Guidelines, OPPTS 860.1000 Background. U.S. Environmental Protection Agency, Office of Prevention Pesticides and Toxic Substances, Washington, D.C.

U.S. FDA. 1992. Statement of policy: foods derived from new plant varieties. Federal Register: 22984-23005.

Ullstrup, A.J. 1972. The impacts of the southern corn leaf blight epidemics of 1970-1971. Annual Review of Phytopathology 10: 37-50.

USDA-ERS. 2006. AREI chapter 4.2: Soil management and conservation. U.S. Department of Agriculture, Economic Research Service, Washington, D.C. http://www.ers.usda.gov/publications/arei/eib16/Chapter4/4.2/ [Accessed October 1, 2010].

USDA-ERS. 2009a. Planted acres-corn-2008-all states. U.S. Department of Agriculture, Economic Research Service, Washington, D.C. http://maps.ers.usda.gov/BaseAcres/index.aspx [Accessed July 14, 2010].

USDA-ERS. 2009b. Briefing rooms/Corn. U.S. Department of Agriculture, Economic Research Services, Washington D.C. http://www.ers.usda.gov/Briefing/Corn/ [Accessed July 13, 2010].

USDA-ERS. 2010. Feed grains database: custom queries. U.S. Department of Agriculture, Economic Research Service, Washington, D.C. http://www.ers.usda.gov/Data/FeedGrains/CustomQuery/Default.aspx [Accessed August 17, 2010].

USDA-FAS. 2010. Production, supply and distribution online. U.S. Department of Agriculture, Foreign Agricultural Service, Washington, D.C. http://www.fas.usda.gov/psdonline/psdHome.aspx [Accessed August 17, 2010].

USDA-NASS. 2004. Agricultural chemical usage - 2003 field crops summary. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C. . http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1560 [Accessed May 12, 2010].

USDA-NASS. 2006. Agricultural chemical usage - 2005 field crops summary. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C. http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1560 [Accessed May 12, 2010].

USDA-NASS. 2009. Acreage. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C. http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1000 [Accessed May 12, 2010].

USDA-NASS. 2010. Data and statistics - Quick stats. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C. http://www.nass.usda.gov/Data_and_Statistics/Quick_Stats/index.asp [Accessed October 4, 2010].

USDA-NRCS. 2010. The PLANTS database. U.S. Department of Agriculture, Natural Resources Conservation Service, Washington, D.C. http://www.plants.usda.gov/index.html [Accessed August 11, 2010].

USDA. 1986. Coordinated framework for regulation of biotechnology, announcement of policy and notice for public comment, office of science and technology policy. U.S. Department of Agriculture, Washington, D.C.

Weekes, R., T. Allnutt, C. Boffey, S. Morgan, M. Bilton, R. Daniels and C. Henry. 2007. A study of crop-to-crop gene flow using farm scale sites of fodder maize (*Zea mays* L.) in the UK. Transgenic Research 16: 203-211.

Widstrom, N.W. 1967. An evaluation of methods for measuring corn earworm injury. Journal of Economic Entomology 60: 791-794.

Wilkes, H.G. 1972. Maize and its wild relatives. Science 177: 1071-1077.

Wych, R.D. 1988. Production of hybrid seed corn. Pages 565-607 in Corn and Corn Improvement. Third Edition. G.F. Sprague and J.W. Dudley (eds.). American Society of Agronomy Inc., Crop Science Society of America Inc., and Soil Science Society of America Inc., Madison, Wisconsin.

Yousef, G.G. and J.A. Juvik. 2001. Crop breeding, genetics & cytology: Comparison of phenotypic and marker-assisted selection for quantitative traits in sweet corn. Crop Science 41: 645-655.

Zambryski, P., A. Depicker, K. Kruger and H.M. Goodman. 1982. Tumor induction by *Agrobacterium tumefaciens*: analysis of the boundaries of T-DNA. Journal of Molecular and Applied Genetics 1: 361-370.

APPENDICES

Appendix A: USDA Notifications

Field trials of MON 87427 have been conducted in the U.S. since 2005. The protocols for these trials include field performance, breeding and observation, agronomics, and generation of field materials and data necessary for this petition. In addition to the phenotypic assessment data provided for MON 87427 observational data on pest and disease stressors were collected from these product development trials. The majority of the final reports have been submitted to the USDA. However, some final reports, mainly from the 2008-2009 seasons, are still in preparation. A list of trials conducted under USDA notification and the status of the final reports for these trials are provided in Table A-1.

Table A-1. USDA Notifications and Permits Approved for MON 87427 and Status of Trials Conducted under These Notifications

USDA No.	Effective Dates	Release State (sites)	Status
2005			
05-292-04n	11/16/05	HI (6), IL (1), PR (2)	Submitted to USDA
05-299-10n	11/04/05	IL (2), PR (2)	Submitted to USDA
2006			
06-039-13n	04/06/06	IA (5), IL (9)	Submitted to USDA
06-052-07n	04/24/06	IA (7), IL (3), NE (1)	Submitted to USDA
06-060-07n	04/24/06	IA (7), IL (7)	Submitted to USDA
06-075-01n	06/12/06	GA (1), IA (6), IL (2), IN (1), MO (1), NE (2), OH (1), TN (1)	Submitted to USDA
06-089-07n	05/15/06	IL (1)	Submitted to USDA
06-089-09n	06/08/06	IL (9), MI (1), MN (2), NE (1), WI (2)	Submitted to USDA
06-142-102n	08/14/06	HI (10)	Submitted to USDA
06-142-105n	06/27/06	PR (2)	Submitted to USDA
06-200-102n	09/11/06	PR (2)	Submitted to USDA
06-200-106n	08/17/06	HI (5)	Submitted to USDA
2007			
07-019-102n	03/17/07	IA (6), IL (7)	Submitted to USDA
07-019-110n	02/18/07	WI (3)	Submitted to USDA
07-019-113n	02/18/07	NE (6)	Submitted to USDA
07-022-104n	02/21/07	MO (2)	Submitted to USDA
07-022-105n	02/21/07	MI (4)	Submitted to USDA
07-022-106n	02/21/07	IL (10)	Submitted to USDA
07-022-107n	02/21/07	IL (8)	Submitted to USDA
07-022-108n	02/21/07	TN (2)	Submitted to USDA
07-024-120n	03/18/07	IA (12)	Submitted to USDA
07-024-121n	03/18/07	MN (4)	Submitted to USDA
07-024-122n	03/28/07	IA (8)	Submitted to USDA
07-024-123n	03/18/07	IA (9)	Submitted to USDA
07-024-125n	03/18/07	IN (4)	Submitted to USDA
07-029-106n	03/19/07	IA (8), IL (3), MI (2), NE (1)	Submitted to USDA
07-040-103n	03/18/07	IL (1)	Submitted to USDA
07-051-117n	03/22/07	IL (2)	Submitted to USDA

Table A-1. (continued) USDA Notifications and Permits Approved for MON 87427 and Status of Trials Conducted under These Notifications

USDA No.	Effective Dates	Release State (sites)	Status
2007 cont.			
07-058-103n	03/29/07	MI (2)	Submitted to USDA
07-065-112n	04/10/07	HI (4)	Submitted to USDA
07-065-113n	04/05/07	PR (3)	Submitted to USDA
07-068-109n	04/25/07	HI (5), PR (2)	Submitted to USDA
07-093-105n	05/02/07	IL (1)	Submitted to USDA
07-173-101n	07/22/07	HI (6)	Submitted to USDA
07-214-103n	09/01/07	HI (3)	Submitted to USDA
07-214-104n	09/01/07	HI (4)	Submitted to USDA
07-214-107n	09/01/07	PR (4)	Submitted to USDA
07-214-109n	09/01/07	PR (4)	Submitted to USDA
07-295-101n	12/06/07	HI (1)	In Process
07-295-105n	12/07/07	PR (1)	Submitted to USDA
07-295-106n	11/21/07	HI (1)	Submitted to USDA
07-295-108n	11/21/07	PR (1)	Submitted to USDA
07-295-111n	11/21/07	HI (1)	Submitted to USDA
07-295-112n	12/07/07	PR (1)	Submitted to USDA
07-295-114n	12/06/07	HI (1), PR (1)	Submitted to USDA
07-296-102n	11/22/07	HI (1), PR (1)	Submitted to USDA
07-299-102n	12/06/07	HI (6), PR (2)	Submitted to USDA
07-333-101n	12/29/07	HI (5), IL (2), PR (2)	Submitted to USDA
2008			
08-014-127n	02/13/08	IA (12), NE (2)	Submitted to USDA
08-014-140n	02/13/08	IL (10), IN (1), OH (1)	Submitted to USDA
08-014-141n	02/13/08	MO (1), TN (1)	Submitted to USDA
08-014-145n	02/20/08	MI (2), MN (6), SD (2)	In Process
08-017-102n	02/16/08	IA (3), IL (6), MI (2), NE (1)	Submitted to USDA
08-035-103n	03/21/08	IL (10)	Submitted to USDA
08-035-104n	03/05/08	IA (11)	Submitted to USDA
08-035-105n	03/05/08	IN (1)	Submitted to USDA
08-035-106n	03/05/08	MI (2)	Submitted to USDA
08-035-107n	03/05/08	MN (6)	In Process
08-036-111n	03/06/08	SD (2)	Submitted to USDA
08-036-114n	03/06/08	WI (2)	Submitted to USDA
08-036-118n	03/28/08	TN (1)	Submitted to USDA
08-036-121n	03/06/08	MO (1)	Submitted to USDA

Table A-1. (continued) USDA Notifications and Permits Approved for MON 87427 and Status of Trials Conducted under These Notifications

USDA No.	Effective Dates	Release State (sites)	Status
2008 cont.			
08-038-103n	03/08/08	NE (2)	In Process
08-038-104n	03/08/08	OH (1)	In Process
08-039-106n	03/10/08	IA (8)	Submitted to USDA
08-039-115n	03/09/08	IL (12)	Submitted to USDA
08-042-106n	03/12/08	IL (12)	Submitted to USDA
08-042-108n	03/12/08	IA (8)	Submitted to USDA
08-050-101n	03/20/08	IA (7), IL (2), MI (1), MO (2), PA (1), WI (2)	Submitted to USDA
08-058-103n	03/28/08	AR (1), IA (2), IL (3), IN (1), NE (1)	Submitted to USDA
08-063-104n	04/02/08	IL (3), IN (2), MI (1), NE (4), WI (2)	Submitted to USDA
08-065-109n	04/08/08	IA (4), MN (8)	Submitted to USDA
08-066-102n	04/05/08	IL (1), IN (3), NE (1), OK (3), TX (1)	Submitted to USDA
08-070-102n	04/09/08	HI (5), IL (2), PR (2)	Submitted to USDA
08-070-103n	04/09/08	IL (2), PR (2)	Submitted to USDA
08-172-103n	07/20/08	HI (4), PR (3)	Submitted to USDA
08-183-102n	07/31/08	HI (5), PR (3)	Submitted to USDA
08-185-105n	08/02/08	HI (5), PR (3)	Submitted to USDA
08-274-101n	10/29/08	KS (1), NE (1), TX (2)	Submitted to USDA
08-294-102n	11/25/08	HI (1)	Submitted to USDA
08-352-101n	01/16/09	HI (6), PR (2)	Submitted to USDA
2009			
09-029-107n	02/28/09	IA (10)	Submitted to USDA
09-029-110n	02/28/09	IA (8), NE (6)	In Process
09-033-104n	03/04/09	IL (15)	In Process
09-033-106n	03/04/09	IL (7)	In Process
09-033-107n	03/04/09	IN (3), KS (1), MI (4), MO (2), TN (2)	In Process
09-033-108n	03/04/09	MN (9), WI (5)	In Process
09-042-111n	03/19/09	IA (3), IN (2), KS (2), OH (3)	In Process
09-043-103n	03/14/09	IL (9)	In Process
09-047-110n	03/17/09	HI (7)	In Process
09-058-101n	03/29/09	AR (1), IA (2), IL (5), IN (2), MO (1), NE (1), PA (1)	In Process

Table A-1. (continued) USDA Notifications and Permits Approved for MON 87427 and Status of Trials Conducted under These Notifications

USDA No.	Effective Dates	Release State (sites)	Status
2009 cont.			
09-079-106n	04/19/09	HI (3)	In Process
09-082-110n	04/22/09	IA (2)	In Process
09-082-112n	04/22/09	PR (3)	In Process
09-082-115n	04/22/09	PR (3)	In Process
09-085-102n	04/25/09	IA (1)	In Process
09-086-107n	04/26/09	MS (1)	In Process
09-225-104n	9/12/09	HI (2), PR (3)	In Process
09-230-102n	09/17/09	HI (6), PR (3)	In Process
09-254-101n	10/11/09	HI (6), PR (3)	In Process
09-286-101n	11/12/09	HI (5), PR (2)	In Process

Appendix B: Materials and Methods Used for Molecular Analyses of MON 87427

B.1. Materials

The genomic DNA used in molecular analyses was isolated from seed of MON 87427 LH198 BC3F4 and the conventional control LH198 × HiII. For generational stability analysis, genomic DNA was extracted from seed of the LH198 BC3F7 × LH287] F1 generation of MON 87427, both conventional controls (LH198 × HiII and LH198 × LH287), and from leaf tissue of the LH198 BC3F3, LH198 BC3F6, and LH198 BC3F7 generations, which were harvested from production plan PPN-09-218. The reference substance, PV-ZMAP1043 (Figure III-1), was used as a positive hybridization control in Southern analyses. Probe templates generated from PV-ZMAP1043 were used as additional positive hybridization controls. As additional reference standards, the 1 Kb DNA Extension Ladder and λ DNA/Hind III Fragments from Invitrogen (Carlsbad, CA) were used for size estimations on Southern blots and agarose gels. The 1 Kb DNA Ladder from Invitrogen was used for size estimations on agarose gels for PCR analyses.

B.2. Characterization of the Materials

The identities of the source materials were verified by methods used in molecular characterization to confirm presence or absence of MON 87427. The stability of the genomic DNA was confirmed by observation of interpretable signals from digested DNA samples on ethidium bromide stained agarose gels and/or specific PCR products, and the samples did not appear visibly degraded on the ethidium bromide stained gels.

B.3. DNA Isolation for Southern Blot and PCR Analyses

Genomic DNA was isolated from MON 87427 maize seed according to a hexadecyltrimethylammonium bromide (CTAB) based method. First, the seed was processed to a fine powder using a Harbil paint shaker for three minutes. Briefly, approximately 16 ml of CTAB extraction buffer [1.5% (w/v) CTAB, 75 mM Tris-HCl pH 8.0, 100 mM EDTA pH 8.0, 1.05 M NaCl, and 0.75% (w/v) PVP (MW 40,000)] and 10 µl of RNase (10 mg/ml, Roche) were added to approximately 6 grams of the processed seed. The samples were incubated at 65ºC for ~35 minutes with intermittent mixing and then allowed to cool to room temperature. Approximately 16 ml of chloroform:isoamyl alcohol (CIA) (24:1 (v/v)) was added to the samples, mixed for 5 minutes, and the two phases separated by centrifugation at ~16,000 x g for 5 minutes at room temperature. The aqueous (upper) layer was transferred to a clean tube. The CIA extraction was repeated twice. Approximately 1/10 volume (~1.6 ml) of 10% CTAB buffer [10% (w/v) CTAB and 0.7 M NaCl] and an equal volume of chloroform:isoamyl alcohol [24:1 (v/v)] was added to the aqueous phase, which was then mixed for 5 minutes. To separate the phases, the samples were centrifuged at ~16,000 x g for 5 minutes at room temperature. The aqueous (upper) layer was removed, mixed with an equal volume (~15 ml) of CTAB precipitation buffer [1% (w/v) CTAB, 50 mM Tris pH 8.0, and 10 mM EDTA pH 8.0] and allowed to stand at room temperature for 1 hour. The samples were centrifuged at ~16,000 x g for 10 minutes at room temperature to pellet the

DNA. The supernatant was discarded, and the pellet was dissolved in approximately 2 ml of high salt TE buffer (10 mM Tris-HCl pH 8.0, 11 mM EDTA pH 8.0, and 1 M NaCl) at 60°C for approximately 15 minutes. Approximately 1/10 volume (0.2 ml) of 3 M NaOAc (pH 5.2) and 2 volumes (~4 ml relative to the supernatant) of 100% ethanol were added to precipitate the DNA. The precipitated DNA was spooled into a microcentrifuge tube containing 70% ethanol. The DNA was pelleted in a microcentrifuge at maximum speed (~14,000 rpm) for ~5 minutes, vacuum-dried, and re-dissolved in TE buffer (pH 8.0). The extracted DNA was stored in a 4ºC refrigerator.

Genomic DNA was also isolated from MON 87427 leaf tissue using a hexadecyltrimethylammonium bromide (CTAB) based method. First, the leaf tissue was ground to a fine powder in liquid nitrogen using a mortar and pestle. Briefly, 10 ml of CTAB buffer (1.5% w/v CTAB, 75 mM Tris HCl, 100 mM EDTA, 1.05 M NaCl, and 0.75% w/v PVP) and 200 µg RNase A were added to approximately 2 ml of ground leaf tissue and incubated at 60-70°C for 40-50 minutes with intermittent mixing. The samples were allowed to come to room temperature and split into two 13 ml tubes. Five ml of chloroform were added to the samples. The samples were mixed by hand for 2-3 minutes, then centrifuged at 10,300 x g for 8-10 minutes at room temperature. The upper aqueous phase was transferred to a clean tube and the chloroform step was repeated twice. After the last chloroform step, the aqueous phase was transferred to a clean tube and the DNA was precipitated with 5 ml of 100% ethanol. The precipitated DNA was spooled into a tube with 5-6 ml of 70% ethanol to wash the DNA pellet. The samples were centrifuged at 5,100 x g for 5 minutes at room temperature to pellet the DNA. DNA pellets were vacuum dried, then re-suspended in TE buffer (10 mM Tris HCl, 1 mM EDTA, pH8.0). The extracted DNA was stored in a 4ºC refrigerator.

B.4. Quantification of Genomic DNA

Genomic DNA was quantified using a DyNA Quant 200 Fluorometer (Hoefer, Inc., Holliston, MA). Molecular Size Marker IX (Roche, Indianapolis, IN) was used as the calibration standard.

B.5. Restriction Enzyme Digestion of Genomic DNA

Approximately ten micrograms (µg) of genomic DNA extracted from MON 87427 and the conventional controls were digested with the restriction enzymes *Nco* I or *Nsi* I (New England Biolabs (Ipswich, MA). All digests were conducted in 1X NEBuffer 3 (New England Biolabs) at 37°C in a total volume of ~500 µl using ~20 units or ~50 units of the appropriate enzyme. For the purpose of running positive hybridization controls, ~10 µg of genomic DNA extracted from the control substance was digested, and the appropriate positive hybridization control(s) were added to these digests.

B.6. Agarose Gel Electrophoresis

Digested DNA was resolved on 0.8% (w/v) agarose gels. For all Southern blot analyses except for generational stability, individual digests containing ~10 µg each of MON 87427 and conventional control DNA were loaded on the same gel in a long

run/short run format. The long run allows for greater resolution of large molecular weight DNA whereas the short run allows for the detection of small molecular weight DNA. The positive hybridization controls were only run in the short run format to ensure that the fragments would be retained on the gel. For the generational stability analysis, individual digests of ~10 µg each of genomic DNA extracted from seed or leaf tissue across five generations of MON 87427 and the conventional controls were loaded on the agarose gel in a single run format.

B.7. DNA Probe Preparation for Southern Blot Analyses

Probe templates were prepared by PCR amplification using PV-ZMAP1043 as the template and purified by agarose gel electrophoresis. The probe templates were designed based on the nucleotide composition (% GC) of the sequence in order to optimize the detection of DNA sequences during hybridization. Where possible, probes possessing similar Tms were combined in the same Southern blot hybridization. Approximately 25 ng of each probe template were radiolabeled with either [α^{32}P] deoxycytidine triphosphate (dCTP) or [α^{32}P] deoxyadenosine triphosphate (dATP) (6000 Ci/mmol) using the random priming method (RadPrime DNA Labeling System, Invitrogen, Carlsbad, CA). Probe locations relative to the genetic elements in PV-ZMAP1043 are depicted in Figure III-1.

B.8. Southern Blot Analyses of Genomic DNA

Digested genomic DNA isolated from MON 87427 and from the conventional maize controls was evaluated using Southern blot analyses. PV-ZMAP1043 DNA digested with *Sph* I was added to the conventional control genomic DNA pre-digested with *Nco* I to serve as a positive hybridization control. When multiple probes were hybridized simultaneously to one Southern blot, the appropriate probe templates generated from PV-ZMAP1043 were mixed with pre-digested conventional control genomic DNA to serve as additional positive hybridization controls (Figure III-1). The digested DNA was then separated by agarose gel electrophoresis and transferred onto a nylon membrane. Southern blots were hybridized and washed at 55°C, 60°C, or 65°C, depending on the melting temperature (Tm) of the probes. Table B-1 lists the radiolabeling conditions and hybridization temperatures of the probes used in this study. Multiple exposures of each blot were then generated using Kodak Biomax MS film in conjunction with one or two Kodak Biomax MS intensifying screen(s) in a -80ºC freezer.

Table B-1. Hybridization Conditions of Utilized Probes

Probe	DNA Probe	Element Sequence Spanned by DNA Probe	Probe labeled with dNTP (^{32}P)	Hybridization/Wash Temperature (°C)
1	T-DNA Probe 1	B-Left Border, P-*e35S*, I-*hsp70* (portion)	dATP	60
2	T-DNA Probe 2	I-*hsp70* (portion), TS-*CTP2* (portion)	dATP	55
3	T-DNA Probe 3	TS-*CTP2* (portion), CS-*cp4 epsps* (portion)	dCTP	65
4	T-DNA Probe 4	CS-*cp4 epsps* (portion), T-*nos*, B-Right Border	dATP	60
5	Backbone Probe	Backbone sequence	dCTP	60
6	Backbone Probe	Backbone sequence	dCTP	60
7	Backbone Probe	Backbone sequence	dCTP	60

B.9. DNA Sequence Analyses of the Insert

Overlapping PCR products were generated that span the insert and adjacent 5' and 3' flanking genomic DNA sequences in MON 87427. These products were sequenced using BigDye® terminator chemistry to determine the nucleotide sequence of the insert in MON 87427 as well as that of the DNA flanking the 5' and 3' ends of the insert.

The PCR analyses for product A and product B were conducted using 50 ng of genomic DNA template in a 50 µl reaction volume containing a final concentration of 2 mM $MgSO_4$, 0.2 µM of each primer, 0.2 mM each dNTP, and 0.02 units/µl of Accuprime Taq DNA Polymerase High Fidelity (Invitrogen, Carlsbad, CA). A primary PCR reaction (product C′) was used in a secondary (nested) reaction to generate product C in order to acquire an adequate amount of template for sequencing. The primary PCR reaction for product C' was conducted using 50 ng of genomic DNA template in a 25 µl reaction volume containing a final concentration of 2 mM $MgSO_4$, 0.2 µM of each primer, 0.2 mM each dNTP, 10% DMSO, and 0.02 units/µl of Accuprime Taq DNA Polymerase High Fidelity. The secondary (nested) reaction was conducted using 1 µl of a 1:10 or 1:100 dilution of product C' as genomic DNA template in a 50 µl reaction volume containing a final concentration of 2 mM $MgSO_4$, 0.2 µM of each primer, 0.2 mM each dNTP, 10% DMSO, and 0.02 units/µl of Accuprime Taq DNA Polymerase High Fidelity.

The amplification of product A and product B were performed under the following cycling conditions: one cycle at 94°C for 2 minutes; 35 cycles at 94°C for 15 seconds, 60°C for 30 seconds, 68°C for 3.25 minutes; and one cycle at 68°C for 5 minutes. The amplification of product C′ was performed under the following touchdown cycling conditions: one cycle at 94°C for 2 minutes; 16 cycles at 94°C for 20 seconds, 62°C decreasing 1°C per cycle for 30 seconds, 68°C for 2 minutes; 20 cycles at 94°C for 20 seconds, 45°C for 30 seconds, 68°C for 2 minutes; and one cycle at 68°C for 7 minutes. The amplification of product C was performed under the following cycling conditions: one cycle at 94°C for 2 minutes; 35 cycles at 94°C for 15 seconds, 60°C for 30 seconds, 68°C for 1.5 minutes; and one cycle at 68°C for 5 minutes.

Aliquots of each PCR product were separated on 1.0% (w/v) agarose gels and visualized by ethidium bromide staining to verify that the products were of the expected size prior to sequencing. To concentrate DNA prior to sequencing, some of the PCR reactions for product B and product C were combined separately and purified with the QIAquick PCR Purification Kit following the manufacturer's instructions (Qiagen, Valencia, CA). The PCR products were sequenced using multiple primers, including primers used for PCR amplification. All sequencing was performed by the Monsanto Genomics Sequencing Center using BigDye® terminator chemistry (Applied Biosystems, Foster City, CA).

B.10. PCR and DNA Sequence Analysis to Examine the MON 87427 Insertion Site

To characterize the MON 87427 insertion site in conventional maize, PCR analysis was performed on genomic DNA from both MON 87427 and the conventional control. The product resulting from the PCR analysis on the conventional control was sequenced. The primers used in this analysis were designed from the DNA sequences flanking the insert

in MON 87427. One primer specific to the 5' flanking end of the insert was paired with a second primer specific to the 3' flanking end of the insert in the genomic DNA sequence.

The PCR analyses were conducted using 50 ng of MON 87427 and conventional control genomic DNA template in separate 50 µl reactions containing a final concentration of 2 mM $MgSO_4$, 0.2 µM of each primer, 0.2 mM each dNTP, 10% DMSO, and 0.02 units/µl of Accuprime Taq DNA Polymerase High Fidelity (Invitrogen). The amplification of the product was performed under the following cycling conditions: one cycle at 94°C for 2 minutes; 30 cycles at 94°C for 15 seconds, 64°C for 30 seconds, 68°C for 1.5 minutes, and one cycle at 68°C for 5 minutes.

Aliquots of each PCR product were separated on 1.0% (w/v) agarose gels and visualized by ethidium bromide staining to verify that the product was of the expected size prior to sequencing. To concentrate DNA prior to sequencing, some of the PCR reactions were purified using the QIAquick PCR Purification Kit following the manufacturer's instructions (Qiagen), and eluates were dried down using a vacufuge. The PCR products were sequenced using multiple primers, including primers used for PCR amplification and primers designed internal to the amplified sequences. All sequencing was performed by the Monsanto Genomics Sequencing Center using BigDye® terminator chemistry (Applied Biosystems, Foster City, CA).

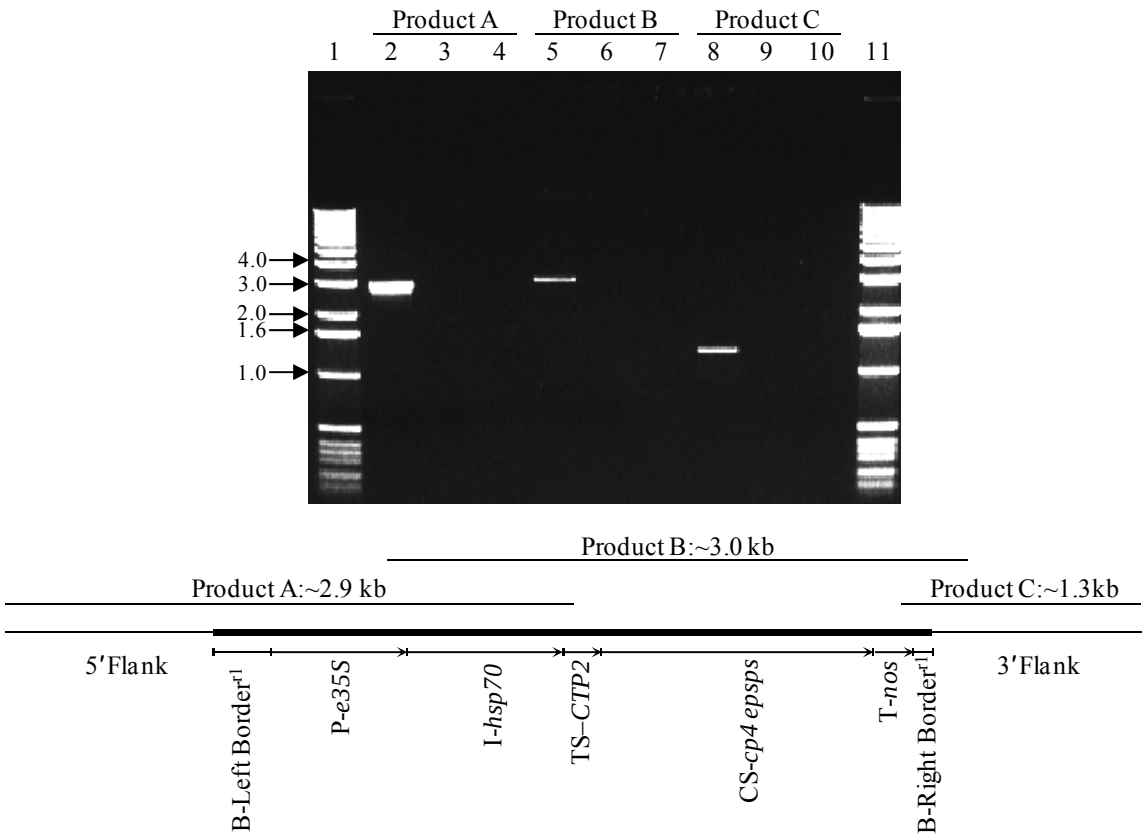

Figure B-1. Overlapping PCR Analysis Across the Insert in MON 87427
PCR was performed on conventional control genomic DNA and MON 87427 genomic DNA extracted from seed tissue. Only lanes containing PCR reactions are shown in the figure. Lanes are marked to show which product has been loaded and is visualized on the agarose gel. The expected product size for each amplicon is provided in the illustration of the insert in MON 87427 that appears at the bottom of the figure. Four to nine microliters of each of the PCR reactions were loaded on the gel. PCR products reported in this figure are representative of the study data.

Lane 1	1 Kb DNA Ladder
2	MON 87427
3	Conventional control
4	No template DNA control
5	MON 87427
6	Conventional control
7	No template DNA control
8	MON 87427
9	Conventional control
10	No template DNA control
11	1 Kb DNA Ladder

The arrows on the agarose gel photograph denote size of DNA, in kilobase pairs, obtained from the 1 Kb DNA Ladder on the ethidium bromide stained gel.

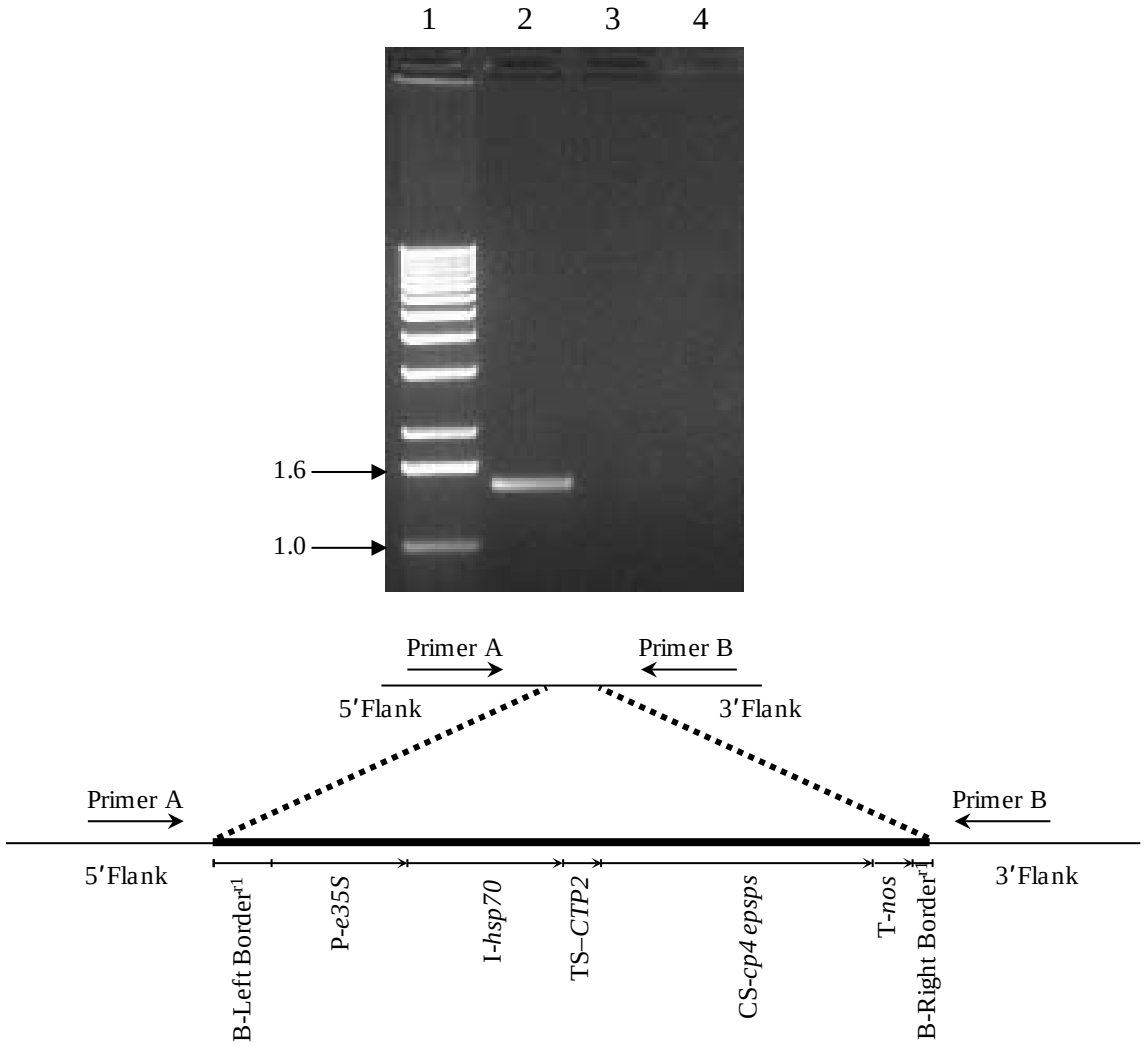

Figure B-2. PCR Amplification of the MON 87427 Insertion Site in Conventional Maize

PCR was performed on conventional control genomic DNA and MON 87427 genomic DNA extracted from seed tissue. Only lanes containing PCR reactions are shown in the figure. Lanes are marked to show which product has been loaded and is visualized on the agarose gel. Depiction of the MON 87427 insertion site in conventional control (upper panel) and the MON 87427 insert (lower panel). PCR amplification was performed using Primer A in the 5' flanking sequence and Primer B in the 3' flanking sequence of the insert in MON 87427. Five microliters of each of the PCR reactions were loaded on the gel. Lane designations are as follows:

Lane 1 1 Kb DNA Ladder
2 Conventional control
3 MON 87427
4 No template DNA control

The arrows on the agarose gel photograph denote size of DNA, in kilobase pairs, obtained from the 1 Kb DNA Ladder on the ethidium stained gel.

Appendix C: Materials, Methods, and Results for Characterization of CP4 EPSPS Protein Produced in MON 87427

C.1. Materials

The MON 87427-produced CP4 EPSPS protein was purified from MON 87427 grain. The MON 87427-produced CP4 EPSPS protein was stored in a -80 ºC freezer in a buffer solution containing 50 mM Tris-HCl, pH 7.5, 50 mM KCl, 2 mM dithiothreitol (DTT), 1 mM benzamidine-HCl, and 25% glycerol.

The *E. coli*-produced CP4 EPSPS protein (, historical APS lot 20-100015) was used as the reference substance. The CP4 EPSPS protein reference substance was generated from cell paste produced by large-scale fermentation of *E. coli* containing the pMON21104 expression plasmid. The coding sequence for *cp4 epsps* contained on the expression plasmid (pMON21104) was confirmed prior to and after fermentation. The *E. coli*-produced CP4 EPSPS protein was previously characterized.

C.2. Description of Assay Control

Protein molecular weight standards (Precision Plus Protein™ Standards Dual color; Bio-Rad, Hercules, CA) were used to calibrate some SDS-PAGE gels and verify protein transfer to polyvinylidene difluoride (PVDF) membranes. Broad range SDS-PAGE molecular weight standards (Bio-Rad, Hercules, CA) were used to generate a standard curve for the apparent molecular weight estimation of the MON 87427-produced CP4 EPSPS protein. The *E. coli*-produced CP4 EPSPS reference standard was used to construct a standard curve for the estimation of total protein concentration using a Bio-Rad protein assay. A PTH-amino acid standard mixture (Applied Biosystems, Foster City, CA) was used to calibrate the instrument for each analysis. This mixture served to verify system suitability criteria such as percent peak resolution and relative amino acid chromatographic retention times. A peptide mixture (Sequazyme™ Peptide Mass Standards kit, Applied Biosystems, Foster City, CA) was used to calibrate the MALDI-TOF mass spectrometer for tryptic mass and a bovine serum albumin (BSA) standard (NIST, Gaithersburg, MD) was used to calibrate the MALDI-TOF mass spectrometer for intact mass analysis. Transferrin (Amersham Biosciences, Piscataway, NJ) and horseradish peroxidase (Sigma-Aldrich, St. Louis, MO) were used as positive controls for glycosylation analysis. CandyCane™ glycoprotein molecular weight standards (Molecular Probes, Eugene, OR) were used as molecular weight markers, as well as, additional positive and negative controls for glycosylation analysis.

C.3. Protein Purification

The plant-produced CP4 EPSPS protein was purified from grain of MON 87427. The CP4 EPSPS protein was purified at ~4 °C from an extract of ground grain using a combination of ammonium sulfate fractionation, hydrophobic interaction chromatography, anion exchange chromatography, and cellulose phosphate affinity chromatography. The purification procedure is briefly described below.

Approximately 400 g of grain of MON 87427 was mixed with 400 g of dry ice and then ground using a laboratory mill (Perten Instruments, model 3100). The ground powder (~400 g) was stored in a -80 ºC freezer until used for extraction of the CP4 EPSPS protein. The ground powder was mixed with extraction buffer (100 mM Tris-HCl, pH 7.5, 2 mM EDTA, 2 mM benzamidine-HCl, 4 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 1% polyvinylpolypyrrolidone and 10% glycerol) for ~1 h at a sample weight (g) to buffer volume (ml) of approximately 1:10. The slurry was centrifuged at 15,000 x *g* for 60 min at ~4 ºC. The supernatant (~3.8 liters) was collected and brought to 40% ammonium sulfate saturation by slow addition of 859 g of ammonium sulfate in a cold room (~ 4 ºC). The solution was stirred for ~1 h at ~ 4 ºC and then centrifuged at 15,000 x *g* for 45 min. The supernatant (~3.8 liters) was again collected and 710 g of ammonium sulfate was added to bring the solution to 70% ammonium sulfate saturation. The solution was stirred for ~ 1 h in a cold room and the pellet was collected by centrifugation at 15,000 x *g* for 60 min. The pellet was re-suspended in 750 ml of PS(A) buffer [50 mM Tris-HCl, pH 7.5, 1 mM DTT, 10% glycerol (v/v), 1.5 M ammonium sulfate]. The sample was loaded onto a 471 ml column (5 cm x 24 cm) of Phenyl Sepharose™ Fast Flow (GE Healthcare, Piscataway, NJ) equilibrated with PS(A) buffer. Proteins were eluted with a linear salt gradient that decreased from 1.5 M to 0 M ammonium sulfate over a volume of 2400 ml. Fractions containing the CP4 EPSPS protein, identified based on Western blot analysis, were pooled to a final volume of ~225 ml. The pooled sample was desalted by dialysis against 4 liters of QS(A) buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 1 mM benzamidine-HCl, 4 mM DTT) at ~4 °C with one additional 4 liters buffer change using a dialysis tubing [Spectrum Laboratories, Inc., Rancho Dominguez, CA; Molecular Weight Cutoff (MWCO): 3.5 kDa] for a total of 24 h.

The desalted sample (310 ml) was loaded onto a 48 ml column (2.6 cm x 9 cm) of Q Sepharose™ Fast Flow anion exchange resin (GE Healthcare, Piscataway, NJ) equilibrated with QS(A) buffer. The bound CP4 EPSPS protein was eluted with a linear salt gradient that increased from 0 M to 0.4 M KCl in QS(A) buffer over 600 ml. Fractions containing CP4 EPSPS, identified by Western blot analysis, were pooled to a final volume of ~ 110 ml. The pooled sample was dialyzed against 2 liters CP2(A) buffer (10 mM sodium citrate, pH 5.0, 1 mM benzamidine-HCl, 2 mM DTT) for a total of 36 h at ~4 °C with 2 additional 2 liters buffer changes using a dialysis tubing (Spectrum Laboratories, Inc. Rancho Dominguez, CA; MWCO: 3.5 kDa).

The dialyzed sample (120 ml) was then loaded onto a 32 ml column (2.6 x 6 cm) of cellulose phosphate P11 cation exchange (Whatman) pre-equilibrated with CP2(A) buffer. After an initial wash with 300 ml of CP2(A), the column was washed with a linear gradient that increased from 0 to 100% UGN50 buffer (10 mM sodium citrate, 1 mM benzamidine, 50 mM NaCl, 0.3 mM UTP, 0.3 mM glucose-1-phosphate, and 4 mM DTT, pH 5.0) over 32 ml and was held at 100% for ~70 ml. The column was further washed with a linear gradient that increased from 0 to 100% PEP buffer (10 mM sodium citrate, 1 mM benzamidine, 50 mM NaCl, 0.3 mM phosphoenolpyruvate (PEP), 4 mM DTT, pH 5.3) over 32 ml and was held at 100% for ~140 ml. The bound CP4 EPSPS protein was eluted with a linear gradient that increased from 0-100% PEP/S3P buffer (10 mM sodium citrate, 1 mM benzamidine, 50 mM NaCl, 0.5 mM PEP, and 0.5 mM

shikimate-3-phosphate (S3P), 4 mM DTT, pH 5.7) over 32 ml and was held at 100% for ~130 ml. Fractions containing CP4 EPSPS protein, based on SDS PAGE analysis and confirmed by Western blot analysis, were pooled (~27 ml). The pooled sample was divided between four iCon™ Concentrators (MWCO: 20 kDa; size: 7 ml; Pierce, Rockford, IL) and concentrated by centrifugation at 4,000 x *g* for 30 min at ~4 °C. Buffer exchange was carried out in the same units by the addition of ~6.5 ml FSB buffer (50 mM Tris-HCl, pH 7.5, 50 mM KCl, 2 mM DTT, 1 mM benzamidine-HCl) followed by centrifugation at 4,000 x *g* for 30 min at ~4 °C. The exchange was conducted a total of four times, and during the final exchange, the sample was concentrated to ~0.2 ml per unit. The samples were pooled (~0.8 ml) and mixed with 0.8 ml FSB buffer (containing 50% glycerol) to final volume of 1.6 ml. Final buffer composition of the sample was: 50 mM Tris-HCl, pH7.5, 50 mM KCl, 2 mM DTT, 1 mM benzamidine-HCl and 25% glycerol. The concentration of the MON 87427-produced CP4 EPSPS protein was determined to be 0.1 mg/ml based on the Bio-Rad protein assay. The CP4 EPSPS protein purified from the grain of MON 87427 was aliquoted and stored at in a -80°C freezer.

C.4. Molecular Weight and Purity Estimation-SDS-PAGE

C.4.1. Methods

An aliquot of the test substance was mixed with 5X Laemmli (LB) to a final total protein concentration of 0.08 µg/µl. Molecular weight markers (Bio-Rad broad-range) and reference substance were diluted to a final total protein concentration of 0.9 and 0.15 µg/µl, respectively. The test substance was analyzed in duplicate at 0.75, 1.5, and 2.25 µg protein per lane. The *E. coli*-produced CP4 EPSPS reference standard was analyzed at 0.75 µg total protein in a single lane. All samples were heated at ~100 °C for 3 min and loaded onto a 10-well pre-cast Tris glycine 4 - 20% polyacrylamide gradient mini-gel (Invitrogen, Carlsbad, CA). Electrophoresis was performed at a constant 150 volts (V) for 95 min. Proteins were fixed by placing the gel in a solution of 40% (v/v) methanol and 7% (v/v) acetic acid for 30 min, stained for 16 h and 40 min with Brilliant Blue G-Colloidal stain (Sigma-Aldrich, St. Louis, MO). Gels were destained for 30 sec with a solution containing 10% (v/v) acetic acid and 25% (v/v) methanol, and for 6 h and 15 min with 25% (v/v) methanol. Analysis of the gel was performed using a Bio-Rad GS-800 densitometer with the supplied Quantity One® software (version 4.4.0, Hercules, CA). The apparent molecular weight of each observed band was estimated from a standard curve generated by the Quantity One software which was based on the molecular weights of the markers and their migration distance on the gel. All visible bands within each lane were quantified using Quantity One software. Apparent molecular weight and purity were reported as an average of all six lanes containing the MON 87427-produced CP4 EPSPS protein.

C.4.2. Results of CP4 EPSPS Protein Molecular Weight Equivalence

For molecular weight and purity analysis, the MON 87427-produced CP4 EPSPS protein was separated using SDS-PAGE. The gel stained with Brilliant Blue G Colloidal stain and analyzed by densitometry (Figure C-1). The MON 87427-produced CP4 EPSPS protein (Figure C-1, lanes 3-8) migrated to the same position on the gel as the *E. coli*-

produced CP4 EPSPS reference standard (Figure C-1, lanes 2) and had an apparent molecular weight of 44.1 kDa (Table C-1). The apparent molecular weight of the *E. coli*-produced CP4 EPSPS reference standard, as reported on the certificate of analysis (COA), is 43.8 kDa. The difference in apparent molecular weight between the MON 87427- and *E. coli*-produced CP4 EPSPS proteins was 0.7% (Table C-1). Because this difference met the previously set acceptance criteria (≤10% difference), the MON 87427- and *E. coli*-produced CP4 EPSPS proteins are considered equivalent based on their experimentally estimated apparent molecular weights.

The purity of the MON 87427-produced CP4 EPSPS protein was calculated based on the six loads on the gel (Figure C-1, lanes 3 to 8). The average purity was determined to be 96%.

Table C-1. Molecular Weight of the MON 87427- and *E. coli*-produced CP4 EPSPS Proteins

Molecular Weight of MON 87427-Produced CP4 EPSPS Protein	Molecular Weight of E. coli-Produced CP4 EPSPS Protein	% Difference from E. coli-Produced CP4 EPSPS Protein[3]
44.1 kDa	43.8 kDa	0.7%

[3]% Difference= | (MW plant - MW *E. coli*)/MW plant | X 100%

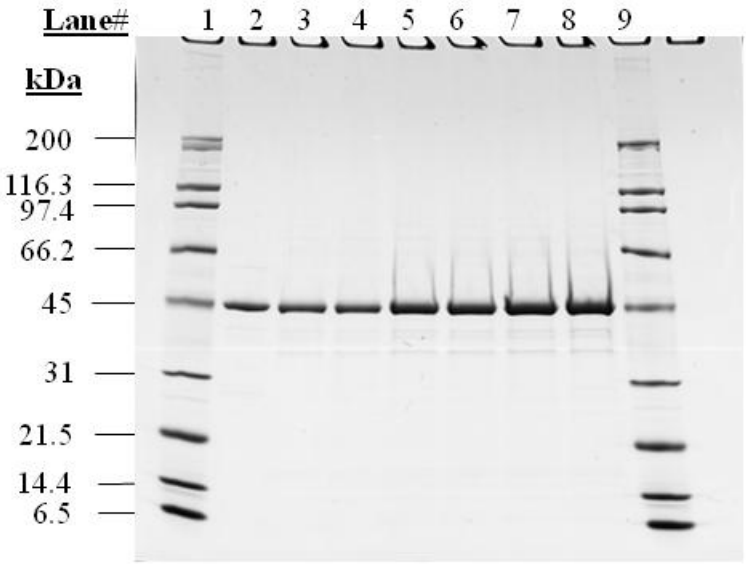

Figure C-1. Molecular Weight and Purity Analysis of the MON 87427-produced CP4 EPSPS Protein
Aliquots of the MON 87427- and the *E. coli*-produced CP4 EPSPS proteins were separated on a 4-20% Tris glycine polyacrylamide gradient gel and then stained with Brilliant Blue G-Colloidal stain. Approximate molecular weights (kDa) are shown on the left and correspond to the markers loaded in Lanes 1 and 9. An empty lane on the right of the gel was partially cropped.

Lane	Sample	Amount (µg)
1	Broad Range MW markers	4.5
2	*E. coli*-produced CP4 EPSPS reference standard	0.75
3	MON 87427-produced CP4 EPSPS protein	0.75
4	MON 87427-produced CP4 EPSPS protein	0.75
5	MON 87427-produced CP4 EPSPS protein	1.5
6	MON 87427-produced CP4 EPSPS protein	1.5
7	MON 87427-produced CP4 EPSPS protein	2.25
8	MON 87427-produced CP4 EPSPS protein	2.25
9	Broad Range MW markers	4.5
10	Empty Lane	

C.5. Western Blot Analysis-Immunoreactivity

C.5.1. Methods

Western blot analysis was performed to confirm the identity of the CP4 EPSPS protein purified from grain of MON 87427 and to compare the immunoreactivity of the MON 87427- and *E. coli*-produced proteins.

The MON 87427- and *E. coli*-produced CP4 EPSPS proteins were analyzed concurrently on the same gel using three loadings of 1, 2, and 3 ng. Loadings of the three concentrations of the test and reference proteins were made in duplicate on the gel. Aliquots of each protein were diluted in water and 5X LB containing 312 mM Tris-HCl, 20% (v/v) 2-mercaptoethanol, 10% (w/v) sodium dodecyl sulfate, 0.025% (w/v) bromophenol blue, 50% (v/v) glycerol, pH 6.8), heated at ~99°C for 3 min, and applied to a 15 well pre-cast Tris-glycine 4 - 20% polyacrylamide gradient gel (Invitrogen, Carlsbad, CA). Pre-stained molecular weight markers (Precision Plus Protein Standards Dual color; Bio-Rad, Hercules, CA) were loaded in parallel to verify electrotransfer of the proteins to the membrane and to estimate the size of the immunoreactive bands observed. Electrophoresis was performed at a constant 150 V for 90 min. Electrotransfer to a 0.45 µm PVDF membrane (Invitrogen, Carlsbad, CA) was performed for 100 min at a constant 25 V. After electrotransfer, the membrane was blocked for 1 h with 5% (w/v) non-fat dried milk (NFDM) in 1X phosphate buffered saline containing 0.05% (v/v) Tween-20 (PBST). The membrane was then probed with a 1:1000 dilution of goat anti-CP4 EPSPS antibody (lot 10000787) in 5% (w/v) NFDM in PBST for 1 h. Excess antibody was removed using three 10 min washes with PBST. Finally, the membrane was probed with horseradish peroxidase-conjugated rabbit anti-goat IgG (Thermo, Rockford, IL) at a dilution of 1:10,000 in 5% (w/v) NFDM in PBST for 1 h. Excess horseradish peroxidase-conjugate was removed using three 10 min washes with PBST. All incubations were performed at room temperature. Immunoreactive bands were visualized using the Amersham ECL™ Western Blotting Detection Reagents (GE, Healthcare, Piscataway, NJ) with exposure (1, 3, and 5 min) to Amersham Hyperfilm ECL™ (GE, Healthcare, Piscataway, NJ). The film was developed using a Konica SRX-101A automated film processor (Tokyo, Japan).

Quantification of the bands on the blot was performed using a Bio-Rad GS-800 densitometer with the supplied Quantity One software (version 4.4.0, Hercules, CA) using the lane finding and contour tool. The signal intensities of the immunoreactive bands observed for the test and reference proteins migrating at the expected position on the blot film were quantified as "contour quantity" values. The raw data was exported to a Microsoft Excel [2007 (12.0.6504.5001) SP1 MSO (12.0.6320.5000)] file for the pair wise comparison of the average of the load replicates. An average difference was calculated for each comparison to assess the immunoreactivity equivalence.

C.5.2. Results of CP4 EPSPS Protein Immunoreactivity Equivalence

Immunoreactive bands of comparable intensity migrating at the expected apparent MW were observed for lanes loaded with either the MON 87427-produced (Figure C-2, lanes

9-14) or *E. coli*-produced CP4 EPSPS proteins (Figure C-2, lanes 2-7). As expected, the signal intensity increased with increasing amounts of the MON 87427- and *E. coli*-produced proteins loaded on the gel. No additional bands were observed in either protein sample. Hence, the western blot analysis confirmed the identity of the MON 87427-produced CP4 EPSPS protein. Densitometric analysis of the bands showed an average difference of 9.6% between the intensity of the signals from the MON 87427-produced CP4 EPSPS protein and the signals from the *E. coli*-produced CP4 EPSPS reference standard (Table C-2). Because the difference was within the previously set acceptance criterion of ± 35%, MON 87427- and *E. coli*-produced proteins are considered to have equivalent immunoreactivity.

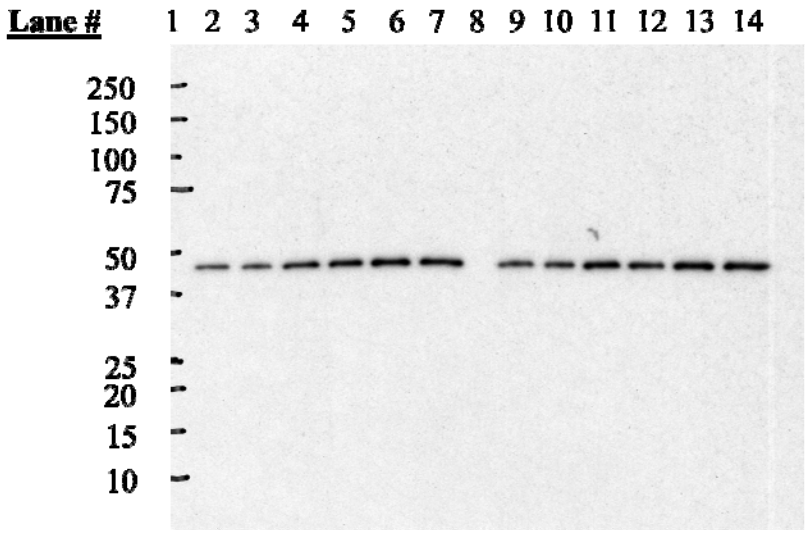

Figure C-2. Western Blot Analysis of MON 87427- and *E. coli* -produced CP4 EPSPS Proteins

Aliquots of the MON 87427-produced CP4 EPSPS protein and the *E. coli*-produced CP4 EPSPS reference standard were separated by SDS-PAGE and electrotransferred to a PVDF membrane. The membrane was incubated with anti-CP4 EPSPS antibodies and immunoreactive bands were visualized using an ECL system and film. Approximate MWs (kDa) are shown on the left and correspond to the markers loaded in lane 1. The 5 min exposure is shown.

Lane	Sample	Amount (ng)
1	Precision Plus Protein Standards Dual color	-
2	*E. coli*-produced CP4 EPSPS reference standard	1
3	*E. coli*-produced CP4 EPSPS reference standard	1
4	*E. coli*-produced CP4 EPSPS reference standard	2
5	*E. coli*-produced CP4 EPSPS reference standard	2
6	*E. coli*-produced CP4 EPSPS reference standard	3
7	*E. coli*-produced CP4 EPSPS reference standard	3
8	Empty	
9	MON 87427-produced CP4 EPSPS protein	1
10	MON 87427-produced CP4 EPSPS protein	1
11	MON 87427-produced CP4 EPSPS protein	2
12	MON 87427-produced CP4 EPSPS protein	2
13	MON 87427-produced CP4 EPSPS protein	3
14	MON 87427-produced CP4 EPSPS protein	3

Table C-2. Comparison of Immunoreactive Signal Between MON 87427- and *E. coli*-produced CP4 EPSPS Proteins.

Sample	*Gel lane*	*Amount (ng)*	*Contour Quantity*	*Average Contour Quantity*[1]	*Percent difference*[2] *(%)*	*Average Difference*[3] *(%)*
E. coli CP4 EPSPS	2	1	1.201	1.106	14.96	9.6
E. coli CP4 EPSPS	3	1	1.011			
				1.3005		
Plant CP4 EPSPS	9	1	1.346			
Plant CP4 EPSPS	10	1	1.255			
				2.308	6.46	
E. coli CP4 EPSPS	4	2	2.130			
E. coli CP4 EPSPS	5	2	2.486			
				2.4675		
Plant CP4 EPSPS	11	2	2.829			
Plant CP4 EPSPS	12	2	2.106			
				3.388	7.37	
E. coli CP4 EPSPS	6	3	3.310			
E. coli CP4 EPSPS	7	3	3.466			
				3.6575		
Plant CP4 EPSPS	13	3	3.433			
Plant CP4 EPSPS	14	3	3.882			

[1]Average Contour Quantity =∑(Contour Quantity)/2

[2]Percent Difference (%) = $\frac{|\text{Average Contour Quantity plant} - \text{Average Contour Quantity E.coli}|}{\text{Average Density plant}}$ X 100%

[3]Average difference (%) =∑ 〖% difference〗 /3.

C.6. MALDI-TOF Tryptic Mass Map Analysis

C.6.1. Methods

MALDI-TOF tryptic mass fingerprint analysis was used to confirm the identity of the MON 87427-produced CP4 EPSPS protein. MON 87427-produced CP4 EPSPS protein was subjected to SDS-PAGE and the gel was stained using Brilliant Blue G Colloidal stain. Each ~44 kDa band was excised, transferred to a microcentrifuge tube, and destained with 40% methanol/10% glacial acetic acid followed by10% acetonitrile in 25 mM ammonium bicarbonate. The gel bands were washed in 100 mM ammonium bicarbonate and then, to reduce the protein in each, gel bands were incubated in 100 µl of 10 mM DTT at ~37°C for 2 h. The protein was then alkylated in the dark for 2 h with 100 µl of 20 mM iodoacetic acid and washed with 200 µl of 25 mM ammonium bicarbonate for 1 h once and for 15 min twice. Gel bands were dried with a Speed-Vac® concentrator and then rehydrated with 20 µl of trypsin solution (20 µg/ml). After 1 h, excess liquid was removed and the gel was incubated at 37.6 °C for 16 h in 40 µl of 10% acetonitrile in 25 mM ammonium bicarbonate. To elute proteolytic fragments, gel bands were sonicated for 5 min. The resulting extracts were transferred to new microcentrifuge tubes labeled Extract 1 and dried using Speed-Vac concentrator. The gel bands were re-extracted twice with 30 µl of a 60% acetonitrile, 0.1% trifluoroacetic acid, 0.1% β-octyl-glucopyranoside solution and sonicated for 5 min. Both 60% acetonitrile, 0.1% trifluoroacetic acid, 0.1% β-octyl-glucopyranoside extracts were pooled into a new tube labeled Extract 2 and dried with a Speed-Vac concentrator. A solution of 0.1% trifluoroacetic acid (TFA) was added to all Extract 1 and 2 tubes and they were dried as before. To acidify the extracts, a solution of 50% acetonitrile, 0.1% trifluoroacetic acid was added to each tube and all were sonicated for 5 min. Each extract (0.3 µl) was spotted to three wells on an analysis plate. For each extract 0.75 µl of 2, 5-dihydroxybenzoic acid (DHB), α-cyano-4-hydroxycinnamic acid (α-Cyano), or 3, 5-dimethoxy-4-hydroxycinnamic acid (Sinapinic acid) (Waters Corp., Milford, MA) was added to one of the spots. The samples in DHB matrix were analyzed in the 300 to 7500 Dalton (Da) range. Samples in α-Cyano and Sinapinic acid were analyzed in the 500 to 5000 and 500 to 7500 Da range, respectively. Protonated (MH+) peptide masses were monoisotopically resolved in reflector mode (Aebersold, 1993; Billecci and Stults, 1993). Calibration mixture 2 was used as the external calibrant (Sequazyme™ Peptide Mass Standards kit, Applied Biosystems, Foster City, CA) for the analysis. GPMAW32 software (Lighthouse Data, Odense M, Denmark) was used to generate a theoretical trypsin digest of the CP4 EPSPS protein sequence. Masses were calculated for each theoretical peptide and compared to the raw mass data. Known autocatalytic fragments from trypsin digestion and apparent modifications were identified in the raw data. The list of experimental masses was then compared to the theoretical list from the GPMAW software. Those experimental masses within 1 Da of a theoretical mass were matched. All matching masses were tallied and a coverage map was generated for the mass fingerprint. The tryptic mass fingerprint coverage was considered acceptable if ≥ 40 % of the protein sequence was identified by matching experimental masses observed for the tryptic peptide fragments to the expected masses for the fragments.

C.6.2. Results of MALDI-TOF Tryptic Mass Map Analysis

The identity of the MON 87427-produced CP4 EPSPS protein was also confirmed by MALDI-TOF mass spectrometry analysis of tryptic peptide fragments prepared from the MON 87427-produced CP4 EPSPS protein. The ability to identify a protein using this method is dependent upon matching a sufficient number of observed tryptic peptide fragment masses with predicted tryptic peptide fragment masses. In general, protein identification made by peptide mapping is considered to be reliable if the measured coverage of the sequence is 15% or higher with a minimum of five matched peptides (Jensen, 1997).

There were 26 unique peptides identified that corresponded to the expected masses of peptides produced from trypsin-digested CP4 EPSPS (Table C-3). The identified masses were used to assemble a mass fingerprint map of the entire CP4 EPSPS protein (Figure C-3). The experimentally determined mass coverage of the CP4 EPSPS protein was 70.3% (320 out of 455 amino acids). This analysis serves as additional identity confirmation for the MON 87427-produced CP4 EPSPS protein.

Table C-3. Summary of the Tryptic Masses Identified for the MON 87427-produced CP4 EPSPS Using MALDI-TOF Mass Spectrometry.

Matrix									
α-Cyano	α-Cyano	DHB	DHB	Sinapinic acid	Sinapinic acid	**Expected Mass[1]**	**Diff.[2]**	**AA Position[3]**	**Fragment**
Extract 1	Extract 2	Extract 1	Extract 2	Extract 1	Extract 2				
506.08						506.22	0.14	354-357	ESDR
599.17						599.33	0.16	29-33	SISHR
616.17	616.32				615.67	616.34	0.17	128-132	RPMGR
629.16						629.29	0.13	201-205	DHTEK
629.16						629.34	0.18	383-388	GRPDGK
711.26	711.43	711.30				711.45	0.19	133-138	VLNPLR
835.17						835.39	0.22	62-69	AMQAMGAR
863.23						863.46	0.23	15-23	SSGLSGTVR
872.21		872.29				872.45	0.24	313-320	GVTVPEDR
872.21		872.29				872.52	0.31	358-366	LSAVANGLK
948.26	948.48	948.32	948.44			948.52	0.26	161-168	TPTPITYR
991.29						991.55	0.26	14-23	KSSGLSGTVR
1115.27		1115.36		1114.83		1115.57	0.30	295-305	LAGGEDVADLR
1357.32	1357.65	1357.44				1357.71	0.39	146-157	SEDGDRLPVTLR
1359.27	1359.58	1359.39	1359.56	1358.90		1359.72	0.45	354-366	ESDRLSAVANGLK
1359.27	1359.58	1359.39	1359.56	1358.90		1359.64	0.37	34-46	SFMFGGLASGETR
		1558.50	1558.65			1558.83	0.35	47-61	ITGLLEGEDVINTGK
1646.34	1646.70	1646.52	1646.92			1646.84	0.50	389-405	GLGNASGAAVATHLDHR
1763.29						1763.81	0.52	367-382	LNGVDCDEGETSLVVR
1993.38	1993.80	1993.60	1993.68	1993.21		1993.97	0.59	206-224	MLQGFGANLTVETDADGVR
2182.54	2183.00	2182.77	2182.92	2182.40	2182.84	2183.17	0.63	275-294	TGLILTLQEMGADIEVINPR
2366.61	2367.14	2366.86	2366.96	2366.66		2367.33	0.72	178-200	SAVLLAGLNTPGITTVIEPIMTR
				2449.44		2450.23	0.79	24-46	IPGDKSISHRSFMFGGLASGETR
				2449.44		2450.22	0.78	105-127	LTMGLVGVYDFDSTFIGDASLTK
3250.78(AVE)		3251.23(AVE)		3250.80(AVE)	3252.37(AVE)	3251.75	0.97	321-351	APSMIDEYPILAVAAAFAEGATVMNGLEELR
		4190.17(AVE)		4190.98(AVE)	4190.14(AVE)	4180.89	0.72	234-274	LTGQVIDVPGDPSSTAFPLVAALLVPGSDVTILNVLMNPTR

[1]Only experimental masses that matched expected masses are listed in the table.

[2]The numbers represent the difference between the expected mass and the experimental mass listed within the first row. Other experimental masses shown within a row also met the criteria of being within 1 Da of the expected mass.

[3]AA position refers to amino acid position within the predicted CP4 EPSPS protein sequence as depicted in Figure C-3.

AVE indicates that the experimental mass average of the observed peptide was compared to the expected peptide masses. For larger peptides the monoisotopic mass is, in general, poorly resolved and therefore the mass average is used for comparison.

001 MLHGASSRPA TARKSSGLSG TVRIPGDKSI SHRSFMFGGL ASGETRITGL

051 LEGEDVINTG KAMQAMGARI RKEGDTWIID GVGNGGLLAP EAPLDFGNAA

101 TGCRLTMGLV GVYDFDSTFI GDASLTKRPM GRVLNPLREM GVQVKSEDGD

151 RLPVTLRGPK TPTPITYRVP MASAQVKSAV LLAGLNTPGI TTVIEPIMTR

201 DHTEKMLQGF GANLTVETDA DGVRTIRLEG RGKLTGQVID VPGDPSSTAF

251 PLVAALLVPG SDVTILNVLM NPTRTGLILT LQEMGADIEV INPRLAGGED

301 VADLRVRSST LKGVTVPEDR APSMIDEYPI LAVAAAFAEG ATVMNGLEEL

351 RVKESDRLSA VANGLKLNGV DCDEGETSLV VRGRPDGKGL GNASGAAVAT

401 HLDHRIAMSF LVMGLVSENP VTVDDATMIA TSFPEFMDLM AGLGAKIELS

451 DTKAA

Figure C-3. MALDI-TOF MS Coverage Map of the MON 87427-produced CP4 EPSPS.
The amino acid sequence of the mature CP4 EPSPS protein was deduced from the *cp4 epsps* gene present in MON 87427. Boxed regions correspond to tryptic peptides that were identified from the MON 87427-produced CP4 EPSPS protein sample using MALDI-TOF MS. In total, 70.3% (320 of 455 total amino acids) of the expected protein sequence was identified.

C.7. MALDI-TOF Mass Analysis of MON 87427-produced CP4 EPSPS Protein

C.7.1. Methods

MALDI-TOF mass spectrometry was used to further characterize the MON 87427-produced CP4 EPSPS. Prior to MALDI-TOF MS analysis, an ethanol precipitation was performed to concentrate the MON 87427-produced CP4 EPSPS protein sample and remove buffer components that interfere with the MALDI-TOF MS analysis. The precipitated protein was re-suspended in 5 µl 60% formic acid. A portion of the MON 87427-produced CP4 EPSPS protein sample, and a BSA protein standard (0.3 µl each), were spotted on an analysis plate, mixed with 0.75 µl of Sinapinic acid solution containing 0.3% TFA and air-dried. Mass spectral analysis of the MON 87427-produced CP4 EPSPS protein was performed using an Applied Biosystems Voyager DE™ Pro Biospectrometry™ Workstation MALDI-TOF MS instrument with the supplied Data Explorer software (version 4.0.0.0, Foster City, CA). Mass calibration of the instrument was performed using the BSA protein standard. The sample was analyzed in the 2,000 to 100,000 Da range using 150 shots at a laser intensity setting of 3316 (unit-less MALDI-TOF instrument specific value). Average protonated (MH+) protein masses were observed in linear mode (Aebersold, 1993; Billecci and Stults, 1993). GPMAW32 software (Lighthouse Data, Odense M, Denmark) was used to generate a theoretical mass of the expected CP4 EPSPS protein sequence based upon the nucleotide sequence. The mass of the MON 87427-produced CP4 EPSPS protein was reported as an average of three separate mass spectral acquisitions.

C.7.2. Results of MALDI-TOF Mass Analysis of MON 87427-produced Protein

The intact mass of the MON 87427-produced CP4 EPSPS protein was also determined by MALDI-TOF MS analysis. The average obtained from three measurements of the intact mass of the MON 87427-produced CP4 EPSPS protein was 47552 Da. The theoretical mass of the full-length protein without N-terminal methionine is 47481 Da. The difference between the measured and theoretical masses is less than 0.15% and within the accuracy window (± 0.4%) of the MALDI-TOF MS instrument.

C.8. N-Terminal Sequencing

C.8.1. Methods

N-terminal sequencing, carried out by automated Edman degradation chemistry, was used to confirm the identity of the MON 87427-produced CP4 EPSPS.

MON 87427-produced CP4 EPSPS was separated by SDS-PAGE and transferred to PVDF membrane. The blot was stained using Coomassie Brilliant Blue R-250 (Bio-rad, Hercules, CA). The major band at ~44 kDa containing the test protein was excised from the blot and was used for N-terminal sequence analysis. The analysis was performed for 15 cycles using automated Edman degradation chemistry (Hunkapillar et al., 1983). An Applied Biosystems 494 Procise® Sequencing System with 140C Microgradient pump and 785 Programmable Absorbance Detector was controlled with Procise Control (version 1.1a) software. Chromatographic data were collected using Atlas 2003 software

(version 3.59a, LabSystems, Altrincham, Cheshire, England). A control protein (10 picomoles of β-lactoglobulin, Applied Biosystems, Foster City, CA) was analyzed before and after the sequence analysis of the CP4 EPSPS protein to verify that the sequencer met performance criteria for repetitive yield and sequence identity. Identity was established if ≥8 amino acids, consistent with the predicted sequence of the N-terminus of the MON 87427-produced CP4 EPSPS, were observed during analysis.

C.8.2. Results of the N-terminal Sequence Analysis

N-terminal sequencing performed on the MON 87427-produced CP4 EPSPS protein resulted in 15 amino acid residues being determined (Table C-4). The sequence obtained is identical to that of the mature CP4 EPSPS protein deduced from the *cp4 epsps* gene present in grain of MON 87427 after processing of the chloroplast transit protein and the N-terminal methionine (Giglione and Meinnel, 2001). The N-terminal sequence information, therefore, confirms the identity of the CP4 EPSPS protein isolated from the grain of MON 87427.

Table C-4. N-Terminal Sequence of the MON 87427-produced CP4 EPSPS

Amino acid residue # from the N-terminus →	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Expected Sequence[1] →	M	L	H	G	A	S	S	R	P	A	T	A	R	K	S	S
	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|
Experimental Sequence[1] →	-	L	H	G	A	S	S	R	P	A	T	A	R	K	S	S

[1]The expected amino acid sequence of the N-terminus of the mature CP4 EPSPS protein was deduced from the cp4 epsps gene present in MON 87427. The experimental sequence obtained from the MON 87427-produced CP4 EPSPS was compared to the expected sequence beginning at position 2.

C.9. Glycosylation Analysis

C.9.1. Methods

Glycosylation analysis was used to determine whether the MON 87427-produced CP4 EPSPS protein was post-translationally modified with covalently bound carbohydrate moieties. Aliquots of the MON 87427-produced CP4 EPSPS protein, the *E. coli*-produced CP4 EPSPS reference standard, and the positive controls, transferrin (GE Healthcare, Piscataway, NJ) and horseradish peroxidase (Sigma-Aldrich, St Louis, MO), were each diluted with water and mixed with 5X LB. These samples were heated at ~98 °C for 3 min, cooled, and each was loaded at approximately 30 and 60 ng per lane on a Tris-glycine 10-well 4 - 20% polyacrylamide gradient mini-gel (Invitrogen, Carlsbad, CA). Precision Plus Protein Dual color Standards (Bio-Rad, Hercules, CA) were also loaded to verify electrotransfer of the proteins to the membrane and CandyCane™ Glycoprotein Molecular Weight Standards (Molecular Probes, Eugene, OR) were loaded as positive controls and markers for molecular weight. Electrophoresis was performed at a constant 150 V for 80 min. Electrotransfer to a 0.45 µm PVDF membrane (Invitrogen, Carlsbad, CA) was performed for 105 min at a constant 25 V.

Carbohydrate detection was performed directly on the PVDF membrane at room temperature using the Pro-Q® Emerald 488 Glycoprotein Gel and Blot Stain Kit (Molecular Probes, Eugene, OR). With this kit, carbohydrate moieties are detected by fluorescence which is produced when Pro-Q Emerald 488 glycoprotein stain reacts with periodate oxidation carbohydrates conjugated to proteins. An image of the final blot containing the fluorescent-labeled glycoproteins was captured using the Bio-Rad PharosFX™ Molecular Imager® System using the Alexa 488 band pass setting and equipped with Quantity One software (version 4.6).

After glycosylation analysis the blot was stained to visualize the proteins present on the membrane. Proteins were stained for 30 sec to 2 min using Coomassie Brilliant Blue R-250 staining solution (Bio-Rad, Hercules, CA) and then destained with 1X destain solution (Bio-Rad, Hercules, CA) for 5 min. After washing with water, the blot was scanned using Bio-Rad GS-800 densitometer with the supplied Quantity One software (version 4.4.0, Hercules, CA).

C.9.2. Results of Glycosylation Analysis

Many eukaryotic proteins undergo post-translational modification with carbohydrate moieties (Rademacher et al., 1988). These carbohydrate moieties may be complex, branched polysaccharide structures or simple monosaccharides. In contrast, glycosylation in prokaryotes is uncommon. In *E. coli*, the organism used to produce the reference protein, only a few specific proteins have been confirmed to be glycosylated (Sherlock et al., 2006). To test whether potential post-translational glycosylation of the MON 87427-produced CP4 EPSPS protein occurred, it was analyzed for the presence of covalently bound carbohydrate moieties. The *E. coli*-produced CP4 EPSPS reference standard was used as a negative control since it has previously been shown to be free of glycosylation (Harrison et al., 1996). Horseradish peroxidase and transferrin were both

used as positive controls. Both the negative and positive controls were analyzed concurrently with the MON 87427-produced CP4 EPSPS protein. The results of this analysis are shown in Figure C-4. The positive controls were clearly detected at the expected molecular weights, in a concentration-dependent manner (Figure C-4A, lanes 2-5). Faint signals at a level slightly above the background noise were observed for the reference protein as well as the test protein at the molecular weight expected for CP4 EPSPS (Figure C-4A, lanes 6-9). Because the *E. coli*-produced CP4 EPSPS protein has previously shown to be free of glycosylation (Harrison et al., 1996), the weak signal observed for both this protein as well as the MON 87427-produced CP4 EPSPS test protein are not indicative of glycosylated species. Other data reported here demonstrated the absence of glycosylation of the MON 87427-produced CP4 EPSPS. In particular, glycosylation would result in an increase in the protein mass relative to the theoretically calculated mass. The agreement of the observed protein mass of the MON 87427-produced CP4 EPSPS protein (47552 Da) as detected by MALDI-TOF mass spectrometric analysis to the theoretical mass (47481 Da) does not support the existence of a glycosylated species, as the addition of even a single sugar would increase the mass by at least 160 Da. Finally, to confirm that the proteins were transferred to the membrane, the same membrane was stained with Coomassie Blue R 250 and scanned again (Figure C-4B). The resulting image demonstrates that the CP4 EPSPS protein was efficiently transferred to the membrane. Thus, the data cited above demonstrate that MON 87427-produced protein is not glycosylated and is equivalent to the *E. coli*-produced CP4 EPSPS reference standard.

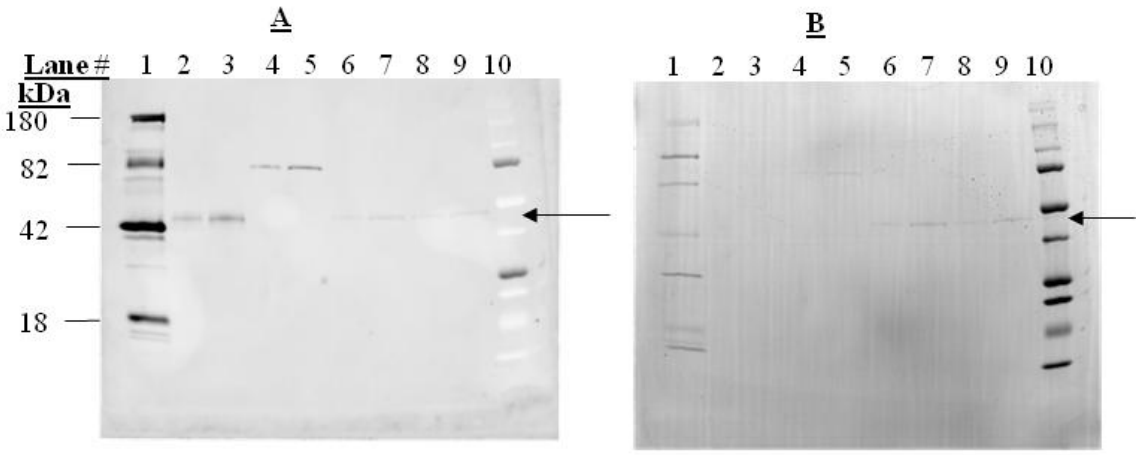

Figure C-4. Glycosylation Analysis of the MON 87427-produced CP4 EPSPS Protein

Aliquots of the MON 87427-produced CP4 EPSPS protein, *E. coli*-produced CP4 EPSPS reference standard (negative control), horseradish peroxidase (positive control) and transferrin (positive control) were separated by SDS-PAGE (4-20%) and electrotransferred to a PVDF membrane. (A) Where present, periodate-oxidized protein-bound carbohydrate moieties reacted with Pro-Q Emerald 488 glycoprotein stain and emitted a fluorescent signal at 488 nm. The signal was captured using a Bio-Rad Molecular Imager FX. (B) The same blot was stained with Coomassie Blue R-250 to confirm the presence of proteins. The signal was captured using a Bio-Rad GS800 with Quantity One software (version 4.4.0). Approximate MWs (kDa) correspond to the glycosylated markers loaded in Lane 1 and the dual color markers (used to verify transfer) in Lane 10. Arrows indicate the band corresponding to CP4 EPSPS proteins.

Lane	Sample	Amount (ng)
1	CandyCane Glycoprotein MW standards	—
2	Horseradish Peroxidase (positive control)	30
3	Horseradish Peroxidase (positive control)	60
4	Transferrin (positive control)	30
5	Transferrin (positive control)	60
6	MON 87427-produced CP4 EPSPS	30
7	MON 87427-produced CP4 EPSPS	60
8	*E. coli*-produced CP4 EPSPS (negative control)	30
9	*E. coli*-produced CP4 EPSPS (negative control)	60
10	Precision Plus Protein™ Standards Dual color	—

C.10. Functional Activity Analysis

C.10.1. Methods

Prior to functional activity analysis, both MON 87427- and *E.coli*-produced proteins were diluted to a purity corrected concentration of ~50 µg/ml with a 50 mM HEPES, pH 7.0 buffer. Assays for both proteins were conducted in triplicate. The reactions were performed in 50 mM HEPES (pH 7.0), 0.1 mM ammonium molybdate, 1 mM PEP and 5 mM potassium fluoride with or without 2 mM S3P for 2 min at ~25 °C. The reactions were initiated by the addition of PEP. After 2 min, the reactions were quenched with malachite green (phosphate assay reagent) and then fixed with 33% (w/v) sodium citrate. A standard curve was prepared using 0 to 10 nmoles of inorganic phosphate in water treated with the malachite green (phosphate assay) reagent and 33% (w/v) sodium citrate. The absorbance of each reaction and each standard was measured in duplicate at 660 nm using a PowerWave™ Xi (BioTek, Richmond, VA) microplate reader. The amount of inorganic phosphate released from PEP in each reaction was determined using the standard curve. For CP4 EPSPS, the specific activity was defined in unit per mg of protein (U/mg), where a unit (U) is defined as 1 µmole of inorganic phosphate released from PEP per min at 25 °C. Calculations of the specific activities were performed using Microsoft Excel 2007 (12.0.6504.5001) SP1 MSO (12.0.6320.5000).

C.10.2. Results of Functional Activity

The results of the functional activity assay are presented in Table C-5. The specific activity of MON 87427- and *E. coli*-produced CP4 EPSPS proteins was measured to be 8.67 U/mg and 5.41 U/mg of CP4 EPSPS, respectively. Because the value of MON 87427-produced CP4 EPSPS protein specific activity falls within 2-fold of the *E. coli*-produced CP4 EPSPS value (between 2.71 U/mg and 10.82 U/mg), the previously set acceptance criteria was met and the MON 87427-produced CP4 EPSPS protein is considered to have equivalent functional activity to that of the *E. coli*-produced protein.

Table C-5. CP4 EPSPS Protein Functional Assay

MON 87427-produced CP4 EPSPS[1] (U/mg)	***E. coli*-produced CP4 EPSPS[1] (U/mg)**	**Previously set acceptance limits[2] (U/mg)**
8.67 ± 0.23	5.41 ± 0.37	2.71-10.82

[1]Value refers to mean and standard deviation calculated based on n = 6 which includes three replicate assays spectrophotometrically analyzed at 660 nm in duplicate.
[2]Within 2-fold of the *E. coli*-produced CP4 EPSPS protein specific activity (5.41 ÷ 2 U/mg to 5.41 x 2 U/mg)

C.11. CP4 EPSPS Protein Identity and Equivalence Summary and Conclusions

A panel of analytical techniques was used to characterize the MON 87427-produced CP4 EPSPS protein purified from grain of MON 87427. Identity of the MON 87427-produced CP4 EPSPS protein was confirmed by recognition with anti-CP4 EPSPS antibodies, identification of the first 15 amino acids of the N-terminus by amino acid sequencing, and mapping of tryptic peptides that yielded a 70.3% overall coverage of the expected protein sequence. The concentration of the MON 87427-produced CP4 EPSPS protein was 0.1 mg/ml. The purity and apparent molecular weight of the MON 87427-produced CP4 EPSPS protein was 96% and 44.1 kDa, respectively. MALDI-TOF mass spectrometry analysis of the intact protein resulted in an average mass of 47552 Da, reflecting the expected mass of the protein minus the N-terminal methionine. The MON 87427-produced CP4 EPSPS protein was not glycosylated and had a specific activity of 8.67 U/mg of CP4 EPSPS.

The equivalence of the MON 87427- and *E. coli*-produced CP4 EPSPS proteins was evaluated by comparing their apparent molecular weight, immunoreactivity with anti-CP4 EPSPS antibodies, glycosylation status, and functional activity. The results obtained demonstrate that the MON 87427-produced CP4 EPSPS protein is equivalent to the *E. coli*-produced CP4 EPSPS protein. This equivalence justifies the use of the previously conducted protein safety studies whereby the *E. coli*-produced CP4 EPSPS protein was used as a test substance.

References for Appendix C

Aebersold, R. 1993. Mass spectrometry of proteins and peptides in biotechnology. Current Opinion in Biotechnology 4: 412-419.

Billeci, T.M. and J.T. Stults. 1993. Tryptic mapping of recombinant proteins by matrix-assisted laser desorption/ionization mass spectrometry. Analytical Chemistry 65: 1709-1716.

Giglione, C. and T. Meinnel. 2001. Organellar peptide deformylases: Universality of the N-terminal methionine cleavage mechanism. Trends in Plant Science 6: 566-572.

Harrison, L.A., M.R. Bailey, M.W. Naylor, J.E. Ream, B.G. Hammond, D.L. Nida, B.L. Burnette, T.E. Nickson, T.A. Mitsky, M.L. Taylor, R.L. Fuchs and S.R. Padgette. 1996. The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. Journal of Nutrition 126: 728-740.

Hunkapiller, M.W., R.M. Hewick, L.E. Hood and W.J. Dreyer. 1983. High-seneitivity sequencing with gas-phase sequenator. Pages 399-413 in Methods in Enzymology. C.H.W. Hirs and S.N. Timasheff (eds.). Academic Press, New York, New York.

Jensen, O.N., A.V. Podtelejnikov and M. Mann. 1997. Identification of the components of simple protein mixtures by high-accuracy peptide mass mapping and database searching. Analytical Chemistry 69: 4741-4750.

Rademacher, T.W., R.B. Parekh and R.A. Dwek. 1988. Glycobiology. Annual Review of Biochemistry 57: 785-838.

Sherlock, O., U. Dobrindt, J.B. Jensen, R. Munk Vejborg and P. Klemm. 2006. Glycosylation of the self-recognizing *Escherichia coli* Ag43 autotransporter protein. Journal of Bacteriology 188: 1798-1807.

Appendix D: Materials and Methods Used for the Analysis of the Levels of CP4 EPSPS Protein in MON 87427

D.1. Materials

Over-season leaf (OSL1-4), grain, pollen, silk, forage, stover, over-season root (OSR1-4), forage-root, senescent root and over-season whole plant (OSWP1-4) tissue samples from MON 87427 were harvested from five field sites in the U.S. during 2008 from plants grown from starting seed lots 10001857 and 10001859, respectively. An *E. coli*-produced CP4 EPSPS protein (lot 20-100015) was used as the analytical reference standard.

D.2. Characterization of the Materials

The identity of MON 87427 was confirmed by verifying the chain of custody documentation prior to analysis. To further confirm the identities of MON 87427 event-specific polymerase chain reaction (PCR) analyses were conducted on the harvested grain from each site. The PCR analyses and the resulting Verification of Identities were archived in the Monsanto Regulatory Archives under the starting seed lot numbers.

D.3. Field Design and Tissue Collection

Field trials were initiated during the 2008 planting season to generate MON 87427 samples at various maize growing locations in the U.S. The OSL1-4, grain, pollen, silk, forage, stover, OSR1-4, forage-root, senescent root and OSWP1-4 tissue samples from the following field sites were analyzed: Jackson County, Arkansas (site code ARNE); Jefferson County, Iowa (site code IARL); Stark County, Illinois (site code ILWY); Parke County, Indiana (site code INRC); and York County Nebraska (site code NEYO). The field sites were representative of maize producing regions suitable for maize commercial production. At the ARNE, IARL and ILWY sites, three replicated plots of plants containing MON 87427 were planted using a randomized complete block field design. The NEYO site contained 3 replicated plots, but was not a randomized complete block field design which has no impact on expression analysis. OSL1-4, grain, pollen, silk, forage, stover, OSR1-4, forage-root, senescent root and OSWP1-4 samples were collected from each replicated plot at each field sites. See Table V-1 for a detailed description of when samples were harvested.

D.4. Tissue Processing and Protein Extraction

All tissue samples were shipped to Monsanto. The processed tissue samples and unprocessed pollen samples were stored in a -80 °C freezer.

CP4 EPSPS protein was extracted from the tissue samples as described in Table D-1. CP4 EPSPS protein was extracted from all grain tissue samples using a Harbil Mixer with the appropriate amount of Tris-borate buffer with L-ascorbic acid and 10 mM deoxycholic acid (TBA with 10 mM DCA) [0.1 M Tris, 0.1 M $Na_2B_4O_7 \bullet 10H_2O$, 0.01 M $MgCl_2$, 0.05% (v:v) Tween®-20 at pH 7.8, 0.2% (w:v) L-ascorbic acid and 10 mM DCA].

CP4 EPSPS protein was extracted from all over season leaf, over season root, forage, pollen, silk, forage root, stover, senescent root, and over season whole plant tissue samples using a Harbil Mixer with the appropriate amount of a phosphate buffered saline buffer (pH 7.4) containing 0.001 M KH_2PO_4, 0.01 M $Na_2HPO_4 \cdot 7H_2O$, 0.137 M NaCl, and 0.0027 M KCl with Tween 20 (1× PBST) and 0.1% (w/v) bovine serum albumin (BSA) (1× PBST with 0.1% (w/v) BSA). Insoluble material was removed from all tissue extracts using a serum filter (Fisher Scientific, Pittsburgh, PA). The extracts were aliquotted and stored frozen in a -80 °C freezer until ELISA analysis.

Table D-1. CP4 EPSPS Extraction[1] Methods for Tissue Samples

Sample Type	Tissue-to-Buffer Ratio	Extraction Buffer
Leaf[2]	1:100	1X PBST with 0.1% (w/v) BSA
Grain	1:100	1X TBA with 10 mM DCA
Pollen	1:100	1X PBST with 0.1% (w/v) BSA
Silk	1:100	1X PBST with 0.1% (w/v) BSA
Root[3]	1:50	1X PBST with 0.1% (w/v) BSA
Forage[4]	1:100	1X PBST with 0.1% (w/v) BSA

[1]The CP4 EPSPS protein was extracted from each tissue by adding the appropriate volume of CP4 EPSPS Extraction Buffer, and shaking in a Harbil mixer. The extracted sample was clarified using a serum filter.
[2]Over- season leaf (OSL1, OSL2, OSL3, and OSL4).
[3]Over- season root (OSR1, OSR2, OSR3, and OSR4, forage-root, and senescent root).
[4]Forage, stover, and over-season whole plant (OSWP1, OSWP2, OSWP3, OSWP4)

D.5. CP4 EPSPS Antibodies

Mouse monoclonal antibody clone 39B6.1 (IgG2a isotype, kappa light chain; lot 7022111) specific for the CP4 EPSPS protein was purified from mouse ascites fluid using Protein-A Sepharose affinity chromatography and was used as the capture antibody in the CP4 EPSPS ELISA. The concentration of the purified IgG was determined to be 2.3 mg/ml by spectrophotometric methods. Production of the 39B6.1 monoclonal antibody was performed by Strategic Biosolutions (Newark, DE). The purified antibody was stored in a buffer (pH 7.2) containing 20 mM sodium phosphate, 150 mM NaCl, and 15 ppm Proclin 300 (Sigma-Aldrich, St. Louis, MO).

The detection reagent was goat anti-CP4 EPSPS antibody, otherwise known as anti-protein 4 (Sigma-Aldrich, catalog number P-5867) conjugated to horseradish peroxidase (HRP).

D.6. CP4 EPSPS ELISA Method

Mouse anti-CP4 EPSPS antibodies were diluted in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, and 150 mM NaCl, pH 9.6) to a final concentration of 2.0 µg/ml, and immobilized onto 96-well microtiter plates followed by incubation in a 4 °C refrigerator for >8 hours. Prior to each step in the assay, plates were washed with 1×PBST.

CP4 EPSPS protein standard or sample extract was added at 100 µl per well and incubated for 1 hour at 37 °C. The captured CP4 EPSPS protein was detected by the addition of 100 µl per well of anti-CP4 EPSPS HRP conjugate. Plates were developed by adding 100 µl per well of 3,3',5,5'-tetramethyl-benzidine (TMB; Kirkegaard & Perry, Gaithersburg, MD). The enzymatic reaction was terminated by the addition of 100 µl per well of 6 M H_3PO_4. Quantification of the CP4 EPSPS protein was accomplished by interpolation from a CP4 EPSPS protein standard curve that ranged from 0.456-14.6 ng/ml.

D.7. Moisture Analysis

Tissue moisture content was determined using an IR-200 Moisture Analyzer (Denver Instrument Company, Arvada, CO). A homogeneous tissue-specific site pool (TSSP) was prepared consisting of samples of a given tissue type grown at a given site. The average percent moisture for each TSSP was calculated from triplicate analyses. A TSSP Dry Weight Conversion Factor (DWCF) was calculated as follows:

$$\text{DWCF} = 1 - \frac{(\text{Mean \% TSSP Moisture})}{(100)}$$

The DWCF was used to convert protein levels assessed on a µg/g fwt basis into levels reported on a µg/g dwt basis using the following calculation:

$$\text{Protein Level in Dry Weight} = \frac{(\text{Protein Level Fresh Weight})}{(\text{DWCF})}$$

The protein levels (ng/ml) that were reported to be less than or equal to the limit of detection (LOD) or less than the limit of quantitation (LOQ) on a fresh weight basis were not reported on a dry weight basis.

D.8. Data Analyses

All CP4 EPSPS ELISA plates were analyzed on a SPECTRAmax® Plus 384 or a SPECTRAmax Plus Molecular Devices, Sunnyvale, CA) microplate spectrophotometer, using a dual wavelength detection method. All protein concentrations were determined by optical absorbance at a wavelength of 450 nm with a simultaneous reference reading of 620-655 nm. Data reduction analyses were performed using Molecular Devices SOFTmax PRO GXP version 5.0.1. Absorbance readings and protein standard concentrations were fitted with a four-parameter logistic curve fit. Following the interpolation from the standard curve, the amount of protein (ng/ml) in the tissue was reported on a "µg/g fwt" basis for data that were greater than or equal to the LOQ. This conversion utilized a sample dilution factor and a tissue-to-buffer ratio. The protein values expressed as "µg/g fwt" were also converted to "µg/g dwt" by applying the DWCF. Microsoft Excel® 2007 (Version (12.0.6504.5001) SP1 MSO (12.0.6320.5000) Microsoft, Redmond, WA) was used to calculate the CP4 EPSPS, protein level in maize tissues. The sample means, standard deviations, and ranges were also calculated by Microsoft Excel 2007.

Any MON 87427 sample extracts that resulted in unexpectedly negative results by ELISA analysis were re-extracted twice for the protein of interest and re-analyzed by ELISA to confirm the results. Samples with confirmed unexpected results were omitted from all calculations. Samples that were not confirmed to be positive were reported as inconclusive and omitted from all calculations.

Appendix E: Materials and Methods, and Individual-Site Results for Compositional Analysis of MON 87427 Maize Forage and Grain

E.1. Materials

Forage and grain from MON 87427 (Seed Lot Number 10001857) were evaluated in this study. Forage and grain from the conventional control (LH198 × LH287) was evaluated. The conventional control was a conventional maize hybrid (Seed Lot Number 10001859) with background genetics similar to that of MON 87427 but does not produce the CP4 EPSPS protein.

The commercial references were 12 conventional maize hybrids. The commercial references were distributed across sites (Table E-1).

Table E-1. Commercial Reference Maize Hybrids

Material Name	Seed Lot Number	Field Site Code
Crows C6501	10001546	ARNE
Midwest Genetics 87801	10000934	ARNE
Fielder's Choice 7864	10001319	ARNE
Fontanelle 5797	10001548	ARNE
Asgrow RX708	10001564	IARL
Dekalb DKC60-15	10000950	IARL
Midwest Genetics G7944	10001571	IARL
NC + 4443	10001572	IARL
Asgrow RX715	10000952	ILWY
Dekalb DKC61-50	10001328	ILWY
Midland 7B15	10001545	ILWY
NK N69-P9	10001544	ILWY

E.2. Characterization of the Materials

The identities of MON 87427, the conventional control, and commercial references were confirmed by verifying the chain of custody documentation prior to analysis. To further confirm the identities of MON 87427, the conventional control, and commercial references, event-specific polymerase chain reaction (PCR) analyses were conducted on the harvested grain from each site. The PCR analyses and the resulting Verification of Identities were archived in the Monsanto Regulatory Archives under the starting seed lot numbers.

E.3. Field Production of the Samples

Forage and grain from MON 87427, the conventional control, and the commercial references were collected from replicated plots at three field sites during the 2008 U.S. growing season. MON 87427, the conventional control, and the commercial references

were planted in a randomized complete block design with three replicates at field sites in Arkansas (ARNE), Iowa (IARL), and Illinois (ILWY). The MON 87427 plots were treated with glyphosate applications, between the V2 – V6 maize growth stages at a target rate of 1.0 lb ai/acre. All samples at the field sites were grown under normal agronomic field conditions for their respective geographic regions. Forage was collected at the R5 plant growth stage and grain was collected at physiological maturity. Forage samples were shipped on dry ice and grain was shipped at ambient temperature from the field sites to Monsanto Company (St. Louis, MO). Sub-samples were ground to a powder, stored in a freezer set to maintain -20°C located at Monsanto Company (St. Louis, MO), and then shipped on dry ice to Covance Laboratories Inc. (Madison, WI) for analysis.

E.4. Summary of Analytical Methods

Ground forage and grain samples were analyzed by Covance Laboratories Inc. Upon receipt, the samples were stored in a freezer set to maintain -20°C until their use. Nutrients assessed in this analysis included proximates (ash, carbohydrates by calculation, moisture, protein, and fat), acid detergent fiber (ADF), neutral detergent fiber (NDF), total dietary fiber (TDF), amino acids, fatty acids (C8-C22), vitamins [A (β-carotene), B1, B2, B6, E, niacin, and folic acid], minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc) in the grain, and proximates, ADF, NDF, calcium and phosphorus in forage. The anti-nutrients assessed in grain included phytic acid and raffinose. Secondary metabolites assessed in grain included furfural, ferulic acid, and p-coumaric acid.

E.4.1. 2-Furaldehyde

The ground sample was extracted with 4% trichloroacetic acid and injected directly on a high-performance liquid chromatography system for quantitation of free furfurals by ultraviolet detection (Albala-Hurtado et al., 1997). The quantitation limit was 0.500 ppm.

Reference Standard:

- Acros, 2 Furaldehyde, 99.7%, Lot Number A0219180

E.4.2. Acid Detergent Fiber

The sample was placed in a fritted vessel and washed with an acidic boiling detergent solution that dissolved the protein, carbohydrate, and ash. An acetone wash removed the fats and pigments. The lignocellulose fraction was collected on the frit and determined gravimetrically (USDA, 1970). The limit of quantitation was 0.100%.

E.4.3. Amino Acid Composition

The following 18 amino acids were analyzed:

Total threonine	Total aspartic acid (including asparagine)
Total serine	Total tyrosine

Total phenylalanine	Total glutamic acid (including glutamine)
Total proline	Total histidine
Total glycine	Total lysine
Total alanine	Total arginine
Total valine	Total tryptophan
Total isoleucine	Total methionine
Total leucine	Total cystine (including cysteine)

The sample was assayed by three methods to obtain the full profile. Tryptophan required a base hydrolysis with sodium hydroxide. The sulfur-containing amino acids required an oxidation with performic acid prior to hydrolysis with hydrochloric acid. Analysis of the samples for the remaining amino acids was accomplished through direct acid hydrolysis with hydrochloric acid. Once hydrolyzed, the individual amino acids were then quantitated using an automated amino acid analyzer (AOAC-International, 2005d). The limit of quantitation was 0.100 mg/g.

Reference Standards:

- Thermo Scientific, K18, 2.5 µmol/mL per constituent (except cystine 1.25 µmol/mL), Lot Number JK126327
- Sigma, L-Tryptophan, 100%, Lot Number 076K0075
- Sigma/BioChemika, L-Cysteic Acid Monohydrate, 99.5% (used as 100%), Lot Number 1305674
- Sigma, L-Methionine Sulfone, 100%, Lot Number 047K1321

E.4.4. Ash

The sample was placed in an electric furnace at 550°C and ignited to drive off all volatile organic matter. The nonvolatile matter remaining was quantitated gravimetrically and calculated to determine percent ash (AOAC-International, 2005a). The limit of quantitation was 0.100%.

E.4.5. Beta Carotene

The sample was saponified and extracted with hexane. The sample was then injected on a reverse phase high-performance liquid chromatography system with ultraviolet light detection. Quantitation was achieved with a linear regression analysis (AOAC-International, 2005j; Quackenbush, 1987). The limit of quantitation for β-carotene was approximately 0.0200 mg/100g.

Reference Standard:

- Sigma-Aldrich, Beta Carotene, Type 1, Purity 96.30% and 94.96% (determined spectrophotometrically), Lot Number 068K2561

E.4.6. Carbohydrates

The total carbohydrate level was calculated by difference using the fresh weight-derived data and the following equation (USDA, 1973):

% carbohydrates = 100 % - (% protein + % fat + % moisture + % ash)

The limit of quantitation was 0.100%.

E.4.7. Fat by Acid Hydrolysis

The sample was hydrolyzed with hydrochloric acid at an elevated temperature. The fat was extracted with ether and hexane. The extract was evaporated on a steambath, re-dissolved in hexane and filtered through a sodium sulfate column. The hexane extract was then evaporated again on a steambath under nitrogen, dried, and weighed (AOAC-International, 2005g). The limit of quantitation was 0.100%.

E.4.8. Fat by Soxhlet Extraction

The sample was weighed into a cellulose thimble containing sodium sulfate and dried to remove excess moisture. Pentane was dripped through the sample to remove the fat. The extract was then evaporated, dried, and weighed (AOAC-International, 2005m). The limit of quantitation was 0.100%.

E.4.9. Fatty Acid Profile with Trans Fat by GC

The lipid was extracted and saponified with 0.5N sodium hydroxide in methanol. The saponification mixture was methylated with 14% boron trifluoride in methanol. The resulting methyl esters were extracted with heptane containing an internal standard. The methyl esters of the fatty acids were analyzed by gas chromatography using external standards for quantitation (AOAC-International, 2005e; AOCS, 1997; AOCS, 2001). The limit of quantitation was 0.00400%.

Reference Standards:

- Nu Chek Prep GLC Reference Standard Hazleton No. 1, *, Lot Number AU18-S
- Nu Chek Prep GLC Reference Standard Hazleton No. 2, *, Lot Number M13-O
- Nu Chek Prep GLC Reference Standard Hazleton No. 3, *, Lot Number MA18-S
- Nu Chek Prep GLC Reference Standard Hazleton No. 4, *, Lot Number JA16-T
- Nu Chek Prep Methyl Gamma Linolenate, used as 100%,
- Lot Number U-63M-JY12-R
- Nu Chek Prep Methyl Tridecanoate, used as 100%, Lot Number N-13M-JA16-T

*Overall purity of the sum of the mixture of components was used as 100%

E.4.10. Folic Acid

The sample was hydrolyzed in a potassium phosphate buffer with the addition of ascorbic acid to protect the folic acid during autoclaving. Following hydrolysis by autoclaving, the sample was treated with a chicken-pancreas enzyme and incubated approximately 18 hours to liberate the bound folic acid. The amount of folic acid was determined by comparing the growth response of the sample, using the bacteria *Lactobacillus casei*, with the growth response of a folic acid standard. This response was measured turbidimetrically (AOAC-International, 2005n; Infant Formula Council, 1985). The limit of quantitation was 0.0600 µg/g.

Reference Standard:

- USP, Folic acid, 98.9%, Lot Number Q0G151

E.4.11. ICP Emission Spectrometry

The sample was dried, precharred, and ignited overnight in a muffle set to maintain 500°C. The resulting ash was dissolved with nitric acid, treated with hydrochloric acid, evaporated to dryness, and put into a solution of 5% hydrochloric acid. The amount of each element was determined at appropriate wavelengths by comparing the emission of the unknown sample, measured on the inductively coupled plasma optical emission spectrometer, with the emission of the standard solutions (AOAC-International, 2005o).

Reference Standards

Inorganic Ventures Reference Standards and Limits of Quantitation:

Mineral	Lot Numbers	Concentration (µg/ml)	Limit of Quantitation (ppm)
Calcium	C2-MEB290078, C2-MEB289124	200, 1000	20.0
Copper	C2-MEB290078, C2-MEB290079	2, 10	0.50
Iron	C2-MEB290078, C2-MEB290080	10, 50	2.00
Magnesium	C2-MEB290078, C2-MEB290079	50, 250	20.0
Manganese	C2-MEB290078, C2-MEB290079	2, 10	0.30
Phosphorus	C2-MEB290078, C2-MEB289124	200, 1000	20.0
Potassium	C2-MEB290078, BB11-203K*	200, 10000*	100
Sodium	C2-MEB290078, C2-MEB289124	200, 1000	100
Zinc	C2-MEB290078, C2-MEB290079	10, 50	0.40

*Used SPEX standard for potassium (1000 µg/ml)

E.4.12. Moisture

The sample was dried in a vacuum oven at approximately 100°C to a constant weight. The moisture weight loss was determined and converted to percent moisture (AOAC-International, 2005h). The limit of quantitation was 0.100%.

E.4.13. Neutral Detergent Fiber

The sample was placed in a fritted vessel and washed with a neutral boiling detergent solution that dissolved the protein, carbohydrate, enzyme, and ash. An acetone wash removed the fats and pigments. Hemicellulose, cellulose, and lignin fractions were collected on the frit and determined gravimetrically (AACC, 1998; USDA, 1970). The limit of quantitation was 0.100%.

E.4.14. Niacin

The sample was hydrolyzed with sulfuric acid and the pH was adjusted to remove interferences. The amount of niacin was determined by comparing the growth response of the sample, using the bacteria *Lactobacillus plantarum*, with the growth response of a niacin standard. This response was measured turbidimetrically (AOAC-International, 2005b). The limit of quantitation was 0.300 µg/g.

Reference Standard:

- USP, Niacin, 99.8%, Lot Number I0E295

E.4.15. p-Coumaric Acid and Ferulic Acid

The sample was extracted with methanol using ultrasonication, hydrolyzed using 4N sodium hydroxide, buffered using acetic acid/sodium hydroxide, acidified with 3N hydrochloric acid, and filtered. The levels of p-coumaric and ferulic acids in the extract were determined by reverse phase high-performance liquid chromatography with ultraviolet detection (Hagerman and Nicholson, 1982). The limit of quantitation for the p-coumaric acid and ferulic acid assays was 50.0 ppm.

Reference Standards:

- Acros Organics, 4-Hydroxy-3-methoxycinnamic (ferulic acid), 99.4%, Lot Number A0248008
- Acros Organics, p-Hydroxycinnamic acid (coumaric acid), 99.4%, Lot Number A0236839

E.4.16. Phytic Acid

The sample was extracted using 0.5M HCl with ultrasonication. Purification and concentration were accomplished on a silica-based anion-exchange column. The sample was analyzed on a polymer high-performance liquid chromatography column PRP-1,

5µm (150 x 4.1mm) with a refractive index detector (Lehrfeld, 1989; Lehrfeld, 1994). The limit of quantitation was 0.100%.

Reference Standard:

- Aldrich, Phytic Acid Dodecasodium Salt Hydrate, 98%, Lot Number 068K0755

E.4.17. Protein

Nitrogenous compounds in the sample were reduced in the presence of boiling sulfuric acid and a mercury catalyst mixture to form ammonia. The acid digest was made alkaline. The ammonia was distilled and then titrated with a previously standardized acid. The percent nitrogen was calculated and converted to equivalent protein using the factor 6.25 (AOAC-International, 2005l; Bradstreet, 1965; Kalthoff and Sandell, 1948). The limit of quantitation was 0.100%.

E.4.18. Raffinose

The sample was extracted with deionized water and the extract treated with a hydroxylamine hydrochloride solution in pyridine, containing phenyl-β-D-glucoside as an internal standard. The resulting oximes were converted to silyl derivatives by treatment with hexamethyldisilazane and trifluoracetic acid and analyzed by gas chromatography using a flame ionization detector (Brobst, 1972; Mason and Slover, 1971). The limit of quantitation was 0.0500%.

Reference Standard:

- Sigma, D-(+)-Raffinose Pentahydrate, 95.5% after correction for degree of hydration, Lot Number 037K1059

E.4.19. Total Dietary Fiber

Duplicate samples were gelatinized with α-amylase and digested with enzymes to break down starch and protein. Ethanol was added to each sample to precipitate the soluble fiber. The samples were filtered, and the residue was rinsed with ethanol and acetone to remove starch and protein degradation products and moisture. Protein content was determined for one of the duplicates; ash content was determined for the other. The total dietary fiber in the sample was calculated using the protein and ash values (AOAC-International, 2005f). The limit of quantitation was 1.00%.

E.4.20. Vitamin B_1 (Thiamine Hydrochloride)

The sample was autoclaved under weak acid conditions to extract the thiamine. The resulting solution was incubated with a buffered enzyme solution to release any bound thiamine. The solution was purified on a cation-exchange column. An aliquot was reacted with potassium ferricyanide to convert thiamine to thiochrome. The thiochrome was extracted into isobutyl alcohol, measured on a fluorometer, and quantitated by

comparison to a known standard (AOAC-International, 2005k). The limit of quantitation was 0.01 mg/100g. Results are reported as thiamine hydrochloride.

Reference Standard:

- USP, Thiamine hydrochloride, 95.9% after correction for moisture content, Lot Number 01F236

E.4.21. Vitamin B_2

The sample was hydrolyzed with dilute hydrochloric acid and the pH was adjusted to remove interferences. The amount of riboflavin was determined by comparing the growth response of the sample, using the bacteria *Lactobacillus casei*, with the growth response of multipoint riboflavin standards. The growth response was measured turbidimetrically (AOAC-International, 2005i; USP, 2005). The limit of quantitation was 0.200 µg/g.

Reference Standard:

- USP, Riboflavin, 100%, Lot Number: N0C021

E.4.22. Vitamin B_6 (Pyridoxine Hydrochloride)

The sample was hydrolyzed with dilute sulfuric acid in the autoclave and the pH was adjusted to remove interferences. The amount of pyridoxine was determined by comparing the growth response of the sample, using the yeast *Saccharomyces carlsbergensis*, with the growth response of a pyridoxine standard. The response was measured turbidimetrically (AOAC-International, 2005c; Atkins et al., 1943). Results are reported as pyridoxine hydrochloride. The limit of quantitation was 0.0700 µg/g.

Reference Standard:

- USP, Pyridoxine hydrochloride, 99.8%, Lot Number: Q0G409

E.4.23. Vitamin E

The product was saponified to break down any fat and release vitamin E. The saponified mixture was extracted with ethyl ether and then quantitated by high-performance liquid chromatography using a silica column (Cort et al., 1983; McMurray et al., 1980; Speek et al., 1985). The limit of quantitation was approximately 0.00500 mg/g.

Reference Standard:

- USP, Alpha Tocopherol, 100%, Lot Number M

E.5. Data Processing and Statistical Analysis

After compositional analyses were performed, data spreadsheets containing individual values for each analysis were sent to Monsanto Company for review. Data were then transferred to Certus International where they were converted into the appropriate units and statistically analyzed. The formulas were used for re-expression of composition data for statistical analysis are listed in Table E-2.

Table E-2. Re-expression Formulas for Statistical Analysis of Composition Data

Component	From (X)	To	Formula[1]
Proximates (excluding Moisture), Fiber, Anti-nutrients	% fw	% dw	X/d
Minerals (Calcium, Magnesium, Phosphorus, Potassium, Sodium)	ppm fw	% dw	$(X/d) \times 10^{-4}$
Grain Minerals (Copper, Iron, Manganese, Zinc)	ppm fw	mg/kg dw	X/d
Vitamin A, Vitamin B_1	mg/100g fw	mg/kg dw	$(X/d) \times 10$
Vitamin E	mg/g fw	mg/kg dw	$(X/d) \times 10^3$
Folic Acid, Niacin, Vitamin B_2, Vitamin B_6	µg/g fw	mg/kg dw	X/d
Secondary Metabolites	ppm fw	µg/g dw	X/d
Fatty Acids (FA)	% fw	% total fa	$(100)X_j/\Sigma X$, for each FA_j where ΣX is over all the FA
Amino Acids (AA)	mg/g fw	% dw	$(X/d) \times 10^{-1}$

[1] 'X' is the individual sample value; 'd' is the fraction of the sample that is dry matter.

In order to complete a statistical analysis for a compositional constituent, at least 50% of the values for an analyte had to be greater than the assay limit of quantitation (LOQ). Analytes with more than 50% of observations below the assay LOQ were excluded from summaries and analysis. The following 16 analytes with more than 50% of observations below the assay LOQ were excluded from statistical analysis: 8:0 caprylic acid, 10:0 capric acid, 12:0 lauric acid, 14:0 myristic acid, 14:1 myristoleic acid, 15:0 pentadecanoic acid, 15:1 pentadecenoic acid, 16:1 palmitoleic acid, 17:0 heptadecanoic acid, 17:1 heptadecenoic acid, 18:3 gamma linolenic acid, 20:2 eicosadienoic acid, 20:3 eicosatrienoic acid, 20:4 arachidonic acid, sodium, and furfural.

Otherwise, results below the LOQ were assigned a value equal to one-half the quantitation limit. Five observations for 22:0 behenic acid were assigned a value equal to one-half of the LOQ (0.002 % FW.)

The data were assessed for potential outliers using a studentized PRESS residuals calculation. A PRESS residual is the difference between any value and its value predicted from a statistical model that excludes the data point. The studentized version scales these residuals so that the values tend to have a standard normal distribution when outliers are absent. Thus, most values are expected to be between ± 3. Extreme data points that are also outside of the ± 6 studentized PRESS residual range are considered for exclusion, as outliers, from the final analyses. Six fatty acids from two conventional

references at the ILWY site had PRESS residual values outside of ± 6 range. As none of the identified values were the extreme highest or lowest values within the dataset, these values were not removed from the statistical analysis.

All maize compositional components were statistically analyzed using a mixed model analysis of variance with the SAS MIXED procedure. The three replicated sites were analyzed both separately and combined. Individual replicated site analyses used model (1).

(1) $Y_{ij} = U + T_i + B_j + e_{ij},$

where Y_{ij} = unique individual observation, U = overall mean, T_i = material effect, B_j = random block effect, and e_{ij} = residual error.

Combined site analyses used model (2).

(2) $Y_{ijk} = U + T_i + L_j + B(L)_{jk} + LT_{ij} + e_{ijk},$

where Y_{ijk} = unique individual observation, U = overall mean, T_i = material effect, L_j = random location effect, $B(L)_{jk}$ = random block within location effect, LT_{ij} = random location by material interaction effect, and e_{ijk} = residual error.
For each component analysis, mean comparison tests of MON 87427 versus the conventional control were conducted.

A tolerance interval is an interval that one can claim, with a specified degree of confidence, contains at least a specified proportion, p, of an entire sampled population for the parameter measured.

For each compositional component, 99% tolerance intervals were calculated using the commercial references that are expected to contain, with 95% confidence, 99% of the quantities expressed in the population of commercial references. Each estimate was based upon the average of all observations per unique reference. Because negative quantities are not possible, negative calculated lower tolerance bounds were set to zero.

Table E-3. Statistical Summary of Site ARNE Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	1.51 (0.029) (1.43 - 1.58)	1.51 (0.029) (1.48 - 1.57)	0.00040 (0.039) (-0.047 - 0.042)	-0.090, 0.091	0.992	1.13, 1.97 (1.18 - 1.82)
Carbohydrates	84.46 (0.29) (84.03 - 84.78)	84.73 (0.29) (84.16 - 85.12)	-0.27 (0.41) (-0.87 - 0.62)	-1.22, 0.69	0.537	80.77, 89.46 (82.26 - 87.17)
Moisture (% fw)	11.40 (0.16) (11.20 - 11.70)	11.60 (0.16) (11.30 - 11.90)	-0.20 (0.16) (-0.30 - -0.10)	-0.56, 0.16	0.233	7.56, 14.80 (9.31 - 12.70)
Protein	10.84 (0.33) (10.47 - 11.33)	10.22 (0.33) (9.91 - 10.62)	0.62 (0.47) (-0.15 - 1.20)	-0.45, 1.70	0.217	5.79, 13.43 (8.07 - 12.13)
Total Fat	3.18 (0.11) (3.13 - 3.23)	3.54 (0.11) (3.47 - 3.65)	-0.36 (0.16) (-0.52 - -0.24)	-0.72, 0.0014	0.050	2.12, 5.35 (2.90 - 4.30)
Fiber (% dw)						
Acid Detergent Fiber	3.34 (0.16) (3.15 - 3.49)	3.41 (0.16) (3.27 - 3.54)	-0.064 (0.21) (-0.27 - 0.22)	-0.54, 0.41	0.766	1.84, 4.39 (2.29 - 4.27)

Table E-3 (continued). Statistical Summary of Site ARNE Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	Test[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% Confidence Interval	Significance (p-Value)	Conventional Tolerance Interval[5] (Range)
Fiber (% dw)						
Neutral Detergent Fiber	10.41 (0.22) (10.16 - 10.92)	10.88 (0.22) (10.32 - 11.27)	-0.47 (0.30) (-0.90 - -0.16)	-1.17, 0.23	0.162	5.69, 11.81 (7.06 - 10.66)
Total Dietary Fiber	13.28 (0.18) (13.14 - 13.51)	13.23 (0.18) (12.67 - 13.75)	0.046 (0.25) (-0.24 - 0.52)	-0.53, 0.63	0.860	8.67, 15.32 (10.25 - 14.30)
Amino Acid (% dw)						
Alanine	0.81 (0.029) (0.78 - 0.83)	0.75 (0.029) (0.72 - 0.77)	0.065 (0.042) (0.054 - 0.080)	-0.032, 0.16	0.160	0.32, 1.12 (0.58 - 0.98)
Arginine	0.53 (0.021) (0.49 - 0.55)	0.52 (0.021) (0.48 - 0.56)	0.0067 (0.029) (-0.068 - 0.065)	-0.061, 0.074	0.825	0.24, 0.68 (0.34 - 0.57)
Aspartic Acid	0.70 (0.020) (0.68 - 0.71)	0.65 (0.020) (0.62 - 0.68)	0.049 (0.028) (0.036 - 0.056)	-0.017, 0.11	0.126	0.34, 0.92 (0.52 - 0.78)
Cystine	0.26 (0.0055) (0.25 - 0.27)	0.25 (0.0055) (0.24 - 0.25)	0.013 (0.0078) (0.0054 - 0.020)	-0.0053, 0.031	0.142	0.14, 0.30 (0.18 - 0.26)

Table E-3 (continued). Statistical Summary of Site ARNE Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Glutamic Acid	1.99 (0.076) (1.90 - 2.04)	1.86 (0.076) (1.78 - 1.92)	0.14 (0.11) (0.12 - 0.17)	-0.11, 0.38	0.244	0.77, 2.84 (1.46 - 2.49)
Glycine	0.43 (0.0087) (0.42 - 0.43)	0.40 (0.0087) (0.39 - 0.42)	0.022 (0.012) (0.011 - 0.032)	-0.0067, 0.050	0.116	0.23, 0.52 (0.32 - 0.43)
Histidine	0.31 (0.0084) (0.30 - 0.31)	0.30 (0.0084) (0.28 - 0.31)	0.011 (0.012) (0.00008 - 0.019)	-0.016, 0.039	0.366	0.16, 0.39 (0.22 - 0.33)
Isoleucine	0.38 (0.015) (0.37 - 0.40)	0.36 (0.015) (0.33 - 0.37)	0.029 (0.021) (0.022 - 0.035)	-0.019, 0.077	0.195	0.16, 0.53 (0.27 - 0.46)
Leucine	1.32 (0.058) (1.24 - 1.36)	1.22 (0.058) (1.16 - 1.27)	0.099 (0.082) (0.078 - 0.13)	-0.091, 0.29	0.264	0.43, 1.95 (0.93 - 1.69)
Lysine	0.33 (0.0055) (0.32 - 0.33)	0.32 (0.0055) (0.31 - 0.33)	0.011 (0.0078) (-0.0011 - 0.019)	-0.0075, 0.029	0.215	0.19, 0.40 (0.26 - 0.34)

Table E-3 (continued). Statistical Summary of Site ARNE Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Tyrosine	0.33 (0.038) (0.25 - 0.38)	0.32 (0.038) (0.25 - 0.36)	0.0090 (0.054) (-0.12 - 0.11)	-0.12, 0.13	0.872	0.077, 0.45 (0.11 - 0.43)
Valine	0.53 (0.017) (0.51 - 0.54)	0.49 (0.017) (0.46 - 0.51)	0.032 (0.023) (0.021 - 0.041)	-0.022, 0.086	0.210	0.25, 0.67 (0.38 - 0.58)
Fatty Acid (% total FA)						
16:0 Palmitic	11.49 (0.056) (11.47 - 11.52)	10.99 (0.056) (10.88 - 11.08)	0.50 (0.080) (0.38 - 0.59)	0.31, 0.68	<0.001	6.42, 15.23 (9.13 - 12.33)
18:0 Stearic	2.17 (0.021) (2.16 - 2.17)	2.04 (0.021) (1.99 - 2.07)	0.13 (0.030) (0.093 - 0.18)	0.063, 0.20	0.002	0.87, 2.88 (1.54 - 2.38)
18:1 Oleic	26.34 (0.14) (26.16 - 26.62)	25.35 (0.14) (25.06 - 25.71)	1.00 (0.17) (0.88 - 1.20)	0.61, 1.38	<0.001	11.30, 43.27 (21.39 - 34.71)
18:2 Linoleic	57.94 (0.16) (57.61 - 58.13)	59.56 (0.16) (59.18 - 59.82)	-1.62 (0.21) (-1.69 - -1.57)	-2.11, -1.13	<0.001	41.35, 74.78 (49.38 - 63.16)

Table E-3 (continued). Statistical Summary of Site ARNE Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% total FA)						
18:3 Linolenic	1.15 (0.014) (1.13 - 1.17)	1.19 (0.014) (1.18 - 1.22)	-0.047 (0.018) (-0.088 - -0.017)	-0.089, -0.0047	0.033	0.78, 1.52 (0.97 - 1.35)
20:0 Arachidic	0.48 (0.0035) (0.47 - 0.48)	0.46 (0.0035) (0.45 - 0.46)	0.021 (0.0050) (0.0092 - 0.030)	0.0096, 0.033	0.002	0.15, 0.67 (0.32 - 0.53)
20:1 Eicosenoic	0.22 (0.0024) (0.22 - 0.23)	0.23 (0.0024) (0.22 - 0.23)	-0.0013 (0.0034) (-0.0045 - 0.0025)	-0.0091, 0.0065	0.711	0.12, 0.36 (0.21 - 0.31)
22:0 Behenic	0.21 (0.0042) (0.21 - 0.23)	0.19 (0.0042) (0.18 - 0.20)	0.021 (0.0059) (0.0054 - 0.031)	0.0075, 0.035	0.007	0, 0.32 (0.057 - 0.23)
Mineral						
Calcium (% dw)	0.0077 (0.00024) (0.0075 - 0.0079)	0.0067 (0.00024) (0.0060 - 0.0076)	0.00095 (0.00034) (-0.00009 - 0.0017)	0.00016, 0.0017	0.024	0.0019, 0.0076 (0.0038 - 0.0068)
Copper (mg/kg dw)	1.86 (0.074) (1.59 - 2.07)	1.84 (0.074) (1.78 - 1.89)	0.022 (0.10) (-0.19 - 0.18)	-0.22, 0.26	0.835	0.17, 3.48 (1.10 - 2.62)

Table E-3 (continued). Statistical Summary of Site ARNE Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Iron (mg/kg dw)	24.12 (0.84) (23.45 - 24.69)	23.57 (0.84) (22.10 - 25.57)	0.55 (1.19) (-2.12 - 2.11)	-2.20, 3.29	0.657	11.42, 28.01 (16.55 - 24.10)
Magnesium (% dw)	0.13 (0.0041) (0.13 - 0.14)	0.12 (0.0041) (0.12 - 0.13)	0.0057 (0.0058) (-0.00043 - 0.010)	-0.0076, 0.019	0.348	0.080, 0.16 (0.11 - 0.15)
Manganese (mg/kg dw)	8.74 (0.27) (8.42 - 9.31)	8.86 (0.27) (8.33 - 9.31)	-0.12 (0.38) (-0.46 - 0.092)	-1.00, 0.76	0.760	0, 12.67 (4.00 - 9.17)
Phosphorus (% dw)	0.33 (0.0070) (0.33 - 0.34)	0.34 (0.0070) (0.33 - 0.35)	-0.0060 (0.0099) (-0.020 - 0.0053)	-0.029, 0.017	0.558	0.24, 0.42 (0.28 - 0.37)
Potassium (% dw)	0.40 (0.0086) (0.39 - 0.40)	0.40 (0.0086) (0.39 - 0.41)	-0.0035 (0.012) (-0.013 - 0.0029)	-0.032, 0.024	0.777	0.24, 0.54 (0.33 - 0.46)
Zinc (mg/kg dw)	23.24 (0.63) (21.98 - 25.42)	22.06 (0.63) (21.65 - 22.40)	1.18 (0.89) (-0.41 - 3.29)	-0.89, 3.24	0.224	11.46, 30.37 (17.30 - 25.45)

Table E-3 (continued). Statistical Summary of Site ARNE Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Vitamin (mg/kg dw)						
Folic Acid	0.38 (0.025) (0.34 - 0.43)	0.41 (0.025) (0.35 - 0.47)	-0.030 (0.036) (-0.097 - 0.073)	-0.11, 0.052	0.423	0.11, 0.61 (0.24 - 0.57)
Niacin	25.77 (2.47) (24.92 - 27.18)	26.05 (2.47) (24.52 - 28.52)	-0.28 (3.49) (-3.30 - 2.66)	-8.33, 7.78	0.938	7.89, 49.83 (20.63 - 43.08)
Vitamin A	0.95 (0.056) (0.88 - 0.99)	0.87 (0.056) (0.76 - 0.98)	0.078 (0.079) (0.013 - 0.21)	-0.10, 0.26	0.349	0.38, 1.68 (0.58 - 1.50)
Vitamin B1	2.90 (0.14) (2.83 - 2.93)	2.53 (0.14) (2.48 - 2.60)	0.37 (0.20) (0.33 - 0.45)	-0.085, 0.83	0.097	2.21, 3.65 (2.41 - 3.48)
Vitamin B2	3.27 (0.17) (3.05 - 3.56)	2.36 (0.17) (2.18 - 2.58)	0.91 (0.23) (0.62 - 1.23)	0.38, 1.43	0.004	0, 4.47 (1.28 - 3.29)
Vitamin B6	8.50 (0.31) (8.21 - 8.69)	8.92 (0.31) (8.23 - 9.61)	-0.42 (0.45) (-1.40 - 0.36)	-1.45, 0.60	0.367	2.57, 12.07 (5.24 - 10.29)

Table E-3 (continued). Statistical Summary of Site ARNE Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Vitamin (mg/kg dw)						
Vitamin E	16.71 (0.85) (16.01 - 17.44)	17.76 (0.85) (17.47 - 18.10)	-1.06 (1.20) (-2.09 - -0.27)	-3.82, 1.71	0.405	0, 25.61 (6.67 - 17.34)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2] MON 87427 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the non near isogenic, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits were set to zero.

Table E-4. Statistical Summary of Site ARNE Grain Anti-nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Anti-nutrient (% dw)						
Phytic Acid	0.89 (0.029) (0.87 - 0.89)	0.97 (0.029) (0.94 - 1.00)	-0.080 (0.036) (-0.12 - -0.054)	-0.16, 0.0038	0.058	0.73, 1.23 (0.82 - 1.07)
Raffinose	0.11 (0.0066) (0.11 - 0.11)	0.13 (0.0066) (0.13 - 0.14)	-0.024 (0.0094) (-0.028 - -0.023)	-0.046, -0.0028	0.031	0.024, 0.29 (0.092 - 0.21)

[1]dw = dry weight.
[2] MON 87427 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the near isogenic, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits were set to zero.

Table E-5. Statistical Summary of Site ARNE Grain Secondary Metabolite Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Secondary Metabolite (µg/g dw)						
Ferulic Acid	2475.67 (73.70) (2342.34 - 2559.19)	2416.91 (73.70) (2315.55 - 2500.00)	58.76 (104.23) (-92.83 - 209.93)	-181.59, 299.11	0.588	1070.41, 2955.86 (1588.35 - 2630.98)
p-Coumaric Acid	243.80 (9.78) (227.48 - 260.43)	245.08 (9.78) (233.83 - 252.26)	-1.28 (11.70) (-21.68 - 9.66)	-28.27, 25.71	0.915	58.74, 313.97 (124.16 - 250.30)

[1]dw = dry weight.
[2] MON 87427 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the near isogenic, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial references. Negative limits were set to zero.

Table E-6. Statistical Summary of Site ARNE Forage Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.60 (0.27) (4.39 - 4.76)	4.56 (0.27) (3.99 - 5.09)	0.043 (0.34) (-0.33 - 0.66)	-0.74, 0.82	0.901	2.66, 6.48 (3.70 - 5.95)
Carbohydrates	87.05 (0.78) (86.68 - 87.25)	86.93 (0.78) (85.44 - 88.52)	0.12 (1.11) (-1.27 - 1.24)	-2.43, 2.67	0.915	80.13, 94.05 (83.23 - 90.37)
Moisture (% fw)	70.73 (1.29) (68.50 - 73.10)	69.37 (1.29) (67.40 - 72.30)	1.37 (1.82) (-3.80 - 5.70)	-2.83, 5.57	0.474	51.70, 86.22 (61.00 - 76.00)
Protein	6.84 (0.55) (6.65 - 7.02)	6.40 (0.55) (5.40 - 7.18)	0.44 (0.72) (-0.17 - 1.26)	-1.22, 2.10	0.560	1.34, 11.57 (4.37 - 9.31)
Total Fat	1.52 (0.28) (1.45 - 1.56)	2.12 (0.28) (1.98 - 2.28)	-0.60 (0.35) (-0.74 - -0.42)	-1.40, 0.20	0.120	0.44, 3.33 (0.78 - 3.16)
Fiber (% dw)						
Acid Detergent Fiber	24.14 (1.62) (21.78 - 26.97)	27.26 (1.62) (25.49 - 28.84)	-3.11 (2.13) (-7.07 - -0.46)	-8.02, 1.79	0.181	14.84, 38.51 (21.33 - 35.92)

Table E-6 (continued). Statistical Summary of Site ARNE Forage Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Neutral Detergent Fiber	38.71 (1.43) (37.17 - 41.50)	34.61 (1.43) (33.07 - 36.71)	4.10 (1.92) (3.13 - 4.79)	-0.33, 8.54	0.065	25.12, 54.99 (29.68 - 60.16)
Mineral						
Calcium (% dw)	0.19 (0.011) (0.18 - 0.21)	0.18 (0.011) (0.15 - 0.20)	0.018 (0.013) (-0.0016 - 0.034)	-0.012, 0.047	0.207	0.075, 0.29 (0.10 - 0.24)
Phosphorus (% dw)	0.24 (0.016) (0.20 - 0.27)	0.22 (0.016) (0.19 - 0.23)	0.022 (0.020) (0.013 - 0.038)	-0.025, 0.068	0.316	0.063, 0.37 (0.16 - 0.31)

[1]dw = dry weight; fw = fresh weight.
[2] MON 87427 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the near isogenic, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits were set to zero.

Table E-7. Statistical Summary of Site IARL Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	1.57 (0.069) (1.46 - 1.76)	1.56 (0.084) (1.53 - 1.60)	0.0092 (0.11) (-0.14 - -0.031)	-0.25, 0.27	0.934	1.13, 1.97 (1.18 - 1.82)
Carbohydrates	84.24 (0.32) (83.60 - 84.96)	83.11 (0.39) (82.96 - 83.33)	1.13 (0.47) (0.63 - 1.63)	0.017, 2.25	0.047	80.77, 89.46 (82.26 - 87.17)
Moisture (% fw)	10.93 (0.14) (10.90 - 11.00)	10.40 (0.17) (10.20 - 10.60)	0.53 (0.22) (0.30 - 0.80)	0.020, 1.05	0.043	7.56, 14.80 (9.31 - 12.70)
Protein	10.60 (0.30) (9.91 - 11.35)	11.73 (0.35) (11.41 - 11.92)	-1.13 (0.38) (-1.50 - -0.57)	-2.02, -0.24	0.019	5.79, 13.43 (8.07 - 12.13)
Total Fat	3.60 (0.058) (3.56 - 3.66)	3.65 (0.071) (3.59 - 3.71)	-0.046 (0.092) (-0.055 - 0.0098)	-0.26, 0.17	0.635	2.12, 5.35 (2.90 - 4.30)
Fiber (% dw)						
Acid Detergent Fiber	2.98 (0.22) (2.67 - 3.31)	3.13 (0.27) (3.02 - 3.23)	-0.15 (0.34) (-0.063 - 0.078)	-0.96, 0.67	0.684	1.84, 4.39 (2.29 - 4.27)

Table E-7 (continued). Statistical Summary of Site IARL Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Neutral Detergent Fiber	9.34 (0.13) (9.17 - 9.43)	9.26 (0.16) (9.21 - 9.26)	0.079 (0.19) (-0.092 - 0.22)	-0.38, 0.54	0.693	5.69, 11.81 (7.06 - 10.66)
Total Dietary Fiber	12.46 (0.29) (12.13 - 12.68)	12.72 (0.35) (12.64 - 12.81)	-0.26 (0.46) (-0.67 - -0.070)	-1.34, 0.82	0.585	8.67, 15.32 (10.25 - 14.30)
Amino Acid (% dw)						
Alanine	0.82 (0.035) (0.74 - 0.89)	0.90 (0.042) (0.89 - 0.90)	-0.084 (0.051) (-0.15 - -0.017)	-0.21, 0.037	0.143	0.32, 1.12 (0.58 - 0.98)
Arginine	0.48 (0.015) (0.45 - 0.49)	0.53 (0.017) (0.51 - 0.53)	-0.048 (0.018) (-0.079 - -0.016)	-0.091, -0.0051	0.033	0.24, 0.68 (0.34 - 0.57)
Aspartic Acid	0.67 (0.026) (0.62 - 0.71)	0.73 (0.031) (0.72 - 0.73)	-0.061 (0.038) (-0.099 - -0.024)	-0.15, 0.030	0.156	0.34, 0.92 (0.52 - 0.78)
Cystine	0.24 (0.0036) (0.24 - 0.25)	0.26 (0.0042) (0.26 - 0.26)	-0.015 (0.0046) (-0.015 - -0.011)	-0.026, -0.0044	0.012	0.14, 0.30 (0.18 - 0.26)

Table E-7 (continued). Statistical Summary of Site IARL Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Glutamic Acid	2.05 (0.092) (1.85 - 2.24)	2.26 (0.11) (2.20 - 2.28)	-0.21 (0.13) (-0.35 - -0.047)	-0.53, 0.11	0.161	0.77, 2.84 (1.46 - 2.49)
Glycine	0.38 (0.012) (0.36 - 0.40)	0.39 (0.014) (0.39 - 0.39)	-0.018 (0.018) (-0.038 - 0.0035)	-0.060, 0.024	0.344	0.23, 0.52 (0.32 - 0.43)
Histidine	0.31 (0.012) (0.29 - 0.34)	0.34 (0.015) (0.33 - 0.34)	-0.022 (0.018) (-0.045 - 0.0030)	-0.065, 0.020	0.251	0.16, 0.39 (0.22 - 0.33)
Isoleucine	0.38 (0.019) (0.34 - 0.42)	0.42 (0.023) (0.41 - 0.42)	-0.036 (0.029) (-0.081 - 0.0093)	-0.10, 0.032	0.249	0.16, 0.53 (0.27 - 0.46)
Leucine	1.38 (0.065) (1.23 - 1.52)	1.55 (0.079) (1.52 - 1.56)	-0.17 (0.095) (-0.29 - -0.042)	-0.40, 0.052	0.112	0.43, 1.95 (0.93 - 1.69)
Lysine	0.29 (0.0093) (0.29 - 0.30)	0.31 (0.011) (0.31 - 0.31)	-0.015 (0.015) (-0.024 - -0.0085)	-0.049, 0.020	0.346	0.19, 0.40 (0.26 - 0.34)

Table E-7 (continued). Statistical Summary of Site IARL Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Methionine	0.23 (0.0038) (0.22 - 0.23)	0.25 (0.0046) (0.24 - 0.25)	-0.018 (0.0059) (-0.015 - -0.013)	-0.032, -0.0040	0.018	0.11, 0.29 (0.17 - 0.25)
Phenylalanine	0.55 (0.025) (0.50 - 0.60)	0.61 (0.030) (0.60 - 0.61)	-0.059 (0.038) (-0.10 - -0.011)	-0.15, 0.030	0.162	0.23, 0.75 (0.39 - 0.66)
Proline	1.00 (0.039) (0.91 - 1.08)	1.07 (0.047) (1.06 - 1.06)	-0.074 (0.055) (-0.15 - 0.023)	-0.20, 0.055	0.217	0.40, 1.24 (0.66 - 1.07)
Serine	0.49 (0.017) (0.46 - 0.51)	0.56 (0.021) (0.52 - 0.58)	-0.062 (0.024) (-0.063 - -0.062)	-0.12, -0.0047	0.037	0.24, 0.66 (0.38 - 0.59)
Threonine	0.36 (0.010) (0.34 - 0.37)	0.38 (0.013) (0.38 - 0.39)	-0.029 (0.016) (-0.042 - -0.016)	-0.066, 0.0085	0.109	0.20, 0.46 (0.28 - 0.41)
Tryptophan	0.053 (0.0035) (0.049 - 0.058)	0.057 (0.0043) (0.050 - 0.065)	-0.0049 (0.0056) (-0.015 - 0.0080)	-0.018, 0.0083	0.408	0.032, 0.069 (0.039 - 0.063)

Table E-7 (continued). Statistical Summary of Site IARL Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Tyrosine	0.32 (0.023) (0.28 - 0.35)	0.36 (0.029) (0.32 - 0.39)	-0.039 (0.036) (-0.11 - 0.00062)	-0.13, 0.047	0.314	0.077, 0.45 (0.11 - 0.43)
Valine	0.51 (0.022) (0.47 - 0.55)	0.55 (0.027) (0.54 - 0.56)	-0.039 (0.035) (-0.089 - 0.010)	-0.12, 0.043	0.298	0.25, 0.67 (0.38 - 0.58)
Fatty Acid (% total FA)						
16:0 Palmitic	10.72 (0.053) (10.58 - 10.85)	10.44 (0.063) (10.44 - 10.46)	0.28 (0.074) (0.14 - 0.39)	0.10, 0.45	0.007	6.42, 15.23 (9.13 - 12.33)
18:0 Stearic	1.84 (0.018) (1.81 - 1.86)	1.79 (0.022) (1.77 - 1.79)	0.052 (0.027) (0.034 - 0.054)	-0.012, 0.12	0.095	0.87, 2.88 (1.54 - 2.38)
18:1 Oleic	22.91 (0.13) (22.84 - 22.98)	21.95 (0.16) (21.74 - 22.15)	0.97 (0.20) (0.83 - 1.10)	0.49, 1.45	0.002	11.30, 43.27 (21.39 - 34.71)
18:2 Linoleic	62.57 (0.14) (62.49 - 62.70)	63.90 (0.17) (63.72 - 64.09)	-1.34 (0.22) (-1.59 - -1.01)	-1.87, -0.81	<0.001	41.35, 74.78 (49.38 - 63.16)

Table E-7 (continued). Statistical Summary of Site IARL Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% total FA)						
18:3 Linolenic	1.24 (0.0078) (1.22 - 1.26)	1.20 (0.0096) (1.20 - 1.20)	0.040 (0.012) (0.019 - 0.043)	0.011, 0.069	0.014	0.78, 1.52 (0.97 - 1.35)
20:0 Arachidic	0.38 (0.0035) (0.37 - 0.39)	0.37 (0.0043) (0.37 - 0.37)	0.011 (0.0055) (0.0017 - 0.010)	-0.0021, 0.024	0.087	0.15, 0.67 (0.32 - 0.53)
20:1 Eicosenoic	0.20 (0.0016) (0.19 - 0.20)	0.20 (0.0020) (0.20 - 0.20)	-0.0016 (0.0026) (-0.0049 - -0.0018)	-0.0077, 0.0045	0.546	0.12, 0.36 (0.21 - 0.31)
22:0 Behenic	0.15 (0.0027) (0.14 - 0.15)	0.15 (0.0033) (0.15 - 0.15)	-0.00002 (0.0040) (-0.0045 - 0.00034)	-0.0094, 0.0093	0.995	0, 0.32 (0.057 - 0.23)
Mineral						
Calcium (% dw)	0.0055 (0.00020) (0.0054 - 0.0057)	0.0049 (0.00024) (0.0046 - 0.0053)	0.00054 (0.00031) (0.00007 - 0.00084)	-0.00019, 0.0013	0.121	0.0019, 0.0076 (0.0038 - 0.0068)
Copper (mg/kg dw)	1.36 (0.18) (1.21 - 1.56)	1.55 (0.23) (1.49 - 1.61)	-0.19 (0.29) (-0.30 - 0.070)	-0.88, 0.50	0.537	0.17, 3.48 (1.10 - 2.62)

Table E-7 (continued). Statistical Summary of Site IARL Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Iron (mg/kg dw)	24.21 (0.67) (22.67 - 25.84)	23.52 (0.77) (23.15 - 23.83)	0.70 (0.82) (-0.48 - 2.01)	-1.23, 2.63	0.419	11.42, 28.01 (16.55 - 24.10)
Magnesium (% dw)	0.13 (0.0025) (0.13 - 0.13)	0.13 (0.0030) (0.13 - 0.13)	-0.0050 (0.0036) (-0.0055 - -0.0040)	-0.014, 0.0036	0.208	0.080, 0.16 (0.11 - 0.15)
Manganese (mg/kg dw)	9.35 (0.39) (9.26 - 9.40)	9.78 (0.47) (9.51 - 9.82)	-0.42 (0.56) (-0.43 - -0.11)	-1.76, 0.91	0.477	0, 12.67 (4.00 - 9.17)
Phosphorus (% dw)	0.33 (0.0060) (0.32 - 0.35)	0.34 (0.0073) (0.34 - 0.35)	-0.014 (0.0091) (-0.018 - -0.0067)	-0.035, 0.0076	0.170	0.24, 0.42 (0.28 - 0.37)
Potassium (% dw)	0.38 (0.010) (0.38 - 0.39)	0.40 (0.012) (0.38 - 0.41)	-0.013 (0.016) (-0.029 - -0.0011)	-0.050, 0.023	0.419	0.24, 0.54 (0.33 - 0.46)
Zinc (mg/kg dw)	23.54 (0.55) (22.45 - 24.61)	26.51 (0.67) (24.94 - 28.08)	-2.97 (0.86) (-5.63 - -0.34)	-5.01, -0.93	0.010	11.46, 30.37 (17.30 - 25.45)

Table E-7 (continued). Statistical Summary of Site IARL Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Vitamin (mg/kg dw)						
Folic Acid	0.36 (0.024) (0.31 - 0.40)	0.45 (0.028) (0.42 - 0.49)	-0.088 (0.029) (-0.088 - -0.058)	-0.16, -0.018	0.020	0.11, 0.61 (0.24 - 0.57)
Niacin	24.74 (1.48) (22.56 - 27.27)	24.08 (1.62) (22.61 - 27.52)	0.65 (1.35) (-0.24 - 1.78)	-2.55, 3.86	0.643	7.89, 49.83 (20.63 - 43.08)
Vitamin A	0.98 (0.042) (0.94 - 1.03)	0.89 (0.052) (0.83 - 0.95)	0.086 (0.067) (-0.0013 - 0.21)	-0.071, 0.24	0.236	0.38, 1.68 (0.58 - 1.50)
Vitamin B1	2.73 (0.17) (2.58 - 3.03)	2.94 (0.21) (2.90 - 3.02)	-0.21 (0.25) (-0.44 - 0.14)	-0.80, 0.38	0.423	2.21, 3.65 (2.41 - 3.48)
Vitamin B2	1.41 (0.13) (1.17 - 1.60)	1.93 (0.16) (1.89 - 1.96)	-0.51 (0.21) (-0.72 - -0.36)	-1.00, -0.024	0.042	0, 4.47 (1.28 - 3.29)
Vitamin B6	7.11 (0.57) (5.91 - 8.29)	7.51 (0.68) (6.51 - 8.14)	-0.39 (0.78) (-0.60 - 0.15)	-2.23, 1.45	0.630	2.57, 12.07 (5.24 - 10.29)

Table E-7 (continued). Statistical Summary of Site IARL Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Vitamin (mg/kg dw)						
Vitamin E	11.09 (1.18) (8.48 - 13.58)	10.93 (1.45) (10.67 - 11.19)	0.17 (1.87) (-2.70 - 0.55)	-4.25, 4.59	0.931	0, 25.61 (6.67 - 17.34)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2] MON 87427 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the near isogenic, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits were set to zero.

Table E-8. Statistical Summary of Site IARL Grain Anti-nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Anti-nutrient (% dw)						
Phytic Acid	0.98 (0.025) (0.89 - 1.03)	1.00 (0.030) (0.98 - 1.02)	-0.023 (0.039) (0.010 - 0.032)	-0.12, 0.070	0.576	0.73, 1.23 (0.82 - 1.07)
Raffinose	0.11 (0.0043) (0.098 - 0.12)	0.11 (0.0051) (0.11 - 0.11)	0.0036 (0.0059) (-0.0073 - 0.013)	-0.010, 0.017	0.560	0.024, 0.29 (0.092 - 0.21)

[1]dw = dry weight.
[2] MON 87427 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the near isogenic, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits were set to zero.

Table E-9. Statistical Summary of Site IARL Grain Secondary Metabolite Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Secondary Metabolite (µg/g dw)						
Ferulic Acid	2253.00 (92.22) (2188.55 - 2289.56)	2377.76 (112.69) (2293.99 - 2460.85)	-124.75 (141.79) (-171.29 - -13.09)	-460.02, 210.52	0.408	1070.41, 2955.86 (1588.35 - 2630.98)
p-Coumaric Acid	177.78 (10.27) (166.11 - 195.51)	178.58 (12.58) (162.58 - 194.63)	-0.81 (16.14) (-28.53 - 32.92)	-38.97, 37.35	0.961	58.74, 313.97 (124.16 - 250.30)

[1]dw = dry weight.
[2] MON 87427 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the near isogenic, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits were set to zero.

Table E-10. Statistical Summary of Site IARL Forage Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.81 (0.29) (4.49 - 5.10)	5.58 (0.35) (5.23 - 5.84)	-0.78 (0.43) (-0.74 - -0.74)	-1.81, 0.25	0.116	2.66, 6.48 (3.70 - 5.95)
Carbohydrates	86.46 (0.60) (86.21 - 86.75)	84.12 (0.72) (83.80 - 84.64)	2.34 (0.85) (2.11 - 2.61)	0.32, 4.35	0.029	80.13, 94.05 (83.23 - 90.37)
Moisture (% fw)	69.90 (1.03) (67.70 - 71.20)	74.71 (1.21) (73.60 - 75.00)	-4.81 (1.33) (-5.90 - -4.20)	-7.96, -1.66	0.008	51.70, 86.22 (61.00 - 76.00)
Protein	7.03 (0.40) (6.75 - 7.40)	8.63 (0.49) (8.32 - 8.94)	-1.60 (0.63) (-2.00 - -1.57)	-3.09, -0.12	0.037	1.34, 11.57 (4.37 - 9.31)
Total Fat	1.71 (0.32) (1.57 - 1.82)	1.61 (0.39) (1.19 - 2.04)	0.097 (0.50) (-0.30 - 0.63)	-1.10, 1.29	0.853	0.44, 3.33 (0.78 - 3.16)
Fiber (% dw)						
Acid Detergent Fiber	22.89 (2.31) (21.08 - 24.01)	26.21 (2.83) (20.27 - 32.16)	-3.32 (3.66) (-8.15 - 0.82)	-11.97, 5.32	0.393	14.84, 38.51 (21.33 - 35.92)

Table E-10 (continued). Statistical Summary of Site IARL Forage Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Neutral Detergent Fiber	39.68 (2.27) (37.33 - 42.71)	39.33 (2.78) (38.88 - 39.77)	0.36 (3.59) (-1.55 - -0.76)	-8.12, 8.83	0.923	25.12, 54.99 (29.68 - 60.16)
Mineral						
Calcium (% dw)	0.16 (0.016) (0.14 - 0.18)	0.19 (0.019) (0.18 - 0.20)	-0.033 (0.025) (-0.049 - -0.042)	-0.093, 0.026	0.228	0.075, 0.29 (0.10 - 0.24)
Phosphorus (% dw)	0.27 (0.020) (0.25 - 0.31)	0.29 (0.024) (0.28 - 0.31)	-0.024 (0.031) (-0.063 - -0.031)	-0.098, 0.049	0.456	0.063, 0.37 (0.16 - 0.31)

[1]dw = dry weight; fw = fresh weight.
[2] MON 87427 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to near isogenic, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits were set to zero.

Table E-11. Statistical Summary of Site ILWY Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	1.65 (0.053) (1.53 - 1.81)	1.62 (0.053) (1.55 - 1.67)	0.025 (0.067) (-0.051 - 0.14)	-0.13, 0.18	0.716	1.13, 1.97 (1.18 - 1.82)
Carbohydrates	85.94 (0.17) (85.55 - 86.33)	85.58 (0.17) (85.24 - 85.76)	0.36 (0.24) (-0.18 - 1.09)	-0.19, 0.91	0.169	80.77, 89.46 (82.26 - 87.17)
Moisture (% fw)	12.53 (0.21) (12.10 - 13.30)	12.17 (0.21) (11.90 - 12.40)	0.37 (0.29) (-0.30 - 1.10)	-0.31, 1.04	0.245	7.56, 14.80 (9.31 - 12.70)
Protein	8.71 (0.15) (8.46 - 8.86)	8.97 (0.15) (8.62 - 9.19)	-0.26 (0.21) (-0.73 - 0.19)	-0.75, 0.23	0.253	5.79, 13.43 (8.07 - 12.13)
Total Fat	3.72 (0.083) (3.62 - 3.83)	3.84 (0.083) (3.60 - 3.98)	-0.12 (0.12) (-0.33 - 0.11)	-0.39, 0.15	0.333	2.12, 5.35 (2.90 - 4.30)
Fiber (% dw)						
Acid Detergent Fiber	3.78 (0.18) (3.33 - 4.27)	3.05 (0.18) (2.80 - 3.18)	0.73 (0.25) (0.15 - 1.09)	0.15, 1.30	0.020	1.84, 4.39 (2.29 - 4.27)

Table E-11 (continued). Statistical Summary of Site ILWY Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Neutral Detergent Fiber	10.25 (0.33) (9.77 - 10.97)	10.27 (0.33) (9.99 - 10.59)	-0.014 (0.46) (-0.82 - 0.98)	-1.08, 1.05	0.975	5.69, 11.81 (7.06 - 10.66)
Total Dietary Fiber	13.26 (0.38) (12.63 - 14.35)	13.28 (0.38) (13.13 - 13.44)	-0.022 (0.51) (-0.64 - 1.07)	-1.20, 1.16	0.966	8.67, 15.32 (10.25 - 14.30)
Amino Acid (% dw)						
Alanine	0.62 (0.020) (0.61 - 0.63)	0.63 (0.020) (0.55 - 0.67)	-0.016 (0.029) (-0.067 - 0.075)	-0.082, 0.051	0.603	0.32, 1.12 (0.58 - 0.98)
Arginine	0.42 (0.017) (0.40 - 0.45)	0.43 (0.017) (0.39 - 0.45)	-0.0053 (0.020) (-0.035 - 0.015)	-0.051, 0.040	0.796	0.24, 0.68 (0.34 - 0.57)
Aspartic Acid	0.54 (0.016) (0.54 - 0.55)	0.55 (0.016) (0.48 - 0.59)	-0.0075 (0.022) (-0.049 - 0.064)	-0.059, 0.044	0.745	0.34, 0.92 (0.52 - 0.78)
Cystine	0.22 (0.0041) (0.21 - 0.22)	0.22 (0.0041) (0.21 - 0.23)	-0.0048 (0.0051) (-0.013 - 0.0072)	-0.017, 0.0070	0.375	0.14, 0.30 (0.18 - 0.26)

Table E-11 (continued). Statistical Summary of Site ILWY Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Glutamic Acid	1.55 (0.053) (1.53 - 1.58)	1.59 (0.053) (1.38 - 1.70)	-0.035 (0.074) (-0.18 - 0.20)	-0.21, 0.14	0.650	0.77, 2.84 (1.46 - 2.49)
Glycine	0.34 (0.0091) (0.34 - 0.35)	0.35 (0.0091) (0.31 - 0.37)	-0.0046 (0.012) (-0.028 - 0.033)	-0.032, 0.023	0.706	0.23, 0.52 (0.32 - 0.43)
Histidine	0.27 (0.0082) (0.27 - 0.27)	0.27 (0.0082) (0.23 - 0.29)	-0.00074 (0.011) (-0.018 - 0.033)	-0.027, 0.025	0.949	0.16, 0.39 (0.22 - 0.33)
Isoleucine	0.29 (0.011) (0.29 - 0.30)	0.30 (0.011) (0.26 - 0.32)	-0.0075 (0.015) (-0.033 - 0.039)	-0.043, 0.028	0.638	0.16, 0.53 (0.27 - 0.46)
Leucine	1.00 (0.036) (0.97 - 1.02)	1.03 (0.036) (0.89 - 1.10)	-0.028 (0.051) (-0.13 - 0.13)	-0.14, 0.088	0.591	0.43, 1.95 (0.93 - 1.69)
Lysine	0.27 (0.0078) (0.27 - 0.27)	0.28 (0.0078) (0.25 - 0.30)	-0.0041 (0.0094) (-0.021 - 0.026)	-0.026, 0.018	0.671	0.19, 0.40 (0.26 - 0.34)

Table E-11 (continued). Statistical Summary of Site ILWY Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Methionine	0.20 (0.0039) (0.20 - 0.21)	0.20 (0.0039) (0.20 - 0.21)	0.0012 (0.0055) (-0.0089 - 0.012)	-0.011, 0.014	0.829	0.11, 0.29 (0.17 - 0.25)
Phenylalanine	0.42 (0.014) (0.40 - 0.43)	0.43 (0.014) (0.38 - 0.46)	-0.015 (0.020) (-0.063 - 0.052)	-0.062, 0.032	0.474	0.23, 0.75 (0.39 - 0.66)
Proline	0.75 (0.028) (0.74 - 0.77)	0.76 (0.028) (0.65 - 0.83)	-0.015 (0.039) (-0.091 - 0.12)	-0.10, 0.075	0.717	0.40, 1.24 (0.66 - 1.07)
Serine	0.40 (0.014) (0.38 - 0.41)	0.41 (0.014) (0.36 - 0.43)	-0.011 (0.019) (-0.057 - 0.035)	-0.055, 0.033	0.590	0.24, 0.66 (0.38 - 0.59)
Threonine	0.30 (0.0079) (0.29 - 0.30)	0.31 (0.0079) (0.28 - 0.32)	-0.0074 (0.011) (-0.031 - 0.025)	-0.033, 0.018	0.524	0.20, 0.46 (0.28 - 0.41)
Tryptophan	0.047 (0.0030) (0.045 - 0.049)	0.051 (0.0030) (0.042 - 0.056)	-0.0042 (0.0031) (-0.011 - 0.0061)	-0.011, 0.0030	0.215	0.032, 0.069 (0.039 - 0.063)

Table E-11 (continued). Statistical Summary of Site ILWY Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Tyrosine	0.23 (0.032) (0.18 - 0.28)	0.23 (0.032) (0.21 - 0.24)	0.0053 (0.046) (-0.061 - 0.049)	-0.10, 0.11	0.909	0.077, 0.45 (0.11 - 0.43)
Valine	0.42 (0.014) (0.41 - 0.42)	0.42 (0.014) (0.37 - 0.45)	-0.0077 (0.020) (-0.044 - 0.049)	-0.054, 0.038	0.707	0.25, 0.67 (0.38 - 0.58)
Fatty Acid (% total FA)						
16:0 Palmitic	10.54 (0.054) (10.44 - 10.65)	10.21 (0.054) (10.15 - 10.24)	0.33 (0.056) (0.28 - 0.40)	0.20, 0.46	<0.001	6.42, 15.23 (9.13 - 12.33)
18:0 Stearic	1.90 (0.018) (1.89 - 1.91)	1.88 (0.018) (1.82 - 1.93)	0.021 (0.025) (-0.028 - 0.096)	-0.037, 0.080	0.424	0.87, 2.88 (1.54 - 2.38)
18:1 Oleic	23.58 (0.12) (23.29 - 23.78)	23.24 (0.12) (23.17 - 23.39)	0.34 (0.14) (0.13 - 0.49)	0.012, 0.66	0.043	11.30, 43.27 (21.39 - 34.71)
18:2 Linoleic	62.01 (0.18) (61.68 - 62.32)	62.72 (0.18) (62.45 - 62.92)	-0.71 (0.19) (-0.89 - -0.46)	-1.15, -0.27	0.005	41.35, 74.78 (49.38 - 63.16)

Table E-11 (continued). Statistical Summary of Site ILWY Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fatty Acid (% total FA)						
18:3 Linolenic	1.20 (0.012) (1.17 - 1.22)	1.20 (0.012) (1.19 - 1.21)	0.0051 (0.017) (-0.030 - 0.024)	-0.033, 0.044	0.767	0.78, 1.52 (0.97 - 1.35)
20:0 Arachidic	0.41 (0.0052) (0.40 - 0.42)	0.40 (0.0052) (0.38 - 0.41)	0.015 (0.0071) (-0.0022 - 0.034)	-0.0015, 0.031	0.068	0.15, 0.67 (0.32 - 0.53)
20:1 Eicosenoic	0.21 (0.0019) (0.20 - 0.21)	0.21 (0.0019) (0.21 - 0.21)	0 (0.0027) (-0.0045 - 0.0033)	-0.0062, 0.0062	0.999	0.12, 0.36 (0.21 - 0.31)
22:0 Behenic	0.15 (0.0036) (0.15 - 0.16)	0.15 (0.0036) (0.14 - 0.16)	0.00005 (0.0050) (-0.0099 - 0.016)	-0.011, 0.012	0.992	0, 0.32 (0.057 - 0.23)
Mineral						
Calcium (% dw)	0.0049 (0.00014) (0.0048 - 0.0050)	0.0049 (0.00014) (0.0047 - 0.0052)	0 (0.00019) (-0.00037 - 0.00030)	-0.00045, 0.00045	0.994	0.0019, 0.0076 (0.0038 - 0.0068)
Copper (mg/kg dw)	1.66 (0.093) (1.56 - 1.79)	1.75 (0.093) (1.63 - 1.99)	-0.086 (0.13) (-0.42 - 0.16)	-0.39, 0.22	0.530	0.17, 3.48 (1.10 - 2.62)

Table E-11 (continued). Statistical Summary of Site ILWY Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Mineral						
Iron (mg/kg dw)	22.51 (0.41) (22.21 - 22.95)	21.84 (0.41) (20.66 - 22.49)	0.66 (0.57) (-0.019 - 1.55)	-0.66, 1.99	0.280	11.42, 28.01 (16.55 - 24.10)
Magnesium (% dw)	0.13 (0.0021) (0.13 - 0.13)	0.13 (0.0021) (0.13 - 0.14)	-0.0017 (0.0028) (-0.0062 - 0.0039)	-0.0082, 0.0047	0.550	0.080, 0.16 (0.11 - 0.15)
Manganese (mg/kg dw)	5.63 (0.32) (5.52 - 5.72)	5.74 (0.32) (4.89 - 6.49)	-0.10 (0.45) (-0.83 - 0.83)	-1.14, 0.94	0.829	0, 12.67 (4.00 - 9.17)
Phosphorus (% dw)	0.34 (0.0033) (0.34 - 0.35)	0.34 (0.0033) (0.34 - 0.35)	-0.0020 (0.0046) (-0.0049 - 0.00002)	-0.013, 0.0086	0.673	0.24, 0.42 (0.28 - 0.37)
Potassium (% dw)	0.41 (0.0074) (0.40 - 0.42)	0.41 (0.0074) (0.40 - 0.43)	0.0028 (0.010) (-0.017 - 0.021)	-0.021, 0.027	0.796	0.24, 0.54 (0.33 - 0.46)
Zinc (mg/kg dw)	21.25 (0.76) (20.99 - 21.56)	23.55 (0.76) (22.61 - 25.00)	-2.31 (1.07) (-3.44 - -1.62)	-4.78, 0.17	0.063	11.46, 30.37 (17.30 - 25.45)

Table E-11 (continued). Statistical Summary of Site ILWY Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Vitamin (mg/kg dw)						
Folic Acid	0.33 (0.025) (0.28 - 0.39)	0.31 (0.025) (0.29 - 0.32)	0.024 (0.036) (-0.039 - 0.078)	-0.059, 0.11	0.529	0.11, 0.61 (0.24 - 0.57)
Niacin	31.16 (0.90) (28.72 - 33.37)	32.07 (0.90) (31.16 - 33.26)	-0.90 (1.27) (-3.06 - 0.23)	-3.83, 2.03	0.497	7.89, 49.83 (20.63 - 43.08)
Vitamin A	1.12 (0.058) (1.07 - 1.21)	1.10 (0.058) (1.07 - 1.16)	0.019 (0.082) (-0.094 - 0.14)	-0.17, 0.21	0.824	0.38, 1.68 (0.58 - 1.50)
Vitamin B1	3.28 (0.11) (3.08 - 3.41)	3.23 (0.11) (3.08 - 3.41)	0.052 (0.16) (-0.33 - 0.27)	-0.32, 0.43	0.755	2.21, 3.65 (2.41 - 3.48)
Vitamin B2	1.60 (0.10) (1.36 - 1.80)	1.51 (0.10) (1.32 - 1.70)	0.091 (0.15) (-0.35 - 0.34)	-0.25, 0.43	0.555	0, 4.47 (1.28 - 3.29)
Vitamin B6	6.83 (0.41) (6.51 - 7.43)	6.90 (0.41) (5.67 - 7.63)	-0.063 (0.58) (-1.11 - 1.76)	-1.40, 1.28	0.915	2.57, 12.07 (5.24 - 10.29)

Table E-11 (continued). Statistical Summary of Site ILWY Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Vitamin (mg/kg dw)						
Vitamin E	11.63 (1.15) (7.04 - 14.65)	11.84 (1.15) (10.13 - 13.58)	-0.20 (1.63) (-6.54 - 4.52)	-3.97, 3.56	0.903	0, 25.61 (6.67 - 17.34)

[1]dw = dry weight; fw = fresh weight; FA = fatty acid.
[2] MON 87427 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the near isogenic, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits were set to zero.

Table E-12. Statistical Summary of Site IARL Grain Anti-nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Anti-nutrient (% dw)						
Phytic Acid	1.02 (0.024) (1.00 - 1.04)	1.09 (0.024) (1.03 - 1.12)	-0.071 (0.034) (-0.11 - -0.032)	-0.15, 0.0081	0.072	0.73, 1.23 (0.82 - 1.07)
Raffinose	0.20 (0.0076) (0.19 - 0.21)	0.20 (0.0076) (0.18 - 0.21)	0.0046 (0.011) (-0.017 - 0.025)	-0.020, 0.029	0.671	0.024, 0.29 (0.092 - 0.21)

[1]dw = dry weight.
[2] MON 87427 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the near isogenic, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial references. Negative limits were set to zero.

Table E-13. Statistical Summary of Site IARL Grain Secondary Metabolite Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Secondary Metabolite (µg/g dw)						
Ferulic Acid	2317.21 (50.54) (2243.74 - 2354.95)	2368.37 (50.54) (2236.10 - 2500.00)	-51.16 (41.17) (-145.05 - 7.64)	-146.09, 43.77	0.249	1070.41, 2955.86 (1588.35 - 2630.98)
p-Coumaric Acid	193.24 (4.64) (184.51 - 198.39)	191.67 (4.64) (183.88 - 203.20)	1.57 (4.42) (-6.38 - 10.46)	-8.63, 11.76	0.731	58.74, 313.97 (124.16 - 250.30)

[1]dw = dry weight.
[2] MON 87427 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the near isogenic, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits were set to zero.

Table E-14. Statistical Summary of Site IARL Forage Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.79 (0.16) (4.53 - 5.13)	4.58 (0.16) (4.40 - 4.80)	0.21 (0.22) (-0.0096 - 0.33)	-0.31, 0.72	0.378	2.66, 6.48 (3.70 - 5.95)
Carbohydrates	88.18 (0.46) (87.27 - 89.23)	88.56 (0.46) (87.89 - 88.92)	-0.38 (0.47) (-1.59 - 0.31)	-1.45, 0.70	0.442	80.13, 94.05 (83.23 - 90.37)
Moisture (% fw)	65.50 (1.37) (62.70 - 67.90)	66.00 (1.37) (64.10 - 67.30)	-0.50 (1.54) (-1.40 - 1.30)	-4.04, 3.04	0.753	51.70, 86.22 (61.00 - 76.00)
Protein	5.46 (0.40) (4.48 - 6.17)	5.55 (0.40) (5.17 - 5.96)	-0.082 (0.50) (-1.48 - 0.66)	-1.23, 1.07	0.873	1.34, 11.57 (4.37 - 9.31)
Total Fat	1.57 (0.27) (1.09 - 1.85)	1.32 (0.27) (0.58 - 2.20)	0.25 (0.39) (-1.11 - 1.18)	-0.64, 1.14	0.535	0.44, 3.33 (0.78 - 3.16)
Fiber (% dw)						
Acid Detergent Fiber	27.86 (1.16) (26.42 - 29.00)	26.59 (1.16) (24.57 - 27.71)	1.27 (1.61) (-1.28 - 3.58)	-2.45, 4.99	0.452	14.84, 38.51 (21.33 - 35.92)

Table E-14 (continued). Statistical Summary of Site ILWY Forage Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Fiber (% dw)						
Neutral Detergent Fiber	40.98 (2.55) (36.14 - 43.70)	40.76 (2.55) (36.53 - 43.43)	0.22 (2.54) (-0.39 - 1.36)	-5.64, 6.08	0.933	25.12, 54.99 (29.68 - 60.16)
Mineral						
Calcium (% dw)	0.21 (0.013) (0.19 - 0.22)	0.22 (0.013) (0.18 - 0.25)	-0.011 (0.018) (-0.063 - 0.036)	-0.054, 0.032	0.568	0.075, 0.29 (0.10 - 0.24)
Phosphorus (% dw)	0.21 (0.017) (0.20 - 0.21)	0.23 (0.017) (0.19 - 0.27)	-0.019 (0.019) (-0.074 - 0.023)	-0.062, 0.025	0.345	0.063, 0.37 (0.16 - 0.31)

[1]dw = dry weight; fw = fresh weight.
[2] MON 87427 treated with glyphosate.
[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.
[4]Control refers to the near isogenic, conventional control.
[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits were set to zero.

References for Appendix E

AACC. 1998. Official Method 32.20. Approved Methods of the American Association of Cereal Chemists (9th ed.).

Albala-Hurtado, S., M.T. Veciana-Nogues, M. Izquierdo-Pulido, and M.C. Vidal-Carou. 1997. Determination of free and total furfural compounds in infact milk formulas by high-performance liquid chromatography. Journal of Agricultural and Food Chemistry 45:2128-2133.

AOAC-International. 2005a. Method 923.03. Official Methods of Analysis of AOAC International, AOAC-International, (18th ed.) AOAC International, Gaithersburg, Maryland.

AOAC-International. 2005b. Method 944.13. Official Methods of Analysis of AOAC International, AOAC-International, (18th ed.), Gaithersburg, Maryland.

AOAC-International. 2005c. Method 961.15. Official Methods of Analysis of AOAC International, AOAC-International, (18th ed.), Gaithersburg, Maryland.

AOAC-International. 2005d. Method 982.30. Official Methods of Analysis of AOAC International, AOAC-International, (18th ed.), Gaithersburg, Maryland.

AOAC-International. 2005e. Method 983.23. Official Methods of Analysis of AOAC International, AOAC-International, (18th ed.) AOAC International, Gaithersburg, Maryland.

AOAC-International. 2005f. Method 985.29. Official Methods of Analysis of AOAC International, AOAC-International, (18th ed.) AOAC International, Gaithersburg, Maryland.

AOAC-International. 2005g. Methods 922.06 and 954.02. Official Methods of Analysis of AOAC International, AOAC-International, (18th ed.) AOAC International, Gaithersburg, Maryland.

AOAC-International. 2005h. Methods 926.08 and 925.09. Official Methods of Analysis of AOAC International, (18th ed.) AOAC International, Gaithersburg, Maryland.

AOAC-International. 2005i. Methods 940.33 and 960.46. Official Methods of Analysis of AOAC International, AOAC-International, (18th ed.), Gaithersburg, Maryland.

AOAC-International. 2005j. Methods 941.15. Official Methods of Analysis of AOAC International, AOAC International, (18th ed.) Gaithersburg, Maryland.

AOAC-International. 2005k. Methods 942.23, 953.17 and 957.17. Official Methods of Analysis of AOAC International, AOAC-International, (18th ed.), Gaithersburg, Maryland.

AOAC-International. 2005l. Methods 955.04 and 979.09. Official Methods of Analysis of AOAC International, AOAC-International, (18th ed.) AOAC International, Gaithersburg, Maryland.

AOAC-International. 2005m. Methods 960.39 and 948.22. Official Methods of Analysis of AOAC International, AOAC-International, (18th ed.) AOAC International, Gaithersburg, Maryland.

AOAC-International. 2005n. Methods 960.46 and 992.05. Official Methods of Analysis of AOAC International, AOAC-International, (18th ed.), Gaithersburg, Maryland.

AOAC-International. 2005o. Methods 984.27 and 985.01. Official Methods of Analysis of AOAC International, AOAC-International, (18th ed.), Gaithersburg, Maryland.

AOCS. 1997. Official Method Ce 2-66. Official Methods and Recommended Practices of the American Oil Chemists' Society, D. Firestone, (5th ed.) American Oil Chemists Society, Champaign, Illinois.

AOCS. 2001. Method Ce 1e-92. Official Methods and Recommended Practices of the American Oil Chemists Society, (5th ed.) American Oil Chemists Society, Champaign.

Atkins, L., A.S. Schultz, W.L. Williams, and C.N. Frey. 1943. Yeast microbiological methods for determination of vitamins. Industrial and Engineering Chemistry, Analytical Edition 15:141-144.

Bradstreet, R.B. 1965. The Kjeldahl method for organic nitrogen. Academic Press. New York, New York.

Brobst, K.H. 1972. Gas-liquid chromatography of trimethylsilyl derivatives. Methods in carbohydrate chemistry, Vol 6. Academic Press, New York, New York.

Cort, W.M., T.S. Vincente, E.H. Waysek, and B.D. Williams. 1983. Vitamin E content of feedstuffs determined by high-performance liquid chromatographic fluorescence. Journal of Agricultural and Food Chemistry 31:1330-1333.

Hagerman, A.E., and R.L. Nicholson. 1982. High-performace liquid chromatographic determination of hydroxycinnamic acids in the maize mesocotyl. Journal of Agricultural and Food Chemistry 30:1098-1102.

Infant Formula Council. 1985. Section C-2. Methods of Analysis for Infant Formulas, Infant Formula Council, Atlanta, Georgia.

Kalthoff, I.M., and E.B. Sandell. 1948. Quantitative inorganic analysis. MacMillan, New York, New York.

Lehrfeld, J. 1989. High-performance liquid chromatography analysis of phytic acid on a ph-stable, macroporous polymer column. Cereal Chemistry 66:510-515.

Lehrfeld, J. 1994. HPLC separation and quantitation of phytic acid and some inositol phosphates in foods: problem and solutions. Journal of Agricultural and Food Chemistry 42:2726-2731.

Mason, B.S., and H.T. Slover. 1971. Gas-chromatographic method for the determination of sugars in foods. Journal of Agricultural and Food Chemistry 19:551-554.

McMurray, C.H., W.J. Blanchflower, and D.A. Rice. 1980. Influence of extraction techniques on determination of α-Tocopherol in animal feedstuffs. Journal of the Association of Official Analytical Chemists 63:1258-1261.

USP. 1995. Page 1913 in The National Formulary. The United States Pharmacopeia, Washington, D.C.

Quackenbush, F.W. 1987. Reverse Phase HPLC Separation of cis and trans carotenoids and its application to beta carotenes in food materials. Journal of Liquid Chromatography 10:643-654.

Speek, A.J., J. Schrijver, and W.H.P. Schreurs. 1985. Vitamin E composition of some seed oils as determined by high-performance liquid chromatography with fluorometric detection. Journal of Food Science 50:121-124.

USDA. 1970. Forage and fiber analyses. Agricultural Handbook No. 379. U.S. Department of Agriculture, Washington, DC.

USDA. 1973. Energy value of foods. Pages 2-11 in Agriculture Handbook No. 74. U.S. Department of Agriculture, Washington, D.C.

Appendix F: Materials, Methods and Indivdual-Site Results for Seed Dormancy and Germination Analyses of MON 87427

F.1. Materials

MON 87427, the conventional control, and commercial maize reference hybrid starting seed were produced in Jefferson County, IA (IA) and Stark County, IL (IL) in 2008 (Table F-1). Evaluations were conducted on seed from non glyphosate-treated plants.

F.2. Characterization of the Materials

The identities of MON 87427 and conventional control were confirmed by verifying the chain of custody documentation prior to analysis. To further confirm the identities of MON 87427 and conventional control, event-specific polymerase chain reaction (PCR) analyses were conducted on the harvested grain from each site. The PCR analyses and the resulting Verification of Identities were archived in the Monsanto Regulatory Archives under the starting seed lot numbers.

F.3. Performing Facility and Experimental Methods

Dormancy and germination evaluations were conducted at BioDiagnostics, Inc. in River Falls, WI. The principal investigator was certified to conduct seed dormancy and germination testing consistent with the standards established by the Association of Official Seed Analysts (AOSA), a seed trade association (AOSA, 2000; AOSA, 2006; AOSA, 2007).

Seven germination chambers were used in the study and each chamber was maintained dark under one of the following seven temperature regimes: constant temperature of approximately 5, 10, 20, or 30 °C or alternating temperatures of approximately 10/20, 10/30, or 20/30° C. The alternating temperature regimes were maintained at the lower temperature for 16 hours and the higher temperature for 8 hours. The temperature inside each germination chamber was monitored and recorded throughout the duration of the study.

Germination towels for MON 87427, the conventional control, and reference hybrids were prepared per the facility SOPs. Each germination towel represented one replication. The types of data collected depended on the temperature regime. Each rolled germination towel in the AOSA-recommended temperature regime (i.e., alternating 20/30 °C) was assessed periodically during the study for normally germinated, abnormally germinated, hard (viable and nonviable), dead, and firm swollen (viable and nonviable) seed as defined by AOSA guidelines (AOSA, 2006). Each rolled germination towel in the additional temperature regimes (i.e., 5, 10, 20, 30, alternating 10/20, and 10/30 °C) was assessed periodically during the study for germinated, hard (viable and nonviable), dead, and firm swollen (viable and nonviable) seed.

F.4. Statistical Analysis

Statistical analyses were performed by Monsanto Statistics Technology Center. The data were analyzed according to a split-plot design, with production site as the whole plot and starting seed entry as the sub-plot. SAS® (SAS Version 9.2) was used to compare MON 87427 and the conventional control for each characteristic with a level of statistical significance of 5% ($p \leq 0.05$) across sites (combined-site analysis) and within sites (individual-site analysis). Summary statistics were provided for each production site and temperature regime combination. MON 87427 was not statistically compared to the commercial references, and no comparisons were made across temperature regimes. The reference range was calculated from the minimum and maximum mean values observed in the reference hybrids.

F.5. Individual Site Seed Dormancy and Germination Analysis

In the individual-site analysis at 5 °C, MON 87427 had statistically significant fewer percent dead seed than the control at IA (6.8 vs. 15.3%), and higher percent viable firm swollen seed compared to the conventional control (93.3 vs. 84.8%; Table F-2). While a difference was also detected in the combined site analysis for percent dead seed and percent viable firm swollen seed at 5 °C, this mean value of MON 87427 is within the reference range (Section VII, Table VII-2) and is unlikely to be meaningful in terms of increased pest potential (Section VII, Figure VII-1, Step 3).

In the individual site analysis at 10 °C, MON 87427 had higher percent germinated seed than the conventional control (87.8 vs. 81.8%) at IA. This difference was not detected in the combined site analysis (Section VII, Table VII-2) and is unlikely to biologically meaningful in terms of increased pest potential (Section VII, Figure VII-1, Step 2).

In the individual site analysis at 20 °C, MON 87427 exhibited fewer percent dead seed than the conventional control (0.8 vs. 3.3%) at IA and greater percent germinated seed than the conventional control (99.3 vs. 96.8%) at IA. While differences were also detected in the combined site analysis for percent dead seed and percent germinated seed at 20 °C, the mean values of MON 87427 were within the reference range (Section VII, Table VII-2) and are unlikely to be biologically meaningful in terms of increased pest potential (Section VII, Figure VII-1, Step 3).

In the individual site analysis at 30 °C, MON 87427 exhibited greater percent dead seed than the conventional control (3.3 vs. 1.0%) at IL and fewer percent germinated seed than the conventional control (96.8 vs. 99.0%) at IL. These differences were not detected in the combined site analysis (Section VII, Table VII-2) and are unlikely to be biologically meaningful in terms of increased pest potential (Section VII, Figure VII-1, Step 2).

Statistically significant differences detected between MON 87427 and the conventional control for germination characteristics in the individual-site analysis were not consistently detected across temperature regimes or seed production sites. While some differences were detected in the combined-site analysis, the assessed dormancy and germination values of MON 87427 were within the range of values expected for

commercial maize, indicating that MON 87427 seed dormancy and germination characteristics have not been altered compared to conventional maize.

Table F-1. Starting Seed of MON 87427, Conventional Control and Commercial Maize Reference Hybrids Used in Dormancy Assessment

Site[1]	Substance Type	Starting Seed Name	Phenotype
IA	Control	Control	Conventional
IA	Reference	Asgrow RX708	Conventional
IA	Reference	Dekalb DKC60-15	Conventional
IA	Reference	Midwest Genetics G7944	Conventional
IA	Test	MON 87427	Glyphosate-induced non-viable pollen
IL	Control	Control	Conventional
IL	Reference	Asgrow RX715	Conventional
IL	Reference	Dekalb DKC61-50	Conventional
IL	Reference	Midland 7B15	Conventional
IL	Reference	NK N69-P9	Conventional
IL	Test	MON 87427	Glyphosate-induced non-viable pollen

[1] Site codes are as follows: IA = Jefferson County, IA; IL = Stark County, IL.

Table F-2. Comparison of MON 87427 to the Conventional Control for Dormancy and Germination Characteristics

Temperature (°C)	Germination Category	Mean[1] (S.E.) IA[2] MON 87427	IA Control	IL MON 87427	IL Control
5	Germinated†	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Viable hard†	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Dead	6.8* (1.4)	15.3 (4.0)	6.3 (3.0)	7.0 (1.2)
	Viable firm swollen	93.3* (1.4)	84.8 (4.0)	93.8 (3.0)	93.0 (1.2)
10	Germinated	87.8* (2.1)	81.8 (1.9)	77.0 (1.9)	76.3 (2.4)
	Viable hard†	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Dead	2.8 (1.2)	4.8 (0.9)	5.0 (0.7)	4.0 (0.8)
	Viable firm swollen	9.5 (1.2)	13.5 (1.9)	18.0 (1.7)	19.8 (2.3)
20	Germinated	99.3* (0.5)	96.8 (1.0)	98.5 (0.3)	97.5 (0.9)
	Viable hard†	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Dead	0.8* (0.5)	3.3 (1.0)	1.5 (0.3)	2.5 (0.9)
	Viable firm swollen†	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
30	Germinated	98.0 (0.6)	97.8 (0.9)	96.8* (0.8)	99.0 (0.4)
	Viable hard†	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Dead	2.0 (0.6)	2.3 (0.9)	3.3* (0.8)	1.0 (0.4)
	Viable firm swollen†	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

Table F-2 (continued). Comparison of MON 87427 to the Conventional Control for Dormancy and Germination Characteristics

Temperature (°C)	Germination Category	Mean[1] (S.E.) IA[2] MON 87427	IA Control	IL MON 87427	IL Control
10/20	Germinated	98.3 (0.6)	98.5 (0.7)	97.8 (0.9)	98.3 (1.2)
	Viable hard[†]	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Dead	1.8 (0.6)	1.5 (0.7)	2.3 (0.9)	1.8 (1.2)
	Viable firm swollen[†]	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
10/30	Germinated	98.3 (0.3)	97.0 (0.9)	98.8 (0.5)	98.8 (0.6)
	Viable hard[†]	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Dead	1.8 (0.3)	3.0 (0.9)	1.3 (0.5)	1.3 (0.6)
	Viable firm swollen[†]	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
20/30 (AOSA)	Normal germinated	92.8 (1.4)	92.8 (0.8)	96.0 (0.6)	97.0 (0.7)
	Abnormal germinated	6.0 (1.5)	6.3 (0.8)	1.5 (0.9)	1.0 (0.4)
	Viable hard[†]	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
	Dead	1.3 (0.3)	1.0 (0.0)	2.5 (0.9)	2.0 (0.4)
	Viable firm swollen[†]	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

Note: experimental design was a split-plot with four replications (n = 4).
*Indicates significant difference ($p \leq 0.05$) between MON 87427 and the conventional control using analysis of variance.
[†]No statistical comparison could be made due to lack of variability in the data.
[1]In some instances, the total percentage of both MON 84727 and the conventional control did not equal 100% due to numerical rounding of the means. S.E. = standard error.
[2]Site code designations are as follows: IA = Jefferson County, Iowa; IL = Stark County, IL.

References for Appendix F

AOSA. 2000. Tetrazolium testing handbook. Contribution no. 29. The Handbook on Seed Testing, Association of Official Seed Analysts, Lincoln, Nebraska.

AOSA. 2006. Seedling evaluation handbook. Contribution no. 35. The Handbook on Seed Testing, Association of Official Seed Analysts, Lincoln, Nebraska.

AOSA. 2007. Rules for testing seeds. The Handbook on Seed Testing, Association of Official Seed Analysis, Ithaca, New York.

Appendix G: Materials, Methods, and Individual Site Results from Phenotypic, Agronomic and Environmental Interactions Analyses of MON 87427

G.1. Materials

The materials for phenotypic assessments include: MON 87427, a conventional control, and 38 unique, commercial references. The references contain both conventional maize and Roundup Ready® maize hybrids. The list of hybrids planted in each site is presented in Table G-1. The identities of MON 87427 and the conventional control seed were confirmed by PCR analysis prior to use.

G.2. Field Sites and Plot Design

The experiment was established at each of 16 sites in a randomized complete block design with three replications. Arthropods were collected at sites in Stark County, IL (IL1), Boone County, IN (IN2), Butler County, MO (MO) and Berks County, PA (PA). Each plot was 30 feet long and 30 feet wide (12 rows spaced 30 inches apart). Arthropods were not collected at sites in Wapello County, IA (IA1), Benton County, IA (IA2), Clinton County, IL (IL2), Montgomery County, IN (IN1), Ottawa County, MI (MI), Caddo County, OK (OK), Armstrong County, TX (TX), and Walworth County, WI (WI). Each plot was 20 feet long and 10 feet wide (4 rows spaced 30 inches apart). Arthropods were not collected at sites in Jackson County, AR (ARNE), Jefferson County, IA (IARL), Stark County, IL (ILWY), and Parke County, IN (INRC). Each plot was 20 feet long and 15 feet wide (6 rows spaced 30 inches apart).

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Table G-1. Starting Seed for Phenotypic Assessments

Site[1]	Starting Seed	Substance	Phenotype
All	MON 87427	Test	Glyphosate-induced non-viable pollen
All	Conventional Control	Control	Conventional
ARNE	Crows C6501	Reference	Conventional
	Fielder's Choice 7864	Reference	Conventional
	Fontanelle 5797	Reference	Conventional
	Midwest Genetics 87801	Reference	Conventional
IARL	Dekalb DKC 60-15	Reference	Conventional
	NC+ 4443	Reference	Conventional
	Midwest Genetics G7944	Reference	Conventional
	Asgrow RX708	Reference	Conventional
ILWY	Dekalb DKC 61-50	Reference	Conventional
	Midland 7B15	Reference	Conventional
	NK N69-P9	Reference	Conventional
	Asgrow RX715	Reference	Conventional
INRC	Crows C5303	Reference	Conventional
	Midwest Genetics 8122	Reference	Conventional
	Pioneer 33M54	Reference	Conventional
	NC+ 5411	Reference	Conventional
IA1	Dekalb DKC64-27	Reference	Glyphosate-tolerant
	Golden Harvest H-8920	Reference	Conventional
	Stewart S650	Reference	Conventional
	Asgrow RX752RR2	Reference	Glyphosate –tolerant
IA2	Stewart S650	Reference	Conventional
	Asgrow RX715	Reference	Conventional
	Midwest Genetics G7944	Reference	Conventional
	Dekalb DKC63-78	Reference	Conventional
	Burrus 645	Reference	Conventional
	Asgrow RX772	Reference	Conventional
	Dekalb DKC63-78	Reference	Conventional
IL1	Garst 8445	Reference	Conventional
	Dekalb DKC 61-50	Reference	Conventional
	Burrus 645	Reference	Conventional
	Dekalb DKC63-78	Reference	Conventional
IL2	NC+ 5411	Reference	Conventional
	Crows C5303	Reference	Conventional
	Asgrow RX772	Reference	Conventional
	Garst 8445	Reference	Conventional
IN1	Burrus 645	Reference	Conventional

Table G-1 (continued). Starting Seed for Phenotypic Assessments

Site[1]	Starting Seed	Substance	Phenotype
	Garst 8424	Reference	Conventional
	Dekalb DKC63-78	Reference	Conventional
	Dekalb DKC61-42	Reference	Conventional
IN2	Pioneer 33H25	Reference	Conventional
	Pioneer 33D11	Reference	Conventional
	Asgrow RX752RR2	Reference	Glyphosate –tolerant
	Midwest Genetics 8122	Reference	Conventional
MI	Dekalb DKC 60-15	Reference	Conventional
	Fontanelle 7R418	Reference	Glyphosate –tolerant
	Legacy L6600	Reference	Conventional
	NK N65-M7	Reference	Conventional
MO	Midwest Genetics 8403	Reference	Conventional
	Dekalb DKC 65-25	Reference	Conventional
	NK N72-G8	Reference	Conventional
	NK N76-H2	Reference	Conventional
OK	Pfister 2730	Reference	Conventional
	Asgrow RX754RR2	Reference	Glyphosate –tolerant
	Dekalb DKC61-42	Reference	Conventional
	Pioneer 33M54	Reference	Conventional
PA	Asgrow RX708	Reference	Conventional
	NK N69-P9	Reference	Conventional
	Pioneer 33H25	Reference	Conventional
	Asgrow RX708	Reference	Conventional
TX	Dekalb DKC63-78	Reference	Conventional
	Asgrow RX752RR2	Reference	Glyphosate –tolerant
	Dekalb DKC 60-15	Reference	Conventional
	NC+ 5411	Reference	Conventional
WI	Pioneer 33K39	Reference	Conventional

[1] Sites produced under Study REG-08-069: ARNE = Jackson County, AR; IARL = Jefferson County, IA; ILWY = Stark County, IL; INRC = Parke County, IN. Sites produced under Study REG-08-166: IA1 = Wapello County, IA; IA2 = Benton County, IA; IL1 = Stark County, IL; IL2 = Clinton County, IL; IN1 = Montgomery County, IN; IN2 = Boone County, IN; MI = Ottawa County, MI; MO = Butler County, MO; OK = Caddo County, OK; PA = Berks County, PA; TX = Armstrong County, TX; WI = Walworth County, WI.

Table G-2. Field and Planting Information

Site	Plot size (ft x ft)	Rows (#)	Planting date	Planting depth (in)	Soil Series	OM[1] (%)	pH	2007 Crop	2006[2] Crop
IA1	10 x 20	4	6/17/2008	1.5	Grundy silt loam	4.3	7.1	Mixed grass	Mixed grass
IA2	10 x 20	4	6/4/2008	2	Tama Muscatine silty clay loam	3.9	6.1	Milk thistle	Soybean
IL1	30 x 30	12	5/17/2008	1.75	Plano silt loam	3.5	6.6	Maize	Soybean
IL2	10 x 20	4	6/12/2008	1.5	Cisne – Huey complex silt loam	1.3	7.1	Milo	Soybean
IN1	10 x 20	4	6/17/2008	1.5	Reesville silty clay loam	2.6	6.9	Maize	Maize
IN2	30 x 30	12	5/26/2008	1.5	Crosby silt loam	2.0	6.6	Soybean	Maize
MI	10 x 20	4	5/20/2008	1.5	Nester loam	1.8	6.4	Oats	Fallow
MO	30 x 30	12	6/6/2008	1	Amagon silt loam	1.3	5.4	Soybean	Soybean
OK	10 x 20	4	5/30/2008	1-1.5	Pond Creek sandy loam	0.9	5.9	Maize	Fallow
PA	30 x 30	12	5/29/2008	1.5-1.75	Philo/Atkins silt loam	2.0	6.2	Soybean	Tomato
TX	10 x 20	4	6/3/2008	1.5	Pullman silty clay loam	1.1	7.9	Soybean	Fallow
WI	10 x 20	4	5/21/2008	1	Radford silt loam	2.2	5.9	Maize	Maize
ARNE	15 x 20	6	5/17/2008	1.5	Bosket sandy loam	1.2	6.4	Cotton	--
IARL	15 x 20	6	5/22/2008	1.8	Otley silty clay loam	3.5	6.5	Maize	--
ILWY	15 x 20	6	5/9/2008	1.8	Drummer silty clay loam	3.4	6.3	Maize	--
INRC	15 x 20	6	5/28/2008	1.4	Reesville silt loam	3.0	7.1	Wheat	--

Note: At each site, planting rate was 2 seeds/foot and 3 replications per treatment. After seedling vigor and early stand count data were collected, all plots at each site were thinned to a uniform density.

[1]OM = organic matter.

[2]Crop history for 2006 was not reported for the ARNE, IARL, ILWY, or INRC sites.

G.3. Planting and Field Operations

Planting information, soil description and cropping history of the study area at each site are listed in Table G-2. Agronomic practices used to prepare and maintain each study site were characteristic of each respective region. Maintenance pesticides were applied as needed to prevent the study from being compromised at the sites. All maintenance operations were performed uniformly over the entire production area as specified in the protocol.

G.4. Phenotypic Observations

The description of the characteristics measured and the designated developmental stages where observations occurred are listed in Section VII, Table VII-1.

G.5. Environmental Observations

Environmental interactions were used to characterize MON 87427 by evaluating plant response to abiotic stressors, disease damage, arthropod damage, and pest and beneficial arthropod abundance in the plots using the methods described in Section G.6.

G.6. Abiotic Stress Response, Disease Damage, and Arthropod Damage

The test and control plants were evaluated at each site for differences in plant response to abiotic stressors, disease damage, and arthropod damage. Three abiotic stressors, three diseases and three arthropod pests were evaluated four times during the growing season at the following growth stages.

Observation 1: V2-V4 growth stage

Observation 2: V10-V15 growth stage

Observation 3: VT-R3 growth stage

Observation 4: R6 growth stage

The principal investigator at each site chose abiotic stressors, diseases, and arthropod pests that were either actively causing plant injury in the study area or were likely to occur in maize during a given observation period. Therefore, abiotic stressors, diseases, and arthropod pests that were assessed often varied between observations at a site and between sites.

Additional disease damage assessments included the evaluation of ear rot disease and stalk rot disease at harvest at each site. Ear rot disease data were collected by evaluating five representative ears (one per plant) from each plot. The husks were pulled back and each ear was examined for disease infection. To evaluate stalk rot disease, five representative stalks in each plot were cut longitudinally. The stalks were then examined for disease infection.

The observations were collected using a continuous 0-9 rating scale of increasing symptomology. Data were collected numerically and then placed into one of the following categories for reporting purposes:

Rating	Severity of plant damage
0	none (no symptoms observed)
1 – 3	slight (symptoms not damaging to plant development)
4 – 6	moderate (intermediate between slight and severe)
7 – 9	severe (symptoms damaging to plant development)

Additional arthropod damage assessments included quantitative evaluations of corn earworm and European corn borer damage at harvest at the IL1, IN2, MO, and PA sites. Corn earworm damage was evaluated by examining ten non-systematically selected ears (one per plant) from each plot using a rating scale adapted from Widstrom (1967) where:

0 = No visible corn earworm damage
1 = Silk shows evidence of feeding; feeding on the ear is < 0.5 in.
2 = Corn earworm feeding to 0.5 in. beyond the ear tip
3 = Corn earworm feeding to 1.0 in. beyond the ear tip
4 = Corn earworm feeding to 1.5 in. beyond the ear tip
5 = Corn earworm feeding to 2.0 in. beyond the ear tip
6 = Corn earworm feeding to 2.5 in. beyond the ear tip
7 = Corn earworm feeding to 3.0 in. beyond the ear tip
8 = Corn earworm feeding to 3.5 in. beyond the ear tip
9 = Corn earworm feeding to 4.0 in. or greater beyond the ear tip.

European corn borer damage was evaluated by examining ten non-systematically selected plants from each plot. Damage was assessed by splitting each of ten plants and counting the number of live larvae, number of entry/exit holes, number of feeding galleries, and total length of all feeding galleries in each stalk.

G.7. Arthropod Abundance

Pest and beneficial arthropods were collected at the IL1, IN2, MO, and PA sites during the growing season at the following intervals:

Observation 1: approximately V10 – V15 growth stage

Observation 2: approximately V18 – VT growth stage

Observation 3: approximately R1 growth stage

Observation 4: approximately R2 growth stage

Observation 5: approximately R3-R4 growth stage

Arthropods were collected using non-baited yellow sticky traps. Two sticky traps were deployed per plot in the middle third of rows six and seven. Traps were initially placed at the approximate midpoint between the ground level and the top of the plant canopy; however, once the main ear was visible, the sticky traps were deployed at the approximate level of the maize ear for the remainder of the arthropod collections. The sticky traps were deployed for approximately seven days. The collected sticky traps were sent to the Department of Entomology at the University of Arkansas in Fayetteville, AR for arthropod identification and enumeration. A maximum of six of the most abundant pests and six of the most abundant beneficial arthropods were determined for each collection at each site. Certain pest and beneficial arthropod taxa were preselected based on their typical relative abundance and common occurrence across sites. The preselected taxa were enumerated at all sites for each collection time and included aphids and corn flea beetles for the pests, and Araneae (spiders), micro-parasitic hymenoptera, ladybird beetles, and Orius spp. for the beneficial arthropods. Additionally, for each individual collection (e.g., collection 1, IN2 site), four non-systematically selected samples were examined to determine presence and relative abundance of additional pest and beneficial arthropods to be enumerated for that particular collection and site to attain a maximum of six total pest and six total beneficial arthropods when combined with the preselected taxa. Thus, the suite of pest and beneficial arthropods assessed often varied between collections from a site and between sites due to differences in temporal activity and geographical distribution of arthropod taxa.

G.8. Environmental Interactions Evaluation Criteria

For data assessed using the observational severity scale, test and control substances were considered different in susceptibility or tolerance to abiotic stressors, diseases, or arthropod pests if the severity of injury to MON 87427 did not overlap with the severity of injury to the control across all three replications. These data were not subjected to statistical analysis. For each observation at a site, the range of injury severity across the reference substances provided data that are representative of commercial maize hybrids. Arthropod abundance, corn earworm damage, and European corn borer damage were quantitatively evaluated and subjected to statistical analysis as indicated in section G.10.

G.9. Data Assessment

Experienced scientists familiar with the experimental design and evaluation criteria were involved in all components of data collection, summarization, and analysis. Personnel assessed that measurements were taken properly, data were consistent with expectations based on experience with the crop, and the experiment was carefully monitored. Prior to analysis, the overall dataset was evaluated for evidence of biologically relevant changes and for possible evidence of an unexpected plant response. Any unexpected observations or issues that would impact the evaluation objectives were noted. Data were then subjected to statistical analysis as indicated below.

G.10. Statistical Analysis

An analysis of variance was conducted according to a randomized complete block design using SAS® (Version 9.2, SAS Institute, Inc. 2002-2008) to compare MON 87427 and conventional control plants for the phenotypic characteristics listed in Table VII-1. Comparisons of the test and conventional control were conducted within site (individual site analysis) and across sites (combined site analysis). The level of statistical significance was predetermined to be 5% (α=0.05). MON 87427 and the conventional control were not statistically compared to the reference hybrids. Minimum and maximum mean values were calculated for each characteristic from the 38 unique reference hybrids that were included in this study.

An analysis of variance was conducted according to a randomized complete block design with three replications using SAS® (Version 9.2, SAS Institute, Inc. 2002-2008) for the corn earworm damage, European corn borer damage, and the arthropod abundance. The level of statistical significance was predetermined to be 5%. MON 87427 was compared to the conventional control at each site for the corn earworm damage, European corn borer damage, and arthropod abundance. Additionally, the corn earworm damage and European corn borer damage data were pooled across sites for a statistical comparison of MON 87427 and the conventional control. The reference range for the abundance of each arthropod evaluated from a given collection and site was determined from the minimum and maximum abundance value collected from the reference hybrids at the site. However, minimum and maximum mean values in the combined site analysis for the corn earworm damage and European corn borer damage were calculated from the 14 unique reference hybrids at the IL1, IN2, MO, and PA sites.

G.11. Individual Field Site Plant Growth and Development Results and Discussion

In the individual site analysis, a total of 17 statistically significant differences between MON 87427 and the conventional control were detected out of 187 comparisons (Table G-3). Seedling vigor was poorer for MON 87427 compared to the conventional control at the MI (3.7 vs. 3.0 rating), PA (2.3 vs. 1.7 rating), and ARNE (4.0 vs. 2.3 rating) sites. MON 87427 had lower early stand than the conventional control at the PA site (73.3 vs. 94.3 plants/plot). Days to 50% pollen shed was greater for MON 87427 compared to the conventional control at the TX site (67.7 vs. 65.7 days). Days to 50% silking was greater for MON 87427 compared to the conventional control at the OK (56.3 vs. 54.0 days) and WI (74.0 vs. 72.0 days) sites. MON 87427 exhibited higher stay green (i.e. less green tissue) than the conventional control at the IN2 site (7.0 vs. 3.3 rating). MON 87427 exhibited shorter ear height (44.3 vs. 50.4 inches) and taller plant height (108.2 vs. 101.3 inches) compared to the conventional control at the IL2 site. Stalk lodging was greater for MON 87427 compared to the conventional control at the MI (1.3 vs. 0.0 plants/plot) and MO (72.0 vs. 48.7 plants/plot) sites. Root lodging was lower for MON 87427 compared to the conventional control at the PA site (0.0 vs. 0.7 plants/plot). MON 87427 had lower final stand (62.7 vs. 64.0 plants per plot) than the conventional control at the MI site. Test weight was lower for MON 87427 than the conventional control (57.4 vs. 59.7 lbs/bushel) at the IL2 site. And yield for MON 87427 was lower compared to the

conventional control at the IN2 (120.5 vs. 164.7 bu/acre) and IARL (167.0 vs. 221.2 bu/acre) sites.

The differences between MON 87427 and the conventional control detected in the individual site analysis for early stand count, days to 50% pollen shed, days to 50% silking, stay green, ear height, plant height, stalk lodging, root lodging, final stand count, test weight, and yield were not detected in the combined site analysis (Section VII, Table VII-4). Thus, the differences detected for these phenotypic characteristics are not indicative of a consistent response associated with the trait and are unlikely to be biologically meaningful in terms of increased pest potential of MON 87427 compared to the conventional control (Figure VII-1, step 2, "no" answer). Differences in seedling vigor between MON 87427 and the conventional control were detected in the individual site and combined site analyses; however, the combined site mean value of MON 87427 was within the reference range (Section VII, Table VII-4), and the difference is unlikely to be biologically meaningful in terms of increased pest potential (Figure VII-1, step 3, "no" answer).

In the individual site analysis of corn earworm and European corn borer damage, no statistically significant differences were detected between MON 87427 and the conventional control for 15 out of 18 comparisons for corn earworm and European corn borer damage (Table G-7). In addition, no numerical differences were observed for two comparisons for which p-values could not be generated due to lack of variability in the data. A single statistically significant difference was detected for corn earworm damage and two differences were detected for European corn borer damage. MON 87427 had a lower corn earworm damage rating compared to the conventional control at the IN2 site (0.50 vs. 1.23 per ear). MON 87427 had a greater gallery length compared to the conventional control at the IL1 site (3.04 vs. 1.31 in. per stalk) and lower at site IN2 (1.01 vs. 2.71 in. per stalk). However, the differences detected in the individual site analysis were not significant in the combined site analysis. Thus, the differences are not indicative of a consistent trend in the data and are unlikely to be biologically meaningful in terms of increased pest potential (Figure VII-1, Step 2, "no" answer).

Table G-3. Phenotypic Comparison of MON 87427 to the Conventional Control within Each Site

	Phenotypic Characteristics (units)							
	Seedling vigor (1-9 scale)		Early stand count (#/plot)		Days to 50% pollen shed		Days to 50% silking	
	Mean (S.E.)[1]		Mean (S.E.)		Mean (S.E.)		Mean (S.E.)	
Site[2]	MON 87427	Control	MON 87427	Control	MON 87427	Control	MON 87427	Control
IA1	1.7 (0.67)	1.3 (0.33)	-	-	64.0 (1.15)	64.0 (0.58)	62.3 (0.88)	63.0 (0.58)
IA2	4.0 (0.58)	3.3 (0.67)	47.7 (3.84)	54.7 (2.33)	66.7 (0.67)	66.3 (0.88)	64.3 (0.33)	64.7 (0.88)
IL1	1.0 (0.00)†	1.0 (0.00)	-	-	71.7 (0.33)	71.7 (0.33)	68.7 (0.33)	69.3 (0.67)
IL2	2.3 (0.33)	2.0 (0.00)	78.0 (1.00)	76.3 (1.45)	56.0 (1.00)	54.3 (0.88)	54.3 (1.45)	53.0 (0.58)
IN1	3.0 (0.00)	3.0 (0.00)	76.3 (1.20)	76.7 (1.67)	58.3 (1.33)	58.0 (1.53)	55.3 (1.86)	55.3 (1.86)
IN2	2.0 (0.58)	2.3 (0.33)	73.3 (2.33)	80.0 (2.52)	63.0 (0.00)†	63.0 (0.00)	66.0 (0.00)	66.0 (0.00)
MI	3.7 (0.33)*	3.0 (0.00)	79.7 (1.45)	78.7 (3.84)	72.7 (0.33)	73.0 (0.00)	72.7 (0.33)	73.0 (0.00)
MO	4.7 (0.33)	4.0 (0.58)	76.7 (1.45)	76.3 (0.67)	48.7 (0.33)	48.3 (1.45)	49.7 (0.33)	49.7 (0.88)
OK	4.3 (0.67)	4.7 (0.33)	-	-	54.7 (1.67)	53.0 (1.00)	56.3 (1.86)*	54.0 (1.00)
PA	2.3 (0.33)*	1.7 (0.33)	73.3 (0.33)*	94.3 (2.85)	60.7 (0.67)	61.7 (0.67)	61.0 (0.58)	61.3 (0.33)
TX	1.7 (0.33)	2.0 (0.00)	59.0 (2.31)	60.0 (3.21)	67.7 (0.33)*	65.7 (0.67)	62.0 (0.00)	62.0 (0.00)
WI	2.3 (0.33)	2.3 (0.33)	-	-	76.0 (0.58)	75.7 (0.67)	74.0 (1.00)*	72.0 (0.00)
ARNE	4.0 (0.00)*	2.3 (0.33)	62.3 (1.33)	59.7 (0.67)	56.0 (0.00)	55.7 (0.33)	56.0 (0.00)	55.7 (0.33)
IARL	1.7 (0.67)	1.0 (0.00)	61.0 (4.00)	57.7 (2.40)	71.3 (2.03)	72.0 (2.08)	69.3 (2.03)	70.0 (2.08)
ILWY	1.0 (0.00)†	1.0 (0.00)	63.0 (3.06)	61.7 (2.85)	74.3 (0.33)	74.0 (0.58)	73.7 (0.67)	73.3 (0.33)
INRC	4.0 (0.00)	3.3 (0.33)	62.3 (1.76)	67.0 (1.73)	60.0 (0.00)†	60.0 (0.00)	61.0 (0.00)†	61.0 (0.00)

Table G-3 (continued). Phenotypic Comparison of MON 87427 to the Conventional Control within Each Site

	Phenotypic Characteristics (units)							
	Stay green (1-9 scale)[3]		Ear height (inches)		Plant height (inches)		Dropped ears (#/plot)	
	Mean (S.E.)		Mean (S.E.)		Mean (S.E.)		Mean (S.E.)	
Site	MON 87427	Control	MON 87427	Control	MON 87427	Control	MON 87427	Control
IA1	5.3 (0.33)	5.0 (0.00)	39.3 (2.27)	38.7 (1.44)	86.5 (1.57)	89.3 (2.73)	0.0 (0.00)†	0.0 (0.00)
IA2	4.3 (0.33)	4.3 (0.33)	44.7 (0.93)	42.3 (1.44)	96.1 (0.87)	93.2 (1.91)	0.0 (0.00)	0.0 (0.00)
IL1	2.3 (0.33)	2.3 (0.33)	48.4 (0.67)	47.3 (1.27)	104.0 (1.10)	104.9 (0.52)	0.0 (0.00)†	0.0 (0.00)
IL2	6.3 (0.33)	6.3 (0.33)	44.3 (0.13)*	50.4 (0.61)	108.2 (1.11)*	101.3 (0.68)	0.7 (0.33)	0.3 (0.33)
IN1	-	-	31.9 (2.52)	31.7 (2.00)	79.1 (5.05)	76.2 (4.56)	1.3 (0.88)	3.3 (1.20)
IN2	7.0 (1.00)*	3.3 (0.33)	50.9 (1.40)	49.7 (0.98)	97.4 (2.20)	97.9 (1.78)	2.3 (0.67)	1.7 (0.33)
MI	5.3 (0.33)	5.3 (0.33)	44.4 (1.70)	46.9 (0.44)	85.0 (0.53)	89.1 (2.03)	0.0 (0.00)†	0.0 (0.00)
MO	6.3 (0.33)	6.7 (0.33)	38.3 (0.68)	34.5 (2.85)	81.7 (1.43)	78.7 (5.16)	2.3 (1.20)	2.0 (1.00)
OK	7.0 (2.00)	7.3 (0.33)	31.5 (1.96)	30.9 (1.73)	88.4 (3.72)	87.9 (0.48)	3.3 (2.40)	0.0 (0.00)
PA	5.0 (0.58)	4.0 (0.58)	43.3 (2.27)	40.2 (0.92)	92.6 (3.30)	90.2 (1.86)	0.0 (0.00)	0.0 (0.00)
TX	9.0 (0.00)†	9.0 (0.00)	41.1 (1.74)	41.3 (0.15)	77.6 (0.92)	77.7 (0.78)	0.0 (0.00)	0.0 (0.00)
WI	7.3 (0.67)	7.7 (0.33)	47.5 (1.44)	46.9 (0.18)	90.6 (2.62)	90.6 (3.90)	0.0 (0.00)†	0.0 (0.00)
ARNE	5.7 (0.33)	6.7 (0.67)	45.7 (0.59)	45.7 (1.40)	96.1 (0.77)	93.7 (2.18)	0.0 (0.00)	0.0 (0.00)
IARL	4.0 (0.58)	4.7 (0.33)	33.1 (2.40)	34.9 (5.56)	78.1 (3.24)	78.8 (6.76)	0.0 (0.00)	0.0 (0.00)
ILWY	9.0 (0.00)	9.0 (0.00)	43.4 (0.61)	42.4 (0.99)	97.7 (1.02)	95.5 (1.00)	0.0 (0.00)†	0.0 (0.00)
INRC	1.7 (0.33)	2.0 (0.00)	44.7 (2.14)	46.3 (0.74)	106.1 (1.77)	108.2 (4.20)	0.0 (0.00)†	0.0 (0.00)

Table G-3 (continued). Phenotypic Comparison of MON 87427 to the Conventional Control within Each Site

	Phenotypic Characteristics (units)							
	Stalk lodged plants (#/plot)		Root lodged plants (#/plot)		Final stand count (#/plot)		Grain moisture (%)	
	Mean (S.E.)		Mean (S.E.)		Mean (S.E.)		Mean (S.E.)	
Site	MON 87427	Control	MON 87427	Control	MON 87427	Control	MON 87427	Control
IA1	0.7 (0.33)	1.0 (0.58)	0.0 (0.00)†	0.0 (0.00)	54.3 (1.33)	54.3 (1.20)	20.3 (0.40)	22.0 (2.05)
IA2[4]	0.0 (0.00)†	0.0 (0.00)	0.0 (0.00)†	0.0 (0.00)	51.0 (.)	53.0 (1.00)	19.2 (.)	19.3 (0.44)
IL1	2.3 (0.88)	2.3 (0.33)	0.0 (0.00)†	0.0 (0.00)	63.3 (0.33)	63.7 (0.33)	21.6 (0.25)	23.3 (1.34)
IL2	1.7 (1.20)	2.3 (1.86)	55.7 (2.19)	50.3 (4.63)	72.0 (0.00)	71.7 (0.33)	19.2 (0.85)	19.8 (0.61)
IN1	5.0 (2.00)	4.0 (1.53)	38.0 (19.22)	25.3 (14.72)	61.7 (3.18)	60.7 (1.67)	23.7 (0.69)	24.8 (0.56)
IN2	22.0 (6.66)	17.0 (2.52)	4.0 (1.53)	2.7 (1.67)	64.3 (2.40)	63.3 (0.33)	15.6 (0.76)	17.4 (0.57)
MI	1.3 (0.67)*	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)	62.7 (0.67)*	64.0 (0.00)	17.7 (0.15)	18.0 (0.29)
MO	72.0 (5.86)*	48.7 (0.67)	10.3 (4.10)	2.7 (1.76)	-	-	-	-
OK	3.7 (0.67)	1.5 (1.50)	0.7 (0.67)	0.0 (0.00)	-	-	-	-
PA	3.0 (1.73)	2.0 (1.15)	0.0 (0.00)*	0.7 (0.33)	59.3 (1.20)	61.7 (0.67)	23.3 (1.19)	25.3 (0.69)
TX	0.0 (0.00)†	0.0 (0.00)	0.0 (0.00)†	0.0 (0.00)	-	-	-	-
WI	4.3 (1.86)	1.3 (0.67)	0.3 (0.33)	0.0 (0.00)	63.0 (0.00)	63.3 (0.67)	23.8 (1.29)	24.7 (1.55)
ARNE	3.0 (1.53)	3.0 (1.00)	10.3 (2.19)	2.3 (2.33)	58.3 (0.88)	60.0 (3.06)	15.4 (0.43)	15.5 (0.91)
IARL	3.0 (2.08)	2.7 (1.45)	0.0 (0.00)†	0.0 (0.00)	56.7 (0.88)	54.5 (1.50)	20.9 (0.75)	20.2 (0.30)
ILWY	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)†	0.0 (0.00)	58.0 (1.00)	58.0 (0.58)	18.6 (0.35)	19.7 (0.82)
INRC	1.0 (1.00)	1.3 (0.33)	0.0 (0.00)	0.0 (0.00)	57.5 (5.50)	54.7 (1.33)	13.9 (0.00)	13.9 (0.09)

Table G-3 (continued). Phenotypic Comparison of MON 87427 to the Conventional Control within Each Site

	Phenotypic Characteristics (units)			
	Test weight (lbs/bushel)		Yield (bushels/acre)	
	Mean (S.E.)		Mean (S.E.)	
Site	MON 87427	Control	MON 87427	Control
IA1	53.3 (0.67)	52.0 (1.73)	104.4 (7.03)	92.4 (12.00)
IA2[4]	54.0 (.)	53.5 (0.50)	227.5 (.)	225.4 (23.29)
IL1	55.1 (0.15)	54.5 (0.47)	192.2 (10.16)	206.3 (4.75)
IL2	57.4 (0.09)*	59.7 (0.75)	150.0 (11.46)	139.6 (12.40)
IN1	55.5 (0.29)	54.5 (0.67)	120.8 (13.84)	115.1 (6.88)
IN2	56.4 (1.60)	56.5 (0.40)	120.5 (11.50)*	164.7 (6.42)
MI	57.8 (0.74)	58.0 (0.31)	181.5 (3.88)	187.5 (6.15)
MO	-	-	-	-
OK	-	-	-	-
PA	54.3 (0.37)	54.2 (0.15)	149.7 (12.25)	164.8 (12.82)
TX	-	-	-	-
WI	52.0 (0.76)	52.2 (0.44)	149.3 (9.42)	146.1 (1.59)
ARNE	56.5 (0.15)	56.1 (0.36)	177.6 (1.76)	191.9 (6.36)
IARL	53.3 (0.13)	54.2 (0.20)	167.0 (9.14)*	221.2 (8.74)
ILWY	56.7 (0.26)	56.0 (0.59)	178.9 (12.22)	162.2 (11.39)
INRC	55.6 (0.71)	56.0 (0.15)	172.2 (13.76)	151.4 (19.45)

Note: The experimental design was a randomized complete block with three replications. * Indicates statistical difference between MON 87427 and the conventional control ($p \leq 0.05$). † Indicates exclusion from statistical analysis due to lack of variability. [1]S.E. = standard error. [2]Site codes are as follows: IA1 = Wapello Co., IA; IA2 = Benton Co., IA; IL1 = Stark Co., IL; IL2 = Clinton Co., IL; IN1 = Montgomery Co., IN; IN2 = Boone Co., IN; MI = Ottawa Co., MI; MO = Butler Co., MO; OK = Caddo Co., OK; PA = Berks Co., PA; TX = Armstrong Co., TX; WI = Walworth Co., WI; ARNE = Jackson Co., AR; IARL = Jefferson Co., IA; ILWY = Stark Co., IL; INRC = Parke Co., IN. [3] The stay green scale used for sites ARNE, IARL, ILWY, and INRC was inverted from the scale used for the remaining sites. [4] Harvest observations (final stand count, grain moisture, test weight, and yield) of MON 87427 at IA2 were taken from 1 replication.

Table G-4. Abiotic Stressor Evaluation Using Observational Severity Scale for MON 87427 and the Conventional Control

Abiotic Stressor	Number of observations across all sites[1]	Number of observations where no differences were detected between MON 87427 and the control
Total	**172**	**172**
Cold	6	6
Drought	13	13
Flood	19	19
Frost	4	4
Hail	26	26
Heat	25	25
Mineral Toxicity	1	1
Nitrogen deficiency	2	2
Nutrient deficiency	13	13
Soil compaction	7	7
Wet soil[2]	7	7
Wind	49	49

No differences were observed between MON 87427 and the conventional control. Data were not subjected to statistical analysis.

Note: The experimental design was a randomized complete block with three replications. Observations were made at four crop developmental stages: Observation 1 at V2 -V4; Observation 2 at V10 –V15; Observation 3 at VT – R3; Observation 4 at R6.

[1]Site are as follows: IA1 = Wapello Co., IA; IA2 = Benton Co., IA; IL1 = Stark Co., IL; IL2 = Clinton Co., IL; IN1 = Montgomery Co., IN; IN2 = Boone Co., IN; MI = Ottawa Co., MI; MO = Butler Co., MO; OK = Caddo Co., OK; PA = Berks Co., PA; TX = Armstrong Co., TX; WI = Walworth Co., WI; ARNE = Jackson Co., AR; IARL = Jefferson Co., IA; ILWY = Stark Co., IL; INRC = Parke Co., IN.

[2]Includes; excess water, saturated soil and soil saturation.

Table G-5. Disease Damage Evaluations Using an Observational Severity Scale for MON 87427 and the Conventional Control

Disease stressor	Number of observations across all sites[1]	Number observations where no differences were detected between MON 87427 and the control
Total	**210**	**210**
Anthracnose	12	12
Bacterial leaf blight	1	1
Bacterial leaf spot	1	1
Black sooty mold	1	1
Brown spot	1	1
Ear rot[4]	17	17
Eyespot	8	8
Fusarium	11	11
Grey leaf spot	32	32
Kernel red streak	2	2
Leaf blight	5	5
Maize dwarf mosaic virus	1	1
Northern corn leaf blight	17	17
Purple corn syndrome	1	1
Pythium[2]	9	9
Rhizoctonia	2	2
Root rot	6	6
Rust[3]	33	33
Seedling blight	12	12
Seedling rot	2	2
Smut (common smut)	7	7
Southern corn leaf blight	8	8
Stalk Rot[4]	16	16
Stewart's wilt	5	5

No differences were observed between MON 87427 and the conventional control. Data were not subjected to statistical analysis.

Note: The experimental design was a randomized complete block with three replications at each site. Observations were made at four crop developmental stages: Observation 1 at V2 -V4; Observation 2 at V10 –V15; Observation 3 at VT – R3; Observation 4 at R6.

[1]Site are as follows: IA1 = Wapello Co., IA; IA2 = Benton Co., IA; IL1 = Stark Co., IL; IL2 = Clinton Co., IL; IN1 = Montgomery Co., IN; IN2 = Boone Co., IN; MI = Ottawa Co., MI; MO = Butler Co., MO; OK = Caddo Co., OK; PA = Berks Co., PA; TX = Armstrong Co., TX; WI = Walworth Co., WI; ARNE = Jackson Co., AR; IARL = Jefferson Co., IA; ILWY = Stark Co., IL; INRC = Parke Co., IN.

[2]Includes Pythium root rot.

[3]Rust includes common rust and leaf rust.

[4]The ear rot and stalk rot assessments at harvest involved splitting the stalks of five non-systematically selected plants and evaluating the disease.

Table G-6. Arthropod Damage Evaluated Using an Observational Severity Scale for MON 87427 and the Conventional Control

Arthropod	Number of observations across all sites[1]	Number observations where no differences were detected between MON 87427 and the control
Total	**167**	**167**
Aphid[2]	10	10
Armyworm	11	11
Bill bug	5	5
Black cutworm	4	4
Chinch bug	2	2
Corn earworm	13	13
Corn rootworm beetle[3]	12	12
Cutworm	3	3
European corn borer[4]	20	20
Fall armyworm	20	20
Flea beetle[5]	9	9
Grasshopper	17	17
Japanese beetle	8	8
Seedcorn beetle	3	3
Southern corn leaf beetle	1	1
Southwestern corn borer	6	6
Spider mite	4	4
Stalk borer	1	1
Stink bug	1	1
Thrips	1	1
White grub	4	4
Wireworm	12	12

No differences were observed between MON 87427 and the conventional control. Data were not subjected to statistical analysis.

Note: The experimental design was a randomized complete block with three replications at each site. Observations were made at four crop developmental stages: Observation 1 at V2 -V4; Observation 2 at V10 –V15; Observation 3 at VT – R3; Observation 4 at R6.

[1]Site are as follows: IA1 = Wapello Co., IA; IA2 = Benton Co., IA; IL1 = Stark Co., IL; IL2 = Clinton Co., IL; IN1 = Montgomery Co., IN; IN2 = Boone Co., IN; MI = Ottawa Co., MI; MO = Butler Co., MO; OK = Caddo Co., OK; PA = Berks Co., PA; TX = Armstrong Co., TX; WI = Walworth Co., WI; ARNE = Jackson Co., AR; IARL = Jefferson Co., IA; ILWY = Stark Co., IL; INRC = Parke Co., IN.

[2]includes corn leaf aphid.

[3]Includes Northern and Western corn rootworm beetle.

[4]Includes corn borer.

[5]Includes corn flea beetle.

Table G-7. Comparison of Corn Earworm and European Corn Borer Damage for MON 87427 Compared to the Conventional Control

Pest	Site[1]	Damage Assessment	Mean (S.E.)	
			MON 87427	Control
Corn earworm	IL1	Mean of 10 ears (0 – 9 rating scale)	0.07 (0.03)	0.03 (0.03)
	IN2	Mean of 10 ears (0 – 9 rating scale)	0.50 (0.06)*	1.23 (0.09)
	MO	Mean of 10 ears (0 – 9 rating scale)	2.47 (0.24)	2.67 (0.27)
	PA	Mean of 10 ears (0 – 9 rating scale)	0.00 (0.00)†	0.00 (0.00)
European corn borer	IL1	Number of larva/10 plants	0.07 (0.03)	0.07 (0.03)
		Number of stalk entry/ exit holes of 10 plants	0.53 (0.38)	0.30 (0.06)
		Number of stalk galleries per plant of 10 plants	0.40 (0.30)	0.30 (0.06)
		Stalk gallery length (in.) per plant of plants with at least one gallery	3.04 (0.51)*	1.31 (0.21)
	IN2	Number of larva/10 plants	0.17 (0.12)	0.20 (0.10)
		Number of stalk entry/ exit holes of 10 plants	1.27 (0.44)	0.93 (0.35)
		Number of stalk galleries per plant of 10 plants	1.00 (0.12)	1.20 (0.32)
		Stalk gallery length (in.) per plant of plants with at least one gallery	1.01 (0.20)*	2.71 (0.70)
	MO	Number of larva/10 plants	0.00 (0.00)†	0.00 (0.00)
		Number of stalk entry/ exit holes of 10 plants	0.97 (0.33)	0.90 (0.20)
		Number of stalk galleries per plant of 10 plants	0.70 (0.21)	0.67 (0.12)
		Stalk gallery length (in.) per plant of plants with at least one gallery	0.68 (0.03)	0.81 (0.11)
	PA	Number of larva/10 plants	0.03 (0.03)	0.07 (0.03)
		Number of stalk entry/ exit holes of 10 plants	0.13 (0.09)	0.17 (0.03)
		Number of stalk galleries per plant of 10 plants	0.30 (0.12)	0.20 (0.06)
		Stalk gallery length (in.) per plant of plants with at least one gallery	1.79 (0.44)	1.83 (0.44)

Note: The experimental design was a randomized complete block with three replications at each site. S.E. = standard error.
*Indicates a statistical difference ($p \leq 0.05$) between MON 87427 and the conventional control.
† No statistical comparisons were made due to lack of variability in the data.
[1] Site codes are as follows: IL1=Stark Co., IL; IN2 = Boone Co., IN; MO = Butler Co., MO; PA = Berks Co., PA.

Table G-8. Abundance of Pest Arthropods in Sticky Trap Samples Collected from MON 87427, the Conventional Control, and the Reference Maize Hybrids

		Aphid			Corn ear worm			Corn flea beetle		
		Mean (S.E.)		Reference	Mean (S.E.)		Reference	Mean (S.E.)		Reference
Coll.	Site[1]	MON 87427	Control	range	MON 87427	Control	range	MON 87427	Control	range
1	IL1	0.3 (0.3)	0.3 (0.3)	1.0 - 2.3	-	-	-	0.0 (0.0)[†]	0.0 (0.0)	0.0 - 0.0
	IN2	1.7 (0.3)	1.0 (0.6)	0.7 - 1.3	-	-	-	1.0 (1.0)	1.7 (0.3)	0.7 - 3.0
	MO	2.0 (1.2)	0.3 (0.3)	0.0 - 1.7	-	-	-	33.7 (4.3)	21.0 (6.4)	17.3 - 42.7
	PA	1.3 (0.9)	1.3 (1.3)	1.3 - 2.7	-	-	-	91.7 (26.3)*	59.0 (13.7)	30.3 - 120.3
2	IL1	2.3 (0.9)	2.3 (0.9)	0.7 - 5.0	-	-	-	0.0 (0.0)[†]	0.0 (0.0)	0.0 - 0.0
	IN2	0.7 (0.3)	0.7 (0.7)	1.3 - 3.3	-	-	-	0.7 (0.3)	1.3 (0.9)	1.3 - 3.3
	MO	0.7 (0.7)	1.3 (0.3)	0.7 - 2.3	0.0 (0.0)	0.0 (0.0)	0.0 - 0.3	24.3 (8.0)	22.7 (4.9)	26.3 - 38.0
	PA	0.7 (0.7)	0.3 (0.3)	0.0 - 0.3	-	-	-	32.7 (9.5)	20.7 (7.4)	6.3 - 51.3
3	IL1	62.0 (37.8)	33.7 (2.0)	2.7 - 29.3	-	-	-	0.0 (0.0)	0.0 (0.0)	0.0 - 0.3
	IN2	6.3 (3.9)	4.3 (2.2)	2.0 - 11.3	-	-	-	1.7 (0.9)	1.0 (0.6)	0.7 - 5.7
	MO	13.0 (5.1)	9.0 (5.5)	1.7 - 33.0	-	-	-	24.0 (5.1)	16.3 (4.4)	13.7 - 32.7
	PA	1.3 (0.7)	0.7 (0.7)	0.7 - 1.3	-	-	-	19.0 (1.5)	10.7 (3.2)	7.7 - 24.3
4	IL1	31.3 (8.1)	81.3 (23.4)	5.0 - 32.0	-	-	-	0.7 (0.7)	0.0 (0.0)	0.0 - 0.0
	IN2	4.7 (2.7)	3.7 (2.7)	1.7 - 3.7	-	-	-	0.3 (0.3)	0.3 (0.3)	0.0 - 0.3
	MO	12.3 (3.2)	6.7 (1.5)	1.7 - 10.0	-	-	-	17.3 (7.8)	13.0 (3.0)	17.7 - 33.7
	PA	0.7 (0.7)	0.3 (0.3)	0.7 - 2.0	-	-	-	17.7 (4.4)	11.7 (1.9)	4.3 - 21.3
5	IL1	11.7 (3.5)	15.7 (6.1)	6.3 - 34.0	-	-	-	0.0 (0.0)[†]	0.0 (0.0)	0.0 - 0.0
	IN2	9.7 (4.6)	6.3 (2.6)	4.7 - 7.7	-	-	-	0.3 (0.3)	0.0 (0.0)	0.0 - 1.3
	MO	10.3 (2.0)	12.0 (4.4)	3.3 - 10.7	-	-	-	14.7 (5.3)	7.7 (3.0)	17.3 - 33.7
	PA	4.0 (0.6)	3.3 (2.0)	0.7 - 6.0	-	-	-	12.0 (2.5)*	5.3 (1.5)	3.3 - 10.3

Table G-8 (continued). Abundance of Pest Arthropods in Sticky Trap Samples Collected from MON 87427, the Conventional Control, and the Reference Maize Hybrids

		Delphacid plant hopper			Grasshopper			Leafhopper		
		Mean (S.E.)		Reference	Mean (S.E.)		Reference	Mean (S.E.)		Reference
Coll.	Site[1]	MON 87427	Control	range	MON 87427	Control	range	MON 87427	Control	range
1	IL1	0.0 (0.0)	0.3 (0.3)	0.0 - 0.7	-	-	-	-	-	-
	IN2	6.3 (0.7)	4.7 (1.2)	1.3 - 6.0	-	-	-	1.7 (0.3)	1.0 (0.0)	1.0 - 2.3
	MO	34.0 (9.7)	36.3 (7.5)	27.3 - 59.3	-	-	-	17.0 (0.6)	14.7 (1.7)	12.3 - 29.0
	PA	6.0 (1.7)	7.7 (3.5)	4.0 - 8.3	-	-	-	15.0 (10.1)	4.7 (1.2)	5.7 - 17.0
2	IL1	0.3 (0.3)	0.7 (0.3)	0.7 - 2.3	0.3 (0.3)	0.0 (0.0)	0.0 - 0.7	-	-	-
	IN2	1.7 (1.2)	3.7 (0.3)	0.3 - 5.0	-	-	-	2.7 (1.5)	2.7 (2.2)	1.7 - 5.3
	MO	38.0 (7.9)	30.0 (6.1)	29.0 - 49.0	-	-	-	73.3 (3.5)	73.3 (9.2)	62.3 - 90.3
	PA	2.3 (0.9)	1.7 (0.7)	0.7 - 2.7	-	-	-	7.7 (2.9)*	2.0 (1.2)	2.3 - 10.7
3	IL1	0.0 (0.0)	0.0 (0.0)	0.0 - 1.0	-	-	-	-	-	-
	IN2	0.3 (0.3)	1.3 (0.7)	0.3 - 1.3	0.3 (0.3)	0.3 (0.3)	0.0 - 0.7	4.0 (2.0)	9.3 (3.0)	3.3 - 9.3
	MO	28.3 (10.2)	20.7 (11.6)	12.0 - 36.3	0.0 (0.0)	0.3 (0.3)	0.0 - 0.3	80.0 (10.6)	73.7 (8.8)	64.7 - 71.3
	PA	4.3 (1.2)	4.0 (1.0)	2.3 - 6.7	-	-	-	17.7 (6.2)	10.7 (2.4)	8.0 - 22.3
4	IL1	0.0 (0.0)	0.0 (0.0)	0.0 - 1.0	0.0 (0.0)	0.3 (0.3)	0.0 - 1.3	-	-	-
	IN2	0.3 (0.3)	0.0 (0.0)	0.0 - 0.3	-	-	-	4.7 (2.2)	6.7 (1.8)	2.0 - 7.3
	MO	9.0 (2.6)	8.3 (0.9)	10.3 - 17.3	1.3 (0.9)*	0.0 (0.0)	0.0 - 1.0	51.3 (6.2)	42.0 (3.2)	37.3 - 50.7
	PA	0.3 (0.3)	0.3 (0.3)	0.0 - 1.3	-	-	-	8.0 (0.6)	4.7 (1.2)	3.0 - 20.3
5	IL1	-	-	-	-	-	-	-	-	-
	IN2	0.0 (0.0)	0.0 (0.0)	0 .0 - 0.3	1.0 (1.0)	0.3 (0.3)	0.0 - 0.3	4.0 (1.2)	5.0 (0.6)	1.3 - 9.3
	MO	13.3 (5.0)	8.0 (3.1)	9.7 - 21.0	-	-	-	82.3 (2.9)	79.3 (20.9)	57.7 - 98.0
	PA	0.0 (0.0)	0.0 (0.0)	0.0 - 1.0	-	-	-	6.0 (1.2)	5.0 (1.2)	2.3 - 12.3

Table G-8 (continued). Abundance of Pest Arthropods in Sticky Trap Samples Collected from MON 87427, the Conventional Control, and the Reference Maize Hybrids

		Northern corn rootworm			Sap beetle			Southern corn rootworm		
		Mean (S.E.)		Reference	Mean (S.E.)		Reference	Mean (S.E.)		Reference
Coll.	Site[1]	MON 87427	Control	range	MON 87427	Control	range	MON 87427	Control	range
1	IL1	-	-	-	-	-	-	-	-	-
	IN2	-	-	-	-	-	-	-	-	-
	MO	-	-	-	-	-	-	-	-	-
	PA	-	-	-	-	-	-	-	-	-
2	IL1	1.0 (0.6)	1.3 (0.9)	0.7 - 3.0	-	-	-	-	-	-
	IN2	-	-	-	-	-	-	-	-	-
	MO	-	-	-	-	-	-	0.7 (0.3)	0.7 (0.3)	0.0 - 0.3
	PA	-	-	-	-	-	-	-	-	-
3	IL1	-	-	-	1.3 (1.3)	1.7 (0.9)	1.3 - 3.0	-	-	-
	IN2	-	-	-	-	-	-	-	-	-
	MO	-	-	-	-	-	-	-	-	-
	PA	-	-	-	-	-	-	-	-	-
4	IL1	0.7 (0.3)	2.3 (0.9)	1.7 - 2.7	-	-	-	-	-	-
	IN2	0.0 (0.0)[†]	0.0 (0.0)	0.0 - 0.0	-	-	-	-	-	-
	MO	-	-	-	-	-	-	-	-	-
	PA	-	-	-	-	-	-	-	-	-
5	IL1	1.3 (0.3)	0.3 (0.3)	0.3 - 5.0	0.0 (0.0)	0.7 (0.3)	0.0 - 0.7	-	-	-
	IN2	-	-	-	-	-	-	-	-	-
	MO	-	-	-	-	-	-	0.7 (0.7)	0.0 (0.0)	0.0 - 0.3
	PA	-	-	-	-	-	-	-	-	-

Table G-8 (continued). Abundance of Pest Arthropods in Sticky Trap Samples Collected from MON 87427, the Conventional Control, and the Reference Maize Hybrids

		Western corn rootworm		
		Mean (S.E.)		Reference
Coll.	Site[1]	MON 87427	Control	range
1	IL1	-	-	-
	IN2	18.7 (1.5)	18.7 (7.4)	11.0 - 36.0
	MO	-	-	-
	PA	0.3 (0.3)	0.7 (0.7)	0.0 - 0.7
2	IL1	77.0 (30.1)	41.7 (6.8)	34.0 - 84.3
	IN2	36.7 (9.8)	28.0 (5.5)	23.7 - 38.0
	MO	-	-	-
	PA	-	-	-
3	IL1	52.0 (12.8)	55.3 (19.5)	37.7 - 67.0
	IN2	37.3 (7.0)	30.3 (3.3)	24.3 - 48.3
	MO	-	-	-
	PA	6.0 (1.0)	3.7 (1.2)	2.3 - 6.0
4	IL1	45.7 (9.7)	41.3 (3.0)	44.3 - 69.3
	IN2	34.7 (3.3)	30.3 (4.2)	25.0 - 40.7
	MO	-	-	-
	PA	3.7 (0.7)	2.0 (1.5)	0.3 - 3.3
5	IL1	27.3 (10.9)	25.0 (2.6)	33.0 - 47.3
	IN2	32.0 (2.9)	46.0 (7.5)	34.0 - 52.0
	MO	-	-	-
	PA	1.3 (0.3)	2.0 (0.6)	1.3 - 2.0

Note: The experimental design was a randomized complete block with three replications at each site. Data were collected at five crop developmental stages: Collection 1 at V10-V15, Collection 2 at V18-VT, Collection 3 at R1, Collection 4 at R2, and Collection 5 at R3-R4. A dash (-) indicates arthropod not evaluated. The reference range is the minimum and maximum values of the reference means. S.E. = standard error. *Indicates a statistically significant difference ($p \leq 0.05$) between MON 87427 and the conventional control.

† No statistical comparisons were made due to lack of variability in the data.

[1] Site codes are as follows; IL1=Stark Co., IL; IN2 = Boone Co., IN; MO = Butler Co., MO; PA = Berks Co., PA.

Table G-9. Abundance of Beneficial Arthropods in Sticky Trap Samples Collected from MON 87427, the Conventional Control, and the Reference Hybrids

		Araneae			Big eyed bug			Brown lacewing		
		Mean (S.E.)		Reference	Mean (S.E.)		Reference	Mean (S.E.)		Reference
Coll.	Site[1]	MON 87427	Control	range	MON 87427	Control	range	MON 87427	Control	range
1	IL1	1.0 (0.6)	1.0 (0.6)	2.0 - 4.0	-	-	-	-	-	-
	IN2	1.7 (0.9)	2.0 (0.0)	0.3 - 2.3	-	-	-	-	-	-
	MO	4.0 (2.5)	0.7 (0.3)	1.7 - 3.3	-	-	-	-	-	-
	PA	1.3 (0.9)	0.7 (0.3)	0.3 - 1.3	-	-	-	0.3 (0.3)	0.0 (0.0)	0.0 - 0.3
2	IL1	0.3 (0.3)	0.0 (0.0)	0.0 - 0.7	-	-	-	-	-	-
	IN2	0.7 (0.3)	0.3 (0.3)	0.3 - 0.7	-	-	-	-	-	-
	MO	1.0 (0.0)	2.7 (0.3)	2.3 - 5.7	1.7 (0.9)	3.3 (1.9)	0.7 - 2.7	-	-	-
	PA	0.0 (0.0)	1.0 (0.6)	0.3 - 1.7	-	-	-	-	-	-
3	IL1	0.3 (0.3)	1.0 (0.6)	0.0 - 0.7	-	-	-	-	-	-
	IN2	0.3 (0.3)	1.0 (0.0)	0.3 - 1.7	-	-	-	-	-	-
	MO	1.3 (0.3)	2.7 (1.3)	2.0 - 6.0	6.0 (1.7)	6.0 (2.1)	3.0 - 8.3	-	-	-
	PA	2.0 (1.5)	1.3 (0.3)	1.0 - 2.3	-	-	-	-	-	-
4	IL1	0.3 (0.3)	0.3 (0.3)	0.3 - 0.7	-	-	-	-	-	-
	IN2	0.3 (0.3)	0.3 (0.3)	0.0 - 0.7	-	-	-	0.0 (0.0)	0.3 (0.3)	0.0 - 0.3
	MO	2.3 (0.7)	1.3 (0.3)	1.0 - 4.3	6.3 (0.7)	5.7 (1.8)	3.7 - 5.7	-	-	-
	PA	2.3 (0.3)*	0.7 (0.7)	1.0 - 3.7	-	-	-	-	-	-
5	IL1	0.3 (0.3)	0.3 (0.3)	0.0 - 0.7	-	-	-	-	-	-
	IN2	0.0 (0.0)	0.7 (0.3)	0.0 - 1.3	-	-	-	-	-	-
	MO	4.0 (1.2)	3.3 (2.0)	3.0 - 6.0	1.7 (1.2)	1.0 (0.6)	1.0 - 3.0	-	-	-
	PA	1.0 (0.6)	0.7 (0.3)	0.3 - 2.3	-	-	-	-	-	-

Table G-9 (continued). Abundance of Beneficial Arthropods in Sticky Trap Samples Collected from MON 87427, the Conventional Control, and the Reference Hybrids

		Green lacewing			Ladybird beetle			Macro-parasitic Hymenoptera		
		Mean (S.E.)		Reference	Mean (S.E.)		Reference	Mean (S.E.)		Reference
Coll.	Site[1]	MON 87427	Control	range	MON 87427	Control	range	MON 87427	Control	range
1	IL1	-	-	-	2.0 (0.0)	1.0 (0.6)	0.0 - 3.0	0.0 (0.0)	0.3 (0.3)	0.0 - 1.0
	IN2	-	-	-	7.3 (1.8)	5.7 (1.2)	3.7 - 4.3	-	-	-
	MO	0.3 (0.3)	0.3 (0.3)	0.3 - 1.3	21.7 (5.8)	16.7 (4.1)	16.7 - 38.0	-	-	-
	PA	-	-	-	66.3 (9.9)	64.0 (7.9)	51.7 - 66.7	-	-	-
2	IL1	0.3 (0.3)	0.7 (0.3)	0.0 - 1.0	0.0 (0.0)	0.3 (0.3)	0.3 - 1.7	-	-	-
	IN2	-	-	-	2.0 (1.2)	2.0 (0.6)	0.3 - 2.7	-	-	-
	MO	6.7 (1.5)	6.7 (2.6)	1.0 - 6.0	35.0 (8.1)	49.3 (14.2)	34.3 - 52.3	-	-	-
	PA	-	-	-	29.0 (1.7)	25.0 (4.7)	22.3 - 28.3	-	-	-
3	IL1	0.7 (0.3)	0.0 (0.0)	0.3 - 1.7	0.3 (0.3)*	1.3 (0.3)	0.0- 0.3	2.3 (1.5)	4.3 (1.9)	1.3 - 5.7
	IN2	1.0 (1.0)	1.3 (0.9)	0.7 - 2.3	10.7 (2.8)*	3.0 (1.0)	2.0 - 7.0	1.3 (0.7)	0.3 (0.3)	0.0 - 1.3
	MO	-	-	-	27.7 (6.8)	27.0 (2.5)	15.7 - 27.0	-	-	-
	PA	-	-	-	39.3 (5.2)	47.0 (5.3)	38.0 - 47.7	-	-	-
4	IL1	-	-	-	2.7 (0.9)	2.3 (0.7)	0.3 - 1.0	8.7 (3.5)	11.0 (1.2)	3.7 - 11.3
	IN2	-	-	-	7.7 (2.7)	6.0 (1.2)	3.0 - 10.7	26.7 (3.2)*	9.7 (2.2)	9.3 - 14.7
	MO	-	-	-	18.0 (5.9)	10.0 (3.5)	9.3 - 14.7	-	-	-
	PA	-	-	-	21.0 (3.5)	28.0 (6.6)	23.3 - 41.0	-	-	-
5	IL1	-	-	-	4.0 (0.6)	5.7 (0.9)	1.7 - 3.7	11.0 (3.2)	8.3 (3.4)	7.7 - 9.7
	IN2	-	-	-	7.7 (4.3)	7.7 (1.3)	4.3 - 9.0	47.7 (5.5)*	27.7 (5.3)	29.7 - 36.3
	MO	-	-	-	11.7 (1.3)	10.0 (2.1)	5.7 - 20.7	-	-	-
	PA	-	-	-	23.7 (5.8)	15.7 (3.2)	13.0 - 24.0	-	-	-

Table G-9 (continued). Abundance of Beneficial Arthropods in Sticky Trap Samples Collected from MON 87427, the Conventional Control, and the Reference Hybrids

		Micro-parasitic Hymenoptera			Nabis			*Orius*		
		Mean (S.E.)		Reference	Mean (S.E.)		Reference	Mean (S.E.)		Reference
Coll.	Site[1]	MON 87427	Control	range	MON 87427	Control	range	MON 87427	Control	range
1	IL1	65.0 (6.7)	63.3 (8.7)	49.7 - 56.3	-	-	-	0.7 (0.3)	0.3 (0.3)	0.0 - 0.0
	IN2	68.3 (9.8)	52.0 (2.1)	56.3 - 70.7	0.3 (0.3)	0.0 (0.0)	0.3 - 1.0	2.3 (0.7)	2.3 (1.2)	1.3 - 6.7
	MO	83.0 (9.1)	59.3 (10.3)	61.3 - 106.7	0.3 (0.3)	0.3 (0.3)	0.0 - 1.0	1.3 (0.7)	0.7 (0.3)	0.7 - 2.3
	PA	54.0 (9.8)	43.7 (14.4)	41.3 - 48.3	0.7 (0.3)*	2.7 (0.7)	1.3 - 4.7	13.0 (4.2)	11.3 (0.9)	5.7 - 11.3
2	IL1	11.7 (1.8)	6.7 (3.2)	8.0 - 19.0	-	-	-	3.0 (0.6)	4.7 (1.5)	5.3 - 6.3
	IN2	35.7 (7.1)	35.3 (2.9)	36.0 - 57.0	0.0 (0.0)	0.0 (0.0)	0.0 - 0.3	0.3 (0.3)*	3.7 (1.5)	0.3 - 3.3
	MO	140.7 (39.6)	109.7 (0.7)	116.0 - 144.7	-	-	-	2.3 (0.3)	4.3 (1.2)	1.7 - 4.3
	PA	28.3 (5.5)	27.0 (7.0)	38.0 - 52.0	-	-	-	3.3 (0.7)	4.0 (0.6)	0.7 - 4.3
3	IL1	18.7 (5.4)	24.0 (7.5)	12.0 - 20.0	-	-	-	6.3 (2.3)	3.7 (2.3)	2.0 - 6.3
	IN2	122.0 (9.5)	126.3 (20.2)	116.7 - 131.3	-	-	-	15.3 (1.5)	12.0 (2.5)	16.3 - 22.3
	MO	117.0 (36.7)	84.0 (19.9)	96.0 - 108.0	0.7 (0.7)	0.0 (0.0)	0.3 - 0.7	3.7 (0.9)	3.0 (1.0)	1.7 - 4.7
	PA	204.3 (40.3)	194.0 (37.2)	150.7 - 207.7	-	-	-	10.0 (1.5)	9.7 (2.2)	4.7 - 9.3
4	IL1	75.7 (8.7)	62.0 (30.5)	20.3 - 78.7	-	-	-	6.3 (2.0)	6.0 (2.1)	2.3 - 10.0
	IN2	125.0 (29.1)	124.3 (13.9)	99.7 - 132.7	-	-	-	9.3 (4.3)	9.3 (3.5)	9.0 - 10.7
	MO	115.0 (22.5)	83.0 (12.1)	94.3 - 123.3	0.0 (0.0)	0.3 (0.3)	0.0 - 0.3	3.0 (0.6)	3.7 (0.3)	1.7 - 4.0
	PA	183.0 (50.8)	148.7 (22.8)	172.0 - 196.0	-	-	-	18.3 (0.7)	10.0 (1.5)	13.3 - 20.7
5	IL1	153.0 (40.2)	126.3 (32.2)	13.3 - 152.3	-	-	-	1.0 (1.0)	2.3 (1.2)	0.7 - 2.7
	IN2	185.0 (61.8)	129.7 (37.9)	73.0 - 194.7	0.0 (0.0)	0.3 (0.3)	0.0 - 0.3	5.0 (2.1)	4.3 (0.7)	3.3 - 12.0
	MO	165.7 (21.5)	151.0 (18.3)	182.3 - 204.7	-	-	-	2.0 (0.0)	1.7 (0.9)	0.7 - 3.3
	PA	82.7 (15.1)	84.3 (16.3)	93.3 - 113.0	-	-	-	14.0 (1.2)*	7.3 (0.7)	8.7 - 17.3

Note: The experimental design was a randomized complete block with three replications at each site. Data were collected at five crop developmental stages: Collection 1 at V10-V15, Collection 2 at V18-VT, Collection 3 at R1, Collection 4 at R2, and Collection 5 at R3-R4. A dash (-) indicates arthropod not evaluated. The reference range is the minimum and maximum values of the reference means. S.E. = standard error. *Indicates a statistically significant difference ($p \leq 0.05$) between MON 87427 and the conventional control. † No statistical comparisons were made due to lack of variability in the data.

[1]Site codes are as follows; IL1=Stark Co., IL; IN2 = Boone Co., IN; MO = Butler Co., MO; PA = Berks Co., PA.

References for Appendix G

Widstrom, N.W. 1967. An evaluation of methods for measuring corn earworm injury. Journal of Economic Entomology 60:791-794.

Appendix H: Materials and Methods for Pollen Morphology and Viability Evaluation

H.1. Plant Production

MON 87427, a conventional control, and four commercial references were grown in Butler County, MO, in a randomized complete block design with three replications. Each plot consisted of 12 rows approximately 30 feet in length. Evaluations were conducted on non glyphosate-treated plants.

H.2. Flower Collection

Tassel bags were placed on non-systematically selected plants during pollen shed. The following morning, pollen was collected from three plants per plot and transferred to a uniquely labeled tube. Within approximately 30 minutes of collection, Alexander's stain solution (Alexander, 1980) in a 1:5 dilution was added to each tube (at least 2:1 (v/v) stain to pollen) to fix and stain the pollen, rendering the pollen non-viable; the tubes were closed and the contents shaken until thoroughly mixed. Subsamples were placed on wet ice within 30 minutes of pollen collection and maintained under those conditions until receipt at the performing laboratory. Pollen collected from each plant in a plot represented a subsample, and three subsamples made up one pollen whole sample.

H.3. Pollen Sample Preparation

Slides were prepared by aliquoting suspended pollen / stain solution onto a slide. Pollen samples were viewed under an Olympus Provis AX70 light/fluorescence microscope with an Olympus DP70 digital color camera. The associated PC computer [Microsoft Windows 2000 Professional (© 1981-1999, Microsoft Corp.)] had microscope and camera software [(DP Controller v1.2.1.108 and DP Manager v1.2.1.107, respectively) (© 2001-2003, Olympus Optical Co., Ltd.)].

H.4. Data Collection

Pollen samples were assessed for viability, diameter and general morphology. To assess pollen viability, a minimum of one hundred pollen grains were evaluated under the 10X ocular lens (100X magnification) for each of the three subsamples per plot, and the mean of each whole sample (calculated from the subsamples) was analyzed. When exposed to the staining solution, viable pollen grains stained purple due to the presence of vital cytoplasmic content, while dead pollen grains stained clear to light blue-green. In addition, viable pollen grains appeared round and turgid, whereas non-viable pollen grains may have appeared flaccid, depending on the degree of hydration.

H.5. Statistical Analysis

Monsanto Statistics Technology Center performed the statistical analysis. The design was a randomized complete block design with three replications. SAS was used to compare MON 87427 to the conventional control for pollen viability and diameter, with a

significance level of 5% ($p \leq 0.05$). A reference range consisting of minimum and maximum mean values of the reference substances was reported for each characteristic.

References for Appendix H

Alexander, M.P. 1980. A versatile stain for pollen fungi, yeast and bacteria. Stain Technology 55:13-18.

Appendix I: Petitioner's Environmental Report

I.1. Introduction

This appendix provides information on four key areas to be covered in an environmental assessment for MON 87427: Purpose and Need, the Affected Environment, Alternatives and Potential Environmental Impacts. This environmental report has been prepared by Monsanto for the U.S. Department of Agriculture, Animal and Plant Health Inspection Services (USDA-APHIS) to facilitate the agency's compliance with the National Environmental Policy Act (NEPA) including compliance with the Council on Environmental Quality (CEQ) regulations that implement NEPA (Title 40 of the Code of Federal Regulation (40 CFR) Parts 1500-1508).

I.1.1 Monsanto's Rationale for Creating MON 87427

Almost all seed maize currently utilized in the U.S. is produced through hybridization. Hybrid maize seed production is based on the use of two maize inbred parents, one designated as a female parent inbred and one as a male parent inbred. In this process the designated male parent inbred produces pollen to fertilize the female parent inbred that has been de-tasseled (pollen control of the female inbred through the removal of male reproductive tissues). The detasseling step must occur prior to when the female parent inbred is expected to produce pollen so that the female parent inbred cannot fertilize itself by shedding pollen onto its female reproductive tissues, the silks. Currently, removal of the tassel from the female parent inbred is accomplished either by hand or by mechanical means. Combinations of both of these removal methods may also be used in production fields. The "window" for hand or mechanical tassel removal is brief, usually an average of 3-4 days and costs are relatively high ranging from USD $130 to $200 per acre (Koetters, 2007).

An alternative form of pollen control is Cytoplasmic Male Sterility (CMS), a genetic method in which maternally inherited mitochondrial DNA genes provide pollen sterility in the female inbred when dominant fertility restoration genes are absent. Pollen fertility is restored in the hybrid maize seed produced from crossing this female parent inbred with a male parent inbred that possesses the dominant fertility restoration genes. An extensive breeding integration process is required for CMS, and, as some detasseling is still required for these inbreds, CMS is used only on limited acres in hybrid seed production in the U.S.

Monsanto Company has developed MON 87427, a maize line exhibiting tissue-selective glyphosate-tolerance in vegetative and female reproductive tissues, but not in male reproductive tissues. Thus MON 87427 when sprayed at late vegetative stages of growth with the herbicide glyphosate produces a male sterile phenotype through tissue-selective glyphosate tolerance that either eliminates or greatly reduces the need for hand or mechanical detasseling. Current detasseling practices may require up to two passes with mechanical detasseling equipment and up to three passes if hand detasseling is used. Further complicating detasseling activity is the logistical planning required for relocating adequate labor and resources to the designated hybrid seed production fields at the

appropriate time. In contrast the "window" for late growth stage application of glyphosate to produce the male sterile phenotype through tissue-selective glyphosate tolerance is approximately two weeks as compared to manual and mechanical detasseling which has an average 3-4 day "window". This timing results in significantly improved flexibility in hybrid seed production. Additionally, detasseling costs associated with labor recruitment and deployment, are one of the single largest cost improvement opportunities in hybrid seed production. The use of MON 87427 will decrease hybrid seed production costs primarily through reduction in direct and associated labor costs.

Typically, in maize hybrid seed production, female parent inbreds are crossed with male parent inbreds to produce hybrid maize seed with desirable characteristics. MON 87427 will be a designated female parent inbred in the hybrid seed production process. Pollen from the corresponding fully glyphosate tolerant male parent inbred will fertilize MON 87427 resulting in hybrid maize seed with glyphosate tolerance. The resulting MON 87427 hybrid seed is fully fertile, glyphosate-tolerant, and can be sold to growers for commercial production resulting in forage and grain.

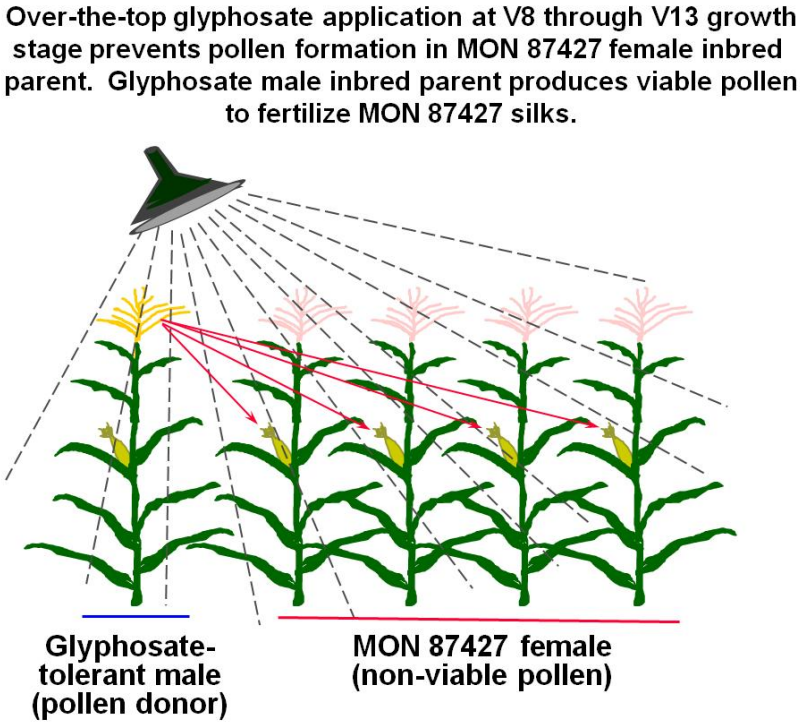


Figure I-1. MON 87427 Production System Concept

Numerous MON 87427 field trials have been conducted in the U.S. under APHIS notifications and permits since 2005 (Appendix A – USDA Planting Notifications). Information has been collected from these field trials, other tests, and the literature to assess whether the lack of tolerance of the male reproductive tissues of MON 87427 to glyphosate and/or the plant transformation process has altered MON 87427 in any way

that would impart plant pest characteristics or cause significant environmental impacts, including cumulative impacts. The purpose of this document is to provide relevant information regarding the potential for reasonably foreseeable, significant environmental impacts.

An analysis of the potential impact of deregulation of MON 87427 on current hybrid maize seed production systems, and related activities such as maize processing, food and feed uses as well as marketing of maize and maize products is presented in this appendix Factors evaluated as part of the assessment include potential impacts to:

- land use patterns, non-agricultural lands, farming practices, commodity and specialty maize production,
- hybrid seed production practices and marketability of maize seed for planting,
- non-target organisms, threatened or endangered species, and biodiversity,
- public health including human consumption and worker safety
- economic impacts associated with environmental impacts.

The analysis conducted considers current and reasonably foreseeable actions, the potential for deregulation of MON 87427 to impact these actions, and potential cumulative impacts. In most cases, there are no impacts relative to current conditions (e.g., no differences between deregulation of MON 87427 versus continuing to regulate). Where differences were noted, these differences are described and their significance evaluated. Also considered in this petition are indirect effects to the environment due to changes in the glyphosate use pattern that would result following the deregulation of MON 87427.

I.2. Purpose and Need

The APHIS Biotechnology Regulatory Service's (BRS) mission is to protect the health and value of American agriculture and natural resources. Under the authority of the Plant Protection Act (PPA), APHIS' Biotechnology Regulatory Services (BRS) regulates the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered (GE) organisms and products that may pose a risk to plant health (U.S. Code § 7701-7772, 7 CFR § 340). A GE organism is considered a regulated article if APHIS has reason to believe it could pose a plant pest risk. An organism is no longer subject to these regulations when APHIS determines that it is unlikely to pose a plant pest risk. A person may petition the agency to evaluate submitted data and determine that a particular regulated article is unlikely to pose a plant pest risk, and, therefore, should no longer be regulated as a potential plant pest (7 CFR § 340.6 "Petition for Determination of Nonregulated Status"). The petitioner is required to provide information related to plant pest risk that APHIS may use to determine whether the regulated article is unlikely to present a plant pest risk (7 CFR § 340.6(c)(4)). If, based

on this information, the USDA determines that the article is unlikely to pose a plant pest risk, the article may be granted nonregulated status.

Monsanto Company (Monsanto) has submitted a petition to APHIS for the determination of nonregulated status for MON 87427, a maize line exhibiting tissue-selective glyphosate-tolerance in vegetative and female reproductive tissues, but not in male reproductive tissues. Thus MON 87427 produces a male sterile phenotype through tissue-selective glyphosate tolerance when sprayed at late vegetative stages of growth with the herbicide glyphosate, eliminating or greatly reducing the need for hand or mechanical detasseling. This results in an increased "window" of approximately two weeks as compared to manual and mechanical detasseling having an average 3-4 day "window" and higher production costs. Monsanto has requested that APHIS make a determination that these maize plants, their progeny and crosses with previously deregulated products will no longer be considered regulated articles under 7 CFR § 340.

APHIS' action in this case is to determine whether or not to grant nonregulated status to MON 87427. APHIS' purpose and need in making this determination is to fulfill its responsibilities under the PPA while complying with NEPA.

I.3. Affected Environment

This section describes the setting for the proposed deregulation and provides the context for evaluating the intensity of the impact due to USDA-APHIS granting deregulated status to MON 87427.

1.3.1. Defining the Affected Environment for MON 87427

MON 87427 may be used as an aid in the production of hybrid maize seed on no greater than the 0.5 M acres comprising hybrid maize seed production in the U.S. This is the maximum number of acres devoted to seed production by various seed producers and MON 87427 may be used on all or some of these acres. Typically, in maize hybrid seed production, female parent inbreds are crossed with male parent inbreds to produce hybrid maize seed with desirable characteristics. MON 87427 will be a designated female parent inbred in the hybrid seed production process. Pollen from the corresponding fully glyphosate tolerant male parent inbred will fertilize MON 87427 resulting in hybrid maize seed with glyphosate tolerance. The resulting MON 87427 hybrid seed is fully fertile, glyphosate-tolerant, and can be sold to growers for commercial production resulting in forage and grain.

The resulting hybrid seed containing MON 87427 has the potential to be planted commercially anywhere in the U.S. where maize is currently grown. Glyphosate-tolerance is not a new trait in maize having been deregulated in 1997 and currently present on approximately 80%[6] of all commercial maize acres in the U.S. (Monsanto

[6] According to the USDA-ERS reference cited in section VIII, 70% of all genetically modified maize grown in the U.S. is herbicide-tolerant. Legitimate alternative references (Monsanto, 2009 and USDA-NASS, 2010a) were used to determine 80% of all genetically modified maize grown in the U.S. is glyphosate-tolerant.

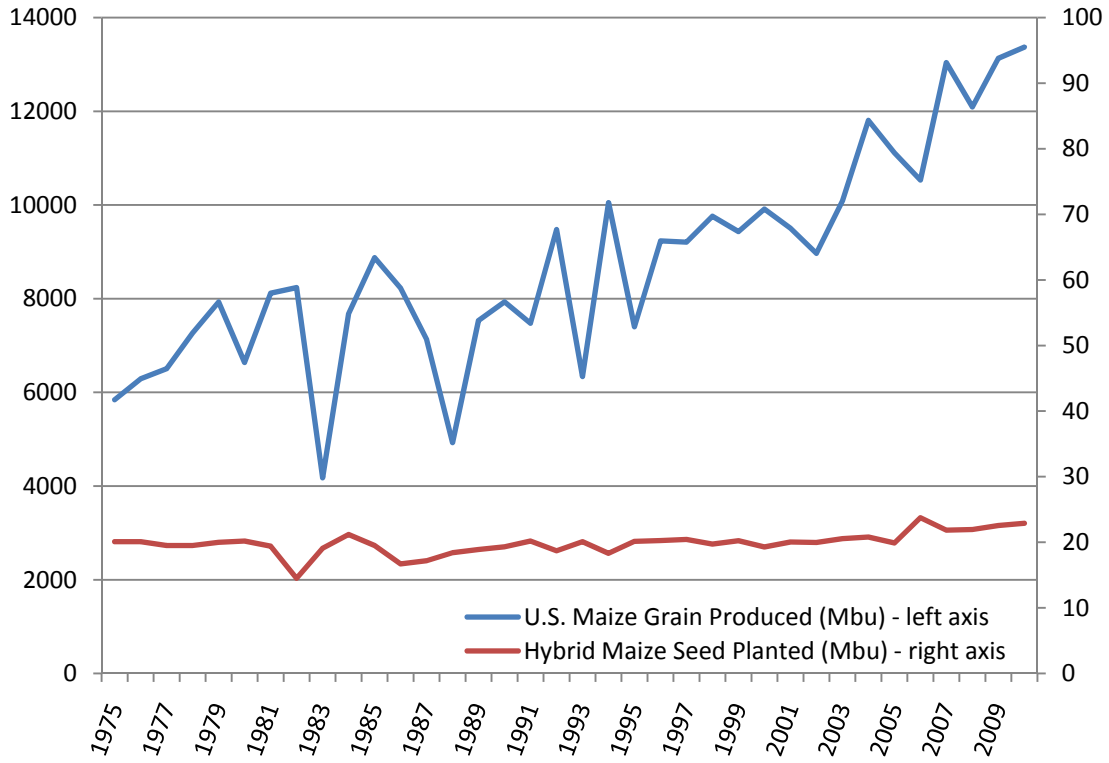
Company, 2009; USDA-NASS, 2010a). Therefore relative to the current commercial production of maize grain no significant changes are anticipated to agricultural practices or inputs, land use, water quality, non-agricultural lands, specialty and organic maize production, maize marketability, human health, farm worker safety, non-target organisms (NTO), threatened and endangered species (TES), or biodiversity from the deregulation of MON 87427 and the planting of MON 87427-containing hybrids. Some non-significant changes based on direct and indirect effects are anticipated in terms of agricultural practices and inputs, and worker safety and economics in the certified hybrid maize seed production process on hybrid maize seed production acres.

I.3.2. Hybrid Seed Production - Overview

In hybrid seed production inbred lines, the result of the transfer of pollen from an individual plant to the silks of the same plant for several generations, produces a line that is stable and can maintain desirable traits to be combined with other inbred lines in the production of hybrid seed. Though crop inputs play a significant role in determining yield and vary based on climate and soil type (Hoeft, et al., 2000a; b; c), the use of hybrid maize has also been important for optimizing crop yield.

Use of hybrid maize seed dates back to the early 1920s when there were approximately 1000 open pollinated inbred lines available in the U.S. (Troyer, 1999). Initially, the adoption of hybrid maize seed was slow, and by 1933 only 1% of the maize grown in the U.S. was produced from hybrid seed. It was under the drought conditions of 1934 and 1936 where farmers noticed the improved performance of hybrid maize seed over the open pollinated inbred varieties, and began to accept and eventually demand access to new hybrids developed for their growing regions (Wych, 1988). Thus hybrid maize almost completely replaced open-pollinated maize varieties in most of the maize belt, and, by 1960, virtually all maize plantings in the U.S. were hybrid (Duvick, 2001).

Today, the production of hybrid maize seed is a multi-billion dollar business. The increase from a one acre hybrid seed production plot in the 1920s to hundreds of thousands of acres of hybrid maize seed production in the 21st century has been driven by improvements in yield and production. Over the last 35 years, the volume of hybrid maize seed planted in the U.S. has changed very little, with 20.10 million bushels (MBu) planted in 1975 and 22.55 MBu planted in 2009 (USDA-ERS, 2010b). However, the yield of harvested grain has increased significantly over that same period (Figure I-2). Total hybrid maize seed production acreage utilizing MON 87427 is anticipated to be no greater than 0.5 M acres, or no greater than 0.6% of the 75-93M acres of maize that have been routinely planted in the U.S. over the last ten years (USDA-NASS, 2010a).



Source: (USDA-ERS, 2010b)

Figure I-2. Hybrid Maize Seed Planted and Grain Produced in the U.S. from 1975-2009.

Commercial hybrid maize seed production is an expensive and labor intensive process. In the case of biotechnology-derived traits, these traits may be found in either the female parent inbred, the male parent inbred or both. The female parent inbred is prevented from shedding pollen to ensure only pollination by the male parent inbred. The male parent inbreds are usually destroyed by mechanical means following pollination to prevent seed mixing during harvest. Ears from the cross-pollinated female parent inbred are harvested, processed, and the seed sold to farmers for planting as hybrid maize seed.

I.3.3. Commercial Maize Production and Uses

Commercial maize production and uses are discussed in Section VIII.B of the petition and summarized here; refer to the petition for more detail. The U.S., China, European Union, Brazil, and Mexico are the top five producers of maize (FAOSTAT, 2009). Globally in 2008, approximately 5.8 million metric tonnes (MMT) of maize seed were used to produce more than 820 MMT of grain harvested from more than 160 million hectares (FAOSTAT, 2009). For this same year, approximately 0.59 MMT of hybrid maize seed were used in the U.S. to produce approximately 37% (307 MMT) of the world's maize crop (FAOSTAT, 2009). Maize is grown in the U.S. almost totally from hybrid seed, and is the largest crop based on acreage planted and net crop value, accounting for >90% of total value and production of feed grains (USDA-ERS, 2009b).

The U.S. is a major player in the world maize trade market, with approximately 20 % of the maize crop exported to other countries (USDA-ERS, 2009b).

The U.S. acreage for cultivating maize has varied. Since 1900, maize acreage ranged from a high of 113 million acres in 1932 to a low of 60.2 million acres in 1983. In the past 10 years (2000-2009), total annual maize acreage planted varied from approximately 75 to 93 million acres. Total annual production during this period ranged from about 9 to 13 billion bushels, and total annual value fluctuated from about 18 to 54 billion dollars depending on production output and commodity prices (USDA-NASS, 2010a). Hybrid maize was planted in almost every state in the continental U.S. in 2008 with the two largest maize producing regions being the Midwest and the Great Plains (USDA-ERS, 2009a). The Midwest region is comprised of eight states: Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Ohio and Wisconsin. The Great Plains includes portions of ten states: Colorado, Kansas, Montana, Nebraska, New Mexico, North Dakota, Oklahoma, South Dakota, Texas and Wyoming (Riebsame, 1990). The Midwest and Great Plains regions contributed 64% and 27%, respectively, of the national maize production total for 2009.

I.3.4. Certified Hybrid Maize Seed Production

By the early 20th century, crop breeders had learned how to develop specific plant varieties with desirable traits. In the U.S., state agricultural experiment stations developed many seed varieties which were distributed to farmers for use. As seeds were saved by farmers and later sold to neighbors, the desirable traits of the varieties often were lost through random genetic changes and contamination with other crop and weed seeds. The value of seed quality (including genetic purity, vigor, weed seed presence, seed borne diseases and inert materials such as dirt) was quickly identified as a major factor in crop yields. States developed seed laws and certification agencies to ensure that purchasers who received certified seed could be assured that the seed met established seed quality standards (Bradford, 2006). The federal government passed the U.S. Federal Seed Act of 1939 to recognize seed certification and official certifying agencies. Regulations first adopted in 1969 under the Federal Seed Act recognize land history, field isolation, and varietal purity standards for foundation, registered, and certified seed. Under international agreements such as the Organization for Economic Co-Operation and Development (OECD) scheme, the U.S. and other countries mutually recognize minimum seed quality standards (Bradford, 2006). The Association of Official Seed Certifying Agencies (AOSCA) represents state and private seed certification in the U.S., and includes international member countries in North and South America, as well as Australia and New Zealand.

Seed certification is based on varietal lineage, as well as quality production and processing standards. Seeds produced for sale to a crop grower (certified seeds) are a limited number of generations from a verified seed stock of the specified variety (Bradford, 2006). Breeder seed is generally produced under the strictest standards and under the supervision of the breeder. Breeder seed is used to produce foundation seed, which is used to produce registered seed, which is then used to produce certified seed that is sold for commercial planting (Bradford, 2006). In addition to documenting the

pedigree of the seed, certification programs also monitor crop rotations, previous crops and weeds in the field, as well as isolation of the field from other varieties of the same genus or species (Bradford, 2006). After seed harvesting and cleaning, the seed is later tested for germination capacity, and analyzed for the presence of seed of other varieties or other crops, weed seeds and inert matter to assure high quality before the seed bags are tagged as "certified" (Bradford, 2006).

Hybrid maize seed must meet state and federal seed standards and labeling requirements. AOSCA is dedicated to assisting companies in the production, identification, distribution and promotion of certified classes of seed. AOSCA establishes minimum standards for quality and identity. Its goal is to standardize certification regulations and procedures internationally so companies compete with one set of standards. The association cooperates with the OECD and other international organizations to develop standards, regulations, procedures, and policies to expedite movement of seed and encourage international commerce in improved hybrids. The AOSCA standards for maize seed are as follows: 98% pure seed (minimum), 2% inert matter (maximum), no weed seed, 0.5% other hybrids, 90% germination (minimum), and 14 % moisture (maximum) (AOSCA, 2009). Certified seeds are produced and officially controlled according to common harmonized procedures. OECD certification provides official worldwide recognition of "quality-guaranteed" seed, facilitating international trade and contributing to removal of technical trade barriers.

In the U.S. almost all maize seed currently used is produced through hybridization. In this process one inbred (male) of maize produces pollen to fertilize a second inbred (female), which has been detasseled (the pollen producing male reproductive tissues are removed) so it cannot fertilize itself. Detasseling currently represents the most widely used method of pollination control. Detasseling involves either the manual removal of tassels or manual detasseling in combination with a mechanical detasseler in production fields. Invariably, using these methods some plants either have a partial tassel present or are totally missed by the mechanical detasseling. To compensate and to preserve the quality of the certified seed, agricultural labor is hired to inspect the plants following mechanical detasseling and complete the detasseling by hand, when necessary (Section VIII.B.2.). Although more cost effective than manual detasseling, it is common to observe greater yield reductions after mechanical detasseling than after manual detasseling. An agricultural study (Craig, 1977), cited unpublished research in which the yield of mechanically detasseled plots was 2 to 40% less than that of hand detasseled treatments, depending upon the inbred involved and the number of mechanical cuttings.

Another method developed to replace manual and/or mechanical detasseling is CMS (Section VIII.B.2.), a genetic method that was widely adopted in the U.S. in the 1950s and 1960s as a means to eliminate pollen from the female parent inbred without the need of manual or mechanical detasseling (Craig, 1977). Results were variable from this approach with cytoplasms in some genetic backgrounds having only partial sterility which must be remedied with detasseling (Wych, 1988) or increased plant susceptibility to certain crop diseases such that CMS systems are used today in only 30% of hybrid maize seed production acres.

Male gametocides are not an option for pollination control due to significant off-target effects, including plant toxicity, that severely limit their use (Loussaert, 2004).

I.3.5. Organic Maize Production

Organic maize typically commands a market premium relative to commodity maize to offset the additional production and record-keeping costs associated with this production method. Organic farming as described by the National Organic Program (USDA-AMS, 2010) [7 C.F.R. § 205], is administered by USDA's Agricultural Marketing Service, requires organic production operations to have distinct, defined boundaries and buffer zones to prevent unintended contact with prohibited substances or products of excluded methods from adjoining land that is not under an organic production management plan. Organic production operations must also develop and maintain an organic production system plan approved by an accredited certifying agent. This plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition of the use of excluded methods. Excluded methods include a variety of methods used to genetically engineer organisms or influence their growth and development by means that are not possible under natural conditions or processes. The use of biotechnology such as that used to produce MON 87427 is an excluded method under the National Organic Program [7 C.F.R. § 205.2]. Buyers recognize that when biotechnology-derived crop varieties are on the market, as with maize, a guarantee that a commodity crop is 100% "free" of biotechnology-derived material is not feasible based on the limitations of testing and sampling methodology and there are some specifications in buyer allowances that permit between 0.1 to 5% biotechnology-derived maize in organic maize (Born, 2005). International regulatory authorities have recognized that testing and sampling methodologies limit the ability to confirm that commodity or specialty maize is 100% free of biotechnology-derived material. Thus, they have set allowable tolerances for biotechnology-derived material in conventional products to support food labeling and traceability laws. These tolerances allow from 0.9% (European Union) up to 5% (Japan) of the food or food ingredients to be biotechnology-derived in products considered "conventional." Levels above the threshold may trigger special labeling.

Organic certification involves oversight by an accredited certifying agent of the materials and practices used to produce or handle an organic agricultural product. This oversight includes an annual review of the certified operation's organic system plan and on-site inspections of the certified operation and its records. Although the National Organic Standards prohibit the use of excluded methods, they do not require testing of inputs or products for the presence of excluded methods. The presence of a detectable residue of a product of excluded methods alone does not necessarily constitute a violation of the National Organic Standards. The unintentional presence of the products of excluded methods will not affect the status of an organic product or operation when the operation has not used excluded methods and has taken reasonable steps to avoid contact with the products of excluded methods as detailed in an approved organic system plan. Organic certification certifies that organic production and handling processes have been followed, not that the product itself is "free" from any particular substance.

I.3.6. Agricultural Practices for Hybrid Maize Seed Production

Agricultural practices for hybrid maize seed production are discussed in Section VIII of the petition and summarized here; refer to the petition for more detail.

I.3.6.1. Production Area and Input Considerations

Hybrid maize seed production (see Section VIII.B. for more detailed discussion) begins with the selection of a suitable growing area. Factors such as temperature, rainfall, day length, and soil nutrient status are important because seed yields may be sensitive to unfavorable conditions during particular periods. For example, extremely high temperatures and dry conditions can affect the timing of silk emergence and growth, pollen shed and pollen viability resulting in poor seed formation and yield. Therefore, limited quantities of hybrid maize seed are produced in the southern states due to high temperatures during pollination, inadequate rainfall during the growing season, and a higher incidence of insects and diseases (C. Peters, Monsanto, Global Operations, personal communication, 2010). Maize seed is also not produced in the most northern portions of the maize belt due to colder soil temperatures where the mean number of growing degree days accumulated during the season may not be sufficient for maize to reach maturity prior to frost (Hoeft et al., 2000a). Maize seed must reach physiological maturity and be harvested prior to damaging frost which can reduce seed viability to unacceptable levels (McDonald and Copeland, 1997). Most hybrid maize seed is produced in the major maize belt states of Nebraska, Iowa, Illinois, Indiana, and Michigan due to the climate and the proximity to market, although irrigation is used in the seed producing areas of Nebraska, Michigan, and certain areas of Illinois and Indiana due to insufficient or timely rainfall and/or sandy soils (C. Peters, Monsanto, Global Operations, personal communication, 2010).

Conservation tillage is most commonly used in maize seed production (C. Peters, Monsanto, Global Operations, personal communication, 2010). No-till is seldom practiced in maize seed production due to poor emergence and growth of the inbred lines, plus higher incidence of insect pests and diseases. Soils tend to stay colder and wetter longer in the spring under no-till systems which are less favorable for maize production (McDonald and Copeland, 1997).

I.3.6.2. Planting Patterns of Production Plots

In maize hybrid seed production, the male and female parent inbreds are physically separated to control pollination within the field. The pollen parent can be planted at two different time points to extend the pollen shedding period, so that the timing of peak pollen shedding coincides with the timing of peak silk exposure in the female parent inbred. Planting patterns in seed production fields include 4:1 (four rows of female parent inbred to one row of male parent inbred), 4:2, 4:1:2:1, 6:2, and solid female with interspersed male. The female parent inbred is never more than two rows from the male parent inbred in the first three patterns. One-half of the female parent inbred rows are adjacent to a male parent inbred in the 4:1 and 4:2 patterns, and two-thirds of the female parent inbred rows are adjacent to a male parent inbred in the 4:1:2:1 pattern. A planting

pattern where every other or every fourth between- row space of a solid planted female parent inbred is interspersed with the male parent inbred fully utilizes the land area for female parent inbred production and achieves closer placement of the male and female parent inbreds (Craig, 1977; Wych, 1988).

I.3.6.3. Production Plot Isolation

Hybrid maize seed producers typically physically isolate production plots from neighboring maize seed or grain production fields to avoid cross-pollination during the flowering stage by wind-borne pollen. The isolation distance from other maize is regulated by seed certification standards, and is typically at least 660 feet from other maize (AOSCA, 2009). Isolation is often enhanced with male parent inbred border rows around the perimeter of the seed production plots, which increases desirable pollen shed from the male parent inbred during the silking period of the female parent inbred and reduces the potential for cross-pollination from external pollen sources. Official seed certification regulations often allow isolation distances between seed production fields to be reduced as the number of border rows increases (Agrawal, et al., 1998).

I.3.6.4. Pollen Control Methods

Pollen control refers to practices that ensure complete pollination of female parent inbreds by male parent inbreds to produce hybrid maize seed. Pollen control in hybrid maize seed production is extremely critical for producing hybrid maize seed with high genetic purity.

Detasseling is the most widely used method of pollen control in the production of hybrid maize seed. The detasseling period begins prior to pollen shedding when tassels have emerged from the leaf sheath. Tassels are physically removed from a female parent inbred maize line after the tassel has fully emerged and before undergoing pollen shed or silk emergence occurs. Removal of all of the tassels from the female parent inbred avoids the risk of self-fertilization of the female parent inbred. Instead, fertilization of the detasseled female parent inbreds is achieved by pollination from a genetically distinct male parent inbred line that is grown in close proximity.

Pollen shed usually occurs in maize over a five to eight day period with the peak production on about the third day (Hoeft et al., 2000a). Pollen shed is not always a continuous process, and can stop and restart depending on climatic conditions or when additional pollen has matured (Hoeft et al., 2000a). As a result, the window for detasseling that averages 3-4 days prior to the initiation of pollen shed is a critical step in maize seed production that, once begun, must be performed on a regular basis, regardless of weather. Removal of the tassel from the female parent inbred in seed production fields is accomplished by a combination of mechanical and manual detasseling methods (Wych, 1988). Mechanical detasseling methods came into widespread use as a way to better control rising production costs that resulted from increasing labor costs and a declining labor supply in the early 1970s (Craig, 1977).

Mechanical detasseling machines either cut or pull the tassels from the maize in all the female parent inbred rows. Mechanical cutters use a rotating blade or knife to remove the top of the maize plant and tassel. Mechanical pullers are complementary to cutters, and use two counter-rotating wheels or rollers to grasp and remove the tassel and upper leaves. Mechanical detasseling is delayed as long as possible before silk emergence, to permit maximum exsertion of tassels and enable their removal with minimum leaf damage. Best results are achieved in a uniform production seed field in which the tassels are well exserted or projecting beyond the tassel ahead of pollen shedding. As conditions become less favorable, the percentage of tassels removed per pass will decrease and leaf damage will increase. Removal of the entire tassel can result in the removal of too much leaf tissue, and reduce maize seed yields by as much as 10% (McDonald and Copeland, 1997). In addition, the tassels that have been removed can become lodged in the leaf canopy and shed pollen, resulting in unwanted self-pollination. This complication is resolved by hand detasseling crews. Crews also hand detassel the maize that was not completely detasseled with the mechanical methods.

Although detasseling is relatively straightforward to accomplish, the production of hybrid maize seed is expensive and labor-intensive, employing tens of thousands of teenage youth and migrant workers each year to hand detassel the maize. The large manual labor force is needed for only a relatively short period of time depending upon the volume of production and the range in female parent inbred maturity dates planted within a seed production area. A detasseling operation is at risk from weather such as heavy rain or windstorms that can lodge or tangle the female parent inbreds just as the tassels begin to emerge, making it difficult to walk or drive through the field. Extreme heat or drought during the onset of flowering can delay the emergence of tassels and silks. Seed fields need to be monitored and inspected closely during the detasseling period, as even a slight mistake can have considerable economic consequences. The labor force must be well trained, closely supervised, and effectively managed, which is complicated because of the reliance on temporary seasonal workers. Liability and worker safety issues associated with employing temporary manual labor are also important considerations.

Another way to achieve pollen control, Cytoplasmic Male Sterility (CMS) is a genetic method that was widely adopted in the U.S. in the 1950s and 1960s as a means to eliminate pollen from the female parent inbred without the need of manual or mechanical detasseling (Craig, 1977). The genetics by which CMS functions is based on the presence of mitochondrial DNA genes that provide pollen sterility when dominant fertility restoration genes are absent in the nuclear genome. Pollen fertility is restored in the hybrid maize seed produced from crossing this female parent inbred with a male parent inbred that possesses the dominant fertility restoration genes in its nuclear DNA.

A number of CMS systems have been identified to facilitate the crossing of two inbreds, and include S-*cms*, C-*cms* and T-*cms*. With the T-*cms* system, detasseling is eliminated entirely through the use of a female parent inbred that is completely male sterile. C and S cytoplasms in certain genetic backgrounds result in only partial male sterility and still require some detasseling.

The T-c*ms* system was adopted by the hybrid maize production industry because more inbreds were completely sterilized and genetic fertility restoration was more easily accomplished with this system (Craig, 1977). In the 1960s in the U.S. nearly all of the female parent inbreds were converted to the T-*cms* genotype used in hybrid maize seed production. Unfortunately, closely linked with this mitochondrial trait was a gene for susceptibility to a pathotoxin produced by *Bipolaris maydis* race T (formerly known as *Helminthosporium maydis* race T) that resulted in destruction of approximately 20% of the U.S. maize acreage from the southern maize leaf blight epidemic in 1969-1970 (Pring and Lonsdale, 1989) although some areas, particularly in southern states sustained greater losses (Ullstrup, 1972). The trait of pollen sterility was inseparable from *H. maydis* disease susceptibility (Levings and Siedow, 1992) making continued use of this genotype problematic.

The C and S cytoplasms are not linked to disease susceptibility, and became important in the late 1970s as a cost-competitive and satisfactory technique for hybrid maize seed production (Duvick, 1972) although some detasseling is still required. However, an extensive breeding integration process is required to move C and S cytoplasms into desirable backgrounds and the progeny may be sterile. Therefore CMS is grown only on limited acres in hybrid seed production.

Finally, chemical hybridization agents, also known as male gametocides, have been developed in the past for use in pollination control, but have had significant off-target effects, including plant toxicity, that severely limit their use (Loussaert, 2004).

I.3.6.5. Control of Weeds, Diseases and Insects

Control of weeds, insects, and diseases within the hybrid maize seed production field is an integral and necessary part of seed production (Wych, 1988). Seed growers rely heavily on herbicides for effective weed control, since inbred maize lines do not compete effectively with weeds.

Volunteer maize commonly occurs in rotational crops in the season following cultivation of either conventional or biotechnology-derived maize. In maize seed production, volunteer maize is the major volunteer management problem and must be controlled to avoid cross pollination with the female parent inbred plants. Effective management is often achieved by planting maize seed following soybean or another crop in the rotation (C. Peters, Monsanto, Global Operations, personal communication, 2010). Under situations where maize seed follows maize seed production or commercial maize grain production, the volunteer maize is controlled by cultivation and hand weeding. Off-type plants or rogue plants that differ phenotypically from inbred maize can grow with inbred seed lines. Though not considered volunteers, these plants are removed from seed fields by hand weeding prior to the pollination.

Insecticides are used to control above and below ground pests, and protect against insect damage to stands, the growing plants, and the female parent inbred ears. Seed companies practice integrated pest management principles and evaluate seed fields to determine if and when insecticide application is justified. Fungicides are also an important

component of hybrid maize seed production and are used to protect susceptible parent lines from damaging fungal diseases. Although genetic resistance to disease is preferred, chemical protection is often needed when resistance is not adequate in the parent line. Spray applications effectively reduce damage from foliar disease in susceptible inbred lines, and seed treatments are widely used to prevent seed and seedling diseases (Smith and White, 1988).

I.3.7. Human Health and Worker Safety

Humans consume maize and have done so for thousands of years with no significant adverse effects. Biotechnology-derived maize is evaluated extensively prior to commercial introduction. All biotechnology-derived maize products on the market today have satisfactorily completed the FDA consultation process established to review the safety of foods and feeds derived from biotechnology-derived crops for human and animal consumption. At this time three biotechnology-derived herbicide-tolerant maize products including a glyphosate/ALS-tolerant male sterile event (DP-32138-1), are currently under review (Table I-1) by the USDA.

Pesticides are used in the production of hybrid seed maize. The use of these pesticides is regulated by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). FIFRA was amended in 1988 to accelerate the re-registration of products with active ingredients registered prior to November 1, 1984. The amended Act calls for the development and submission of data to support the re-registration of an active ingredient, as well as a review of all data submitted to the EPA. During the re-registration process, EPA thoroughly reviews the scientific database underlying a pesticide's registration. The purpose of the Agency's review is to reassess the potential risks arising from the currently registered uses of a pesticide, to determine the need for additional data on health and environmental effects, and to determine whether or not the pesticide meets the "no unreasonable adverse effects" criteria of FIFRA.

In the agricultural production of hybrid seed maize, growers and workers may be exposed to pesticides applied to maize by mixing, loading, or applying chemicals, or by entering a previously treated site. EPA conducts a comprehensive occupational worker safety evaluation and risk assessment of pesticides to assess the risk to agricultural workers during mixing, loading, and applying. Additionally, seed producers train all employees, including seasonal workers, in the Worker Protection Standard (40 CFR § 170) that restricts field re-entry to specified intervals following chemical applications and requires the use of personal protective equipment.

Table I-1. Deregulated or Submitted Biotechnology-derived Herbicide-tolerant Maize Products

Phenotype	*ID Code(s)*	*Institution*	*Date Deregulated*
2,4-D tolerant	DAS-40278-9	Dow	Submitted
Glyphosate tolerant	HCEM485	Stine Seed	Submitted
Glyphosate/ALS- tolerant, male sterile, fertility restored, visual marker	DP-32138-1	Pioneer	Submitted
Glyphosate & imidazolinone tolerant	DP-098140-6	Pioneer	2009
Coleop resistant & glyphosate tolerant	MON 88017	Monsanto	2006
Lepidopteran resistant & glufosinate tolerant	TC-6275	Dow	2004
Lepidopteran resistant & glufosinate tolerant	TC1507	Mycogen	2001
Glyphosate tolerant	NK603	Monsanto	2000
Glufosinate tolerant & male sterile	MS6	AgrEvo	1999
Glufosinate tolerant & male sterile	676,678,680	Pioneer	1998
Lepidopeteran resistant & glufosinate tolerant	CBH-351	AgrEvo	1998
Glyphosate tolerant	GA21	Monsanto[7]	1997
Lepidopteran resistant & glyphosate tolerant	MON802	Monsanto	1997
Glufosinate tolerant & male sterile	MS3	PGS	1996
Glufosinate tolerant	B16 (DLL25)	DeKalb	1995
Glufosinate tolerant	T14, T25	AgrEvo	1995

Source: http://www.aphis.usda.gov/brs/not_reg.html

I.3.8. Adjacent-Agricultural Crop and Non-Agricultural Plants

Modern maize cannot survive outside of cultivation as a weed due to intense selection during domestication, in which traits often associated with weediness such as seed dormancy, dispersal mechanisms, or the ability to form reproducing populations outside

[7] Currently marketed by Syngenta

of cultivation were not selected (Baker, 1965; Galinat, 1988; Keeler, 1989). Maize does not grow and persist in unmanaged habitats and would not be expected to invade and/or persist in nature, including streams, lakes, oceans or other aquatic environments. Additionally the potential for maize gene transfer is limited to sexually compatible species, and occurs only with extreme difficulty and the utilization of special techniques.

Herbicides have been extensively used in U.S. maize commercial production. Off-target movement can occur with all herbicide applications, and can affect non-target plants. The extent of off-target movement from spray drift is a function of the local weather conditions (wind, temperature, humidity, inversion potential), droplet size, and the boom height (height of the application equipment above the crop canopy) (Jordan, et al., 2009). The potential for vapor drift is a function of local weather conditions and the properties of the herbicide and its formulation. The degree of injury to non-target plants that may occur from off-target movement is dependent on the sensitivity of the plant to the herbicide; however these impacts can be managed through good management practices (Jordan et al., 2009; University of Illinois, 2010). For example, growers and commercial applicators are educated by university specialists and industry representatives on the proper application equipment, equipment setup, and climatic conditions to maximize herbicide performance and reduce off-target movement of herbicides. Additionally, equipment manufacturers have developed spray nozzles that provide uniform coverage for effective weed control while applying larger spray droplets to reduce the potential for particle drift.

I.3.9. Animal, Plant and Soil Microbial Communities

I.3.9.1. Animal Communities

Maize production systems in agriculture are host to many animal species. Mammals and birds may seasonally consume grain, and invertebrates can feed on the plant during the entire growing season. Animals that feed primarily on maize are seed-feeding insects and rodents found in agricultural fields. Rodents, such as mice or squirrels, may seasonally feed exclusively on maize grain. Thus, these animals may have a diet containing significant amounts of maize grain. Deer may also browse in maize fields on the forage and on grain left after harvest.

I.3.9.2. Plant Communities

Maize production systems in agriculture contain many plant species typically considered to be weeds as they compete with the crop for resources. Likewise, the environment surrounding a maize field varies in plant composition depending on the region. In certain areas, maize fields may be bordered by other maize, soybean or other crops; fields may also be surrounded by wooded and/or pasture/grassland areas, as well as aquatic environments. Therefore, the types of vegetation, including weeds, around a maize field depend on the area where the maize is planted. A variety of weeds dwell in and around maize fields; those species will also vary depending on the region where the maize is planted. A list of the common weeds found in maize fields can be found in Section VIII of the petition.

I.3.9.3. Soil Microbial Communities

Soil microbial communities that mediate biogeochemical processes and directly impact soil quality are highly complex and are often characterized by high microbial diversity (Tiedje, et al., 1999). Microbial processes are affected by biotic factors (community characteristics and dynamics, specific plant-microorganism interactions) and abiotic factors such as soil structure, hydration, pH and redox potential (Atlas and Bartha, 1997). In agricultural systems, changes in microbial communities have been observed in response to soil disturbance, history of soil amendment, irrigation, tillage, and plant community structure (Buckley and Schmidt, 2001). Consequently, significant variation in microbial populations is expected in agricultural fields.

I.4. Alternatives

The decision-making process of deregulation is governed by 7 CFR § 340.6 (d)(3)(i) which states that APHIS may approve the petition in whole or in part, resulting in three possible outcomes from Monsanto's Petition:

I.4.1. Alternatives Considered and Dismissed

I.4.1.1. Approval in Part Alternative Based on Plant Pest Risk

The 'approval in part" alternative is dependent upon a finding of the potential for a plant pest risk for MON 87427 in certain geographies or under certain conditions. APHIS may impose conditions upon the cultivation or use of MON 87427 in specific geographies or conditions to mitigate potential plant pest risk. For example, APHIS could impose conditions to verify that the material being released into the environment is in fact MON 87427, or conditions that require assurance of the integrity and purity of the material containing MON 87427, or conditions requiring the implementation of stewardship practices in the use of MON 87427 that formed the basis of an APHIS decision that MON 87427 does not pose a plant pest risk. MON 87427 has been thoroughly characterized and extensive information presented in Sections I through IX of this petition demonstrates that MON 87427 does not present a plant pest risk in any of the geographies or under any conditions where MON 87427 may be grown. Therefore, from a plant pest risk perspective, there is no basis for imposing geographic or other restrictions on MON 87427.

We note that herbicides are used widely for production of maize, and their uses are reviewed and approved by EPA. Monsanto has filed an application with the EPA requesting an amendment in the registered use pattern and approval of an end use product label allowing a change in glyphosate use pattern on MON 87427 (See Section I.C.2.). It is EPA's responsibility to review the proposed label amendment and impose mitigating measures for glyphosate use, if needed, to protect the quality of the human environment.

On the basis of this analysis, the "approval in part" alternative will not be considered in this appendix.

I.4.2. Alternatives Studied in Detail

I.4.2.1. No Action Alternative

Under the no action alternative MON 87427 would remain a regulated article. MON 87427 could be grown under USDA notification or permit and confined release conditions. However, a requirement for permits would be so administratively burdensome, that it would not be feasible to provide notifications or permits for planting of commercial hybrids containing MON 87427 making MON 87427 unavailable for use in hybrid maize seed production. If MON 87427 is not deregulated, detasseling would continue to be part of hybrid maize seed production and the problems inherent in that system, including a short "window" for detasseling, and logistical problems with supplying thousands of agricultural workers to the right sites in the needed timeframe as well as increasing costs, would remain.

I.4.2.2. Approval in Whole Alternative

Under this alternative MON 87427 would no longer be a regulated article under 7 CFR § 340. Hybrid maize seed producers would have the option of treating their maize inbreds with glyphosate and the number of maize acres upon which glyphosate would be used would likely increase although the increase would not exceed 0.5 M acres, the extent of total hybrid maize seed production in the U.S. Because MON 87427 does not confer advantages to commercial maize growers beyond the biotechnology-derived maize traits that are already commercially available it is unlikely that the deregulation of MON 87427 would result in increased acres dedicated to maize production.

Monsanto is requesting approval in whole or full deregulated status for MON 87427. Information and assessments presented throughout this environmental report demonstrate that MON 87427 does not present a significant environmental impact if approved in whole.

I.5. Potential Environmental Impacts

The CEQ regulations require that significance be evaluated in terms of context (affected environment) and intensity (the severity of the impact) (40 CFR § 1508.27). Analysis of these factors considered the "no action" and the "approval in whole" alternatives. The differences between the two alternatives address the question of whether deregulation of MON 87427 results in a significant impact to the quality of the human environment. In most cases, there are no differences between the two alternatives. Where differences were noted, these differences are described and their significance evaluated. Factors evaluated as part of the assessment of significance include: potential impacts to land use patterns, farming practices, commercial and organic maize production and to non-agricultural lands, impacts to the marketability of hybrid maize seed for planting and harvested grain for commodity markets, impacts to public health, impacts to non-target organisms, threatened or endangered species, biodiversity and economic impacts associated with any environmental impacts. Finally, cumulative impacts are considered

in light of this action combined with past, present and reasonably foreseeable future actions.

An environmental impacts analysis is greatly dependent on the facts used for estimating effects. Where sufficient information is not available assumptions may be made. Factors and assumptions considered in this analysis include:

- As commercial maize acres have not fluctuated greatly over the last several years (Table VIII-1), acres devoted to the production of hybrid seed maize that support commercial production are not expected to change greatly. Therefore, total hybrid maize seed production acreage for MON 87427 would not exceed 0.5 M acres, or approximately 0.6% of the 75-93M acres of maize that have been routinely planted annually in the U.S. over the last ten years (USDA-NASS, 2010a).

- As determined by field based experimentation, applications of glyphosate to MON 87427 at approximately the V8 through V13 growth stages are effective in generating the male sterile phenotype through tissue-selective glyphosate tolerance in the female parent inbred. The glyphosate application rate on MON 87427 will not exceed six lbs a.e./acre, the maximum over-season application to maize permitted currently by the EPA.

- Under the authority granted by FIFRA, it is EPA's regulatory responsibility to assure that pesticides will not cause unreasonable adverse effects in the environment when used in accordance with the label. Proposed changes to the EPA glyphosate label due to the introduction of MON 87427 are discussed in Appendix K.

- For purposes of evaluating food, feed and environmental safety, there are no practical differences between MON 87427 containing hybrids used for grain production, and inbred maize lines used for seed production. In both instances hybrids and inbreds express the CP4 EPSPS protein and hybrid maize lines contain the genetic material from the parental inbred. Therefore, although glyphosate is applied to MON 87427 inbreds at a relatively later stage during hybrid seed production on limited acres, and MON 87427 hybrids may be treated with glyphosate at various time points for weed control on broad commercial acres, for safety purposes, the evaluation that was conducted on MON 87427 hybrids is appropriate and equally applicable to the inbreds.

I.5.1. Impacts on Land Use, Water Quality, and Climate

I.5.1.1 Approval in Whole Alternative

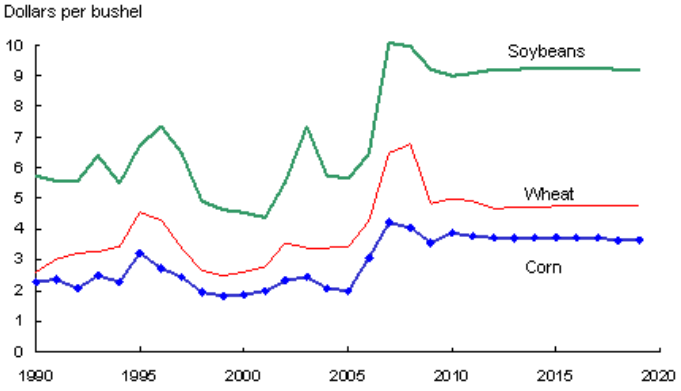
Land Use:

Glyphosate-tolerant maize, including inbreds and hybrids, have been deregulated and grown in the U.S. since 1997 (Table I-1), and hybrids with this trait currently occupy

approximately 80% of total maize commercial acres (Monsanto Company, 2009; USDA-NASS, 2010a). Although the use of MON 87427 will make hybrid maize seed production more efficient and cost effective, it does not confer advantages to commercial growers that would result in increased acres dedicated to maize grain production. If commercial maize acres are not expected to change, hybrid maize seed production acres would also not be expected to change following the introduction of this product.

Despite the rapid adoption of herbicide-tolerant maize products in the past decade, there has been no significant impact on total cropland acreage in the U.S. For example, from 2000 to 2006, the total annual commercial maize acres planted averaged approximately 79M with some minor fluctuations (Table VIII-1), while in the same time frame, the adoption rate for biotechnology-derived herbicide-tolerant maize increased from 25 to 61% (USDA-ERS, 2010a). Agricultural land use, and consequently both crop production and seed production, is dictated by many factors, the most significant of which are commodity prices. As demonstrated in 2007 and 2008, when maize commodity prices were unusually high, the acreage dedicated to maize commercial production was also high (Figure I-3). Accordingly, seed producers may increase acres dedicated to seed production to meet increased need, but they do so in response to commodity prices and market demand, not in response to availability or adoption of biotechnology-derived traits. Additionally, the presence of a tissue-selective glyphosate-tolerance trait in MON 87427 will not facilitate either seed or commercial grain production in areas where maize is not currently grown. It is not anticipated that the introduction of MON 87427 will significantly impact hybrid maize seed production and commercial acres, or have an impact on the geographical range where corn is grown, and thus will not have a significant impact on land use.

U.S. farm-level prices: Corn, wheat, and soybeans



Source: *USDA Agricultural Projections to 2019*, February 2010.
USDA, Economic Research Service.

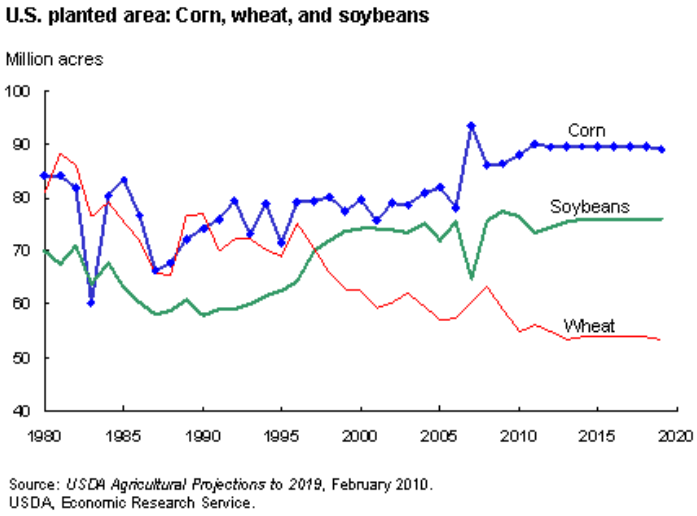


Figure I-3. Comparison of Maize Commodity Prices and Acres to Other Crops Planted in the U.S. Source: (USDA-ERS, 2010b)

Water Quality:

Water quality could be impacted either directly by MON 87427 via plant material impacts on water resources, or indirectly via impacts from the use of glyphosate or tillage practices associated with the planting of MON 87427. Conservation tillage, a system that leaves 30% or more of the previous crop residue covering the soil when planting another crop has been increasingly employed in commercial maize and hybrid maize seed production acres, and helps minimize any impacts of maize production on water quality by reducing soil erosion.

In terms of potential direct impacts on water quality, the CP4 EPSPS protein contained in MON 87427 is a member of the larger family of EPSPS proteins that are ubiquitous in plants and microbes in the environment (CaJacob, et al., 2004). The mode of action of this family of proteins is well known (Alibhai and Stallings, 2001) and the introduced CP4 EPSPS protein itself was derived from a common soil bacterium (*Agrobacterium sp.* strain CP4). The safety of CP4 EPSPS protein present in other glyphosate-tolerant crops has been extensively evaluated (Harrison, et al., 1996), and the U.S. EPA has granted a tolerance exemption for CP4 EPSPS. A history of safe use of CP4 EPSPS is supported by the lack of any documented reports of adverse effects since the introduction of Roundup Ready crops. Under full deregulation of MON 87427, current grower practices related to maize grain production, including weed control and tillage practices would not be altered, as greater than 80% of current maize acres contain the same glyphosate tolerance trait as in MON 87427. Therefore, it is unlikely that the presence of CP4 EPSPS protein in MON 87427 will have a significant impact on water quality.

Under full deregulation of MON 87427 hybrid seed producers would be able to use glyphosate in seed production fields, something they have not been able to do before since usually only one of the inbreds used in hybrid production contained the glyphosate-

tolerance trait. Glyphosate has been thoroughly reviewed by the U.S. EPA and has been determined to "not pose unreasonable risks or adverse effects to humans or the environment" (U.S. EPA, 1993)[8]. With the exception of two additional glyphosate applications at later stages of growth (approximately V8 through V13) to prevent viable pollen production, MON 87427 will be grown under the same conditions as other maize inbred plants used in hybrid seed production. Glyphosate has a proven history of safe use ((Giesy, et al., 2000; Williams, et al., 2000); Appendix K - Impact of Glyphosate on Human Health and the Environment), and its use for MON 87427 on hybrid maize seed production acres (0.6% of total maize acres) will not exceed the maximum over season application of six lbs a.e./acre on maize currently authorized by the EPA. In addition glyphosate is rapidly adsorbed and tightly complexed by soil particles and, even though it is highly water soluble, it does not leach into ground water in most soils. In intensely farmed areas, herbicides have often been found in surface waters due principally to rainfall runoff. With lower application rates, and greater soil sorptivity glyphosate is found at lower concentrations than other herbicides such as atrazine and alachlor, and it has been shown to dissipate more rapidly than other herbicides in surface water (Carpenter, et al., 2002; Cerdeira and Duke, 2006). Therefore, it is unlikely that the use of glyphosate on MON 87427 will have a significant impact on water quality.

Climate:

Conservation tillage is thought to minimize the impacts of agriculture on climate change. The EPA reports conservation tillage as an agricultural practice that "increases carbon storage through enhanced soil sequestration" and that "may reduce energy-related CO_2 emissions from farm equipment" (U.S. EPA, 2010). When carbon is stored, it is not available to be emitted in the form of carbon dioxide (CO_2), a greenhouse gas. Conservation tillage is already an option for growers on 80% of the commercial maize acres that have glyphosate-tolerance trait. Under full deregulation of MON 87427, current grower practices related to tillage in maize grain production would not be altered. Conservation tillage is also used broadly on hybrid maize seed production acres, and it is unlikely that deregulation of MON 87427 would cause seed producers to change this practice. Therefore, considering that tillage practices are unlikely to change with the introduction of MON 87427 and that only 0.6% of total maize acres would be directly affected, it is unlikely that MON 87427 would have a significant effect on the impact that agriculture has on climate change.

I.5.1.2. No Action Alternative

Under the no action alternative MON 87427 would remain a regulated article and would not be available to hybrid maize seed producers. Maize hybrid seed producers would continue to produce seed using current detasseling methods with no anticipated changes in seed production acres or land use. In terms of water quality and climate change,

[8] Glyphosate is currently in the process of EPA Registration Review during which an updated risk assessment will be conducted. Additional risk assessments for glyphosate have been conducted since 1993; some of these are described in Appendix L.

impacts to the environment for the no action alternative would not change from the current conditions and therefore, would not result in significant impacts.

As discussed above, both the approval in whole and no action alternatives would result in no significant changes to current land use, water quality or the impacts of agriculture on climate change.

I.5.2. Potential for Adjacent-Agricultural Crop and Non-agricultural Impacts

I.5.2.1. Approval in Whole Alternative

Apart from limited to no expression of the CP4 EPSPS protein in male reproductive tissues, MON 87427 is similar to other glyphosate-tolerant maize plants currently grown on approximately 80% of U.S. maize acres. MON 87427 displays no altered plant pest characteristics compared to conventional maize and is no more susceptible to insects or diseases that commonly infest maize. The potential for gene transfer from MON 87427 is limited to sexually compatible relatives (teosinte and gamma grass species). As discussed more completely in Sections I.5.8.2., and I.5.8.3., geographical separation, differences in developmental factors and the low reproductive capacity of the seeds resulting from crosses with sexually compatible wild species make the likelihood of survival and spread of MON 87427 and MON 87427-containing hybrids into adjacent-agricultural crop and non-agricultural areas negligible. Therefore, based on information presented in this petition on phenotypic and agronomic characteristics of MON 87427, and knowledge regarding the biology and characteristics of corn, it is concluded that MON 87427 would have no direct impacts to adjacent agricultural crops and non-agricultural vegetation.

Both MON 87427 and commercially grown glyphosate-tolerant maize are tolerant to the herbicide glyphosate due to the presence of the *cp4 epsps* coding sequence. Hence, with the exception of applications of glyphosate in later growth stages to induce the male sterile phenotype, through tissue-selective glyphosate tolerance, on limited (0.6% of total maize acres) hybrid maize seed production acres, herbicide applications on MON 87427 that may result in drift impacting adjacent-agricultural crop and non-agricultural lands would be comparable to those used on commercially grown maize, the majority of which contain the glyphosate tolerance trait.

Seed growers rely heavily on herbicides for effective weed control, since inbred maize lines do not compete effectively with weeds. Broadleaf herbicides and manual weed removal have been used in hybrid maize seed production but, prior to the introduction of MON 87427, glyphosate has not been used in hybrid maize seed production fields because not all inbreds were glyphosate-tolerant. With MON 87427 it is an option to use glyphosate for weed control as well as for pollination control in hybrid maize seed production acres. Glyphosate applications would be on very limited acres (no more than 0.6% of total maize acres), and any drift impacting adjacent-agricultural crop or non-agricultural lands would be comparable to that resulting from glyphosate use on existing commercial maize production acres.

Before the EPA can approve an herbicide for use, FIFRA requires that the agency to conclude that there are no unreasonable adverse effect to human health and the environment, including risks to non-target plants will occur from the authorized use of an herbicide. Glyphosate has been thoroughly reviewed by the EPA and has been determined to "not pose unreasonable risks or adverse effects to humans or the environment" (U.S. EPA, 1993). Herbicide product labels contain application and drift mitigation statements aimed at managing off-target movement impacts, and EPA considers the herbicide product label in its evaluation. Proposed changes to the glyphosate label due to the introduction of MON 87427 that have been submitted to EPA are discussed in Appendix K.

The utilization of MON 87427 in the production of hybrid maize seed will not alter the current practices used to produce maize seed, with the exception of an early glyphosate application to control weeds and additional late season applications of glyphosate to prevent viable pollen production. These applications will not exceed the six lbs a.e./acre maximum over-season application of glyphosate to maize currently permitted by the EPA. Thus it is anticipated that the introduction of MON 87427 will not result in significant indirect impacts to adjacent agricultural crops and non-agricultural vegetation due to the application of glyphosate.

I.5.2.2. No Action Alternative

Under the no action alternative MON 87427 would remain a regulated article and would not be available to hybrid maize seed producers. Maize hybrid seed producers would likely continue to use standard weed control methods including broadleaf herbicides that also have the potential to result in drift to adjacent-agricultural crop or non-agricultural lands. Under this alternative, the potential impact associated with the use of herbicides, including glyphosate, would be unchanged.

I.5.3. Potential Impacts to Agricultural Practices

I.5.3.1. Approval in Whole Alternative

MON 87427 has been shown to be no different from conventional maize in its agronomic and ecological characteristics (Sections VII, VIII and IX), and has the same levels of resistance to insects and diseases as conventional maize. Therefore, other than applications of glyphosate for weed control, and to produce the male sterile phenotype through tissue-selective glyphosate tolerance which will eliminate or greatly reduce the need for mechanical and manual detasseling, there are no other anticipated changes to current agricultural practices due to the introduction of MON 87427. A summary of agronomic practices for maize hybrid seed production is presented in Section VIII of this petition. Also, given that approximately 80% of all commercial maize acres in the U.S. currently are glyphosate-tolerant, agricultural practices are not anticipated to change for commercial acres under full deregulation of MON 87427.

With the deregulation of MON 87427 glyphosate could be used for weed control in seed production acres which may result in a decrease in the application of some currently used

herbicides. However Monsanto recommends, for weed control in hybrid seed production acres, that glyphosate is used in conjunction with pre-emergent herbicides to achieve the best possible control and mitigate concerns over the development of weed resistance (discussed in Appendix J). Two additional glyphosate applications at later stages of growth to induce a male sterile phenotype through tissue-selective glyphosate tolerance will replace or greatly reduce the need for mechanical and manual detasseling. Aside from glyphosate applications, MON 87427 will be grown using the same agricultural inputs as current maize inbreds used in hybrid maize seed production.

I.5.3.2. No Action Alternative

Under the no action alternative MON 87427 would remain a regulated article and would not be available to hybrid maize seed producers. Maize hybrid seed producers would continue to use standard methods including broadleaf herbicides to control weeds and glyphosate would not be applied for either weed or pollination control in hybrid maize seed production acres. Both mechanical and manual detasseling would continue to be the primary form of pollen control and other agricultural inputs and practices would remain the same.

I.5.4. Potential Impact to Commercial Maize Production

I.5.4.1. Approval in Whole Alternative

Biotechnology improved crops are subject to regulation in many countries. In order that maize grain harvested in the U.S. may be freely traded, Monsanto will seek regulatory approval for MON 87427 and its combinations with other biotechnology-derived traits, where required, in all key maize import countries with a functioning regulatory system to support the flow of international trade (Section VIII.I.). Monsanto adheres to the BIO Product Launch Policy[9] including: 1) conducting a market and trade assessment, 2) securing regulatory approvals in key export countries prior to full commercial launch, 3) following generally accepted best seed management practices to prevent unintended low level presence of the event in seed, 4) providing reliable detection methods to growers, processors and buyers prior to commercialization, and 5) communicating to stakeholders the company's product launch stewardship policies. These actions protect against adverse impacts to trade of maize due to the introduction of new biotechnology-derived maize.

An additional consideration is the possibility of an impact on the value of grower's crops due to potential gene transfer in the field. Multiple biotechnology-derived maize products have been introduced and commercialized since 1996, and growers have developed practices to allow for production of a crop to meet customer expectations. Recall that MON 87427-containing inbred lines will not be producing pollen when treated at later growth stages with glyphosate, and therefore the potential for gene transfer to commercial maize during seed production is extremely low. Thus, the introduction of

[9] BIO's Product Launch guidelines can be found at: http://www.excellencethroughstewardship.org/facts/documents/Guide%20for%20Product%20Launch%20Stewardship.pdf.

MON 87427 is not expected to significantly impact commercial maize production due to gene movement from MON 87427 to neighboring maize crops should APHIS grant nonregulated status to MON 87427.

I.5.4.2. No Action Alternative

Under the no action alternative MON 87427 would remain a regulated article and would not be available to seed producers. Growers would continue to use systems that have been developed to maintain genetic identity during commercial production. Therefore, the no action alternative and the approval in whole alternative would not differ in their impact to commercial maize production. The majority of commercial maize grown in the U.S. is already glyphosate-tolerant, and company stewardship policies described above ensure there will be no disruption of trade from the introduction of new biotechnology-derived products.

I.5.5. Potential Impact to Certified Seed Production

I.5.5.1. Approval in Whole Alternative

Certified seed production is a carefully managed process (Section VIII.B.2) for maintaining high quality seed stocks, an essential basis for U.S. agriculture. Seed producers have learned to account for and manage pollen flow both within a seed production field and between nearby fields. For several decades the hybrid maize seed industry has created and adopted systems to maintain and preserve the purity of maize germplasm developed for commodity and specialty uses. To maintain the genetic purity of hybrid maize populations, seed production activities for each maize type are isolated from one another and from commercial grain production (Wych, 1988). Isolation is achieved through various means, but may include physical separation to prevent cross pollination, temporal isolation by planting at different times to stagger pollination times of different materials, detasseling, and the use of cytoplasmic male sterility.

The goal of detasseling and CMS is to produce hybrid seed that meets the necessary purity for hybrid maize seed. Seed must meet state and federal seed standards and labeling requirements. AOSCA is dedicated to assisting companies in the production, identification, distribution and promotion of certified classes of seed. AOSCA establishes minimum standards for quality and identity. Its goal is to standardize certification regulations and procedures internationally so companies compete with one set of standards. The association cooperates with the OECD and other international organizations to develop standards, regulations, procedures, and policies to expedite movement of seed and encourage international commerce in improved seed products. The AOSCA standards for maize seed are described in Section I.3.5. MON 87427 meets or exceeds established seed purity standards (Feng, et al., 2009). Thus, adoption of MON 87427 is not expected to have a significant impact on production of certified hybrid maize seed.

It is anticipated that hybrid seed containing MON 87427 will be produced and marketed in accordance with OECD and AOSCA standards and the U.S. Federal Seed Act, and will

have no adverse impact on current hybrid seed production practices or the ability of breeders and seed producers to meet these standards.

MON 87427 provides an option for producing viable hybrid maize seed as an alternative to detasseling or the use of a CMS systems, using minimal additional agricultural inputs, i.e. late stage applications of glyphosate, (Section I.5.3.). With the introduction of MON 87427, glyphosate can be applied to hybrid maize seed production fields in early growth stage applications as part of the weed control system and applied at later vegetative growth stages (V8 through V13) to produce the male sterile phenotype through tissue-selective glyphosate tolerance in the female parent inbred line. This has not been possible before as usually only one of the inbred lines contained the trait for glyphosate tolerance.

These late stage glyphosate applications will be made only on hybrid maize seed production acres that comprise 0.6% of total maize acres in the U.S. Currently registered uses of glyphosate do "not pose unreasonable risks or adverse effects to humans or the environment" as determined by the EPA (U.S. EPA, 1993). Glyphosate has been authorized by the EPA for in-season, post-emergent use in a variety of crops (U.S. EPA, 1993). Additionally, the EPA's evaluation of glyphosate use on glyphosate-tolerant maize covers all uses in maize to a maximum amount of six pounds acid equivalent per acre (lbs a.e./acre). This rate will not be exceeded in MON 87427 even with the anticipated additional applications of glyphosate at the V8 through V13 growth stage or in hybrid seed production using this trait (Roundup PowerMAX Herbicide, 2007; 2008; Roundup WeatherMAX Herbicide, 2002; 2009).

The safety of CP4 EPSPS protein present in glyphosate-tolerant crops has been extensively evaluated (Harrison et al., 1996). The U.S. EPA has also reviewed the safety of the CP4 EPSPS protein and has established a tolerance exemption for the protein and the genetic material necessary for its production in or on all raw agricultural commodities (40 CFR § 174.523). A history of safe use ((Giesy et al., 2000; Williams et al., 2000); Appendix K – Impact of Glyphosate on Human Health and the Environment) is supported by the lack of any documented reports of adverse effects attributed to the EPSPS protein since the introduction of other Roundup Ready® crops. Therefore, it is not anticipated that the application of glyphosate to hybrid maize seed production acres will significantly impact the production of certified hybrid maize seed.

I.5.5.2. No Action Alternative

Under the no action alternative MON 87427 would remain a regulated article and would not be available to seed producers. Under the no action alternative maize hybrid seed producers would continue to produce seed using hand and mechanical detasseling methods. As direct and associated labor costs continue to increase, (Section VIII.C.) hybrid seed production costs are also likely to increase.

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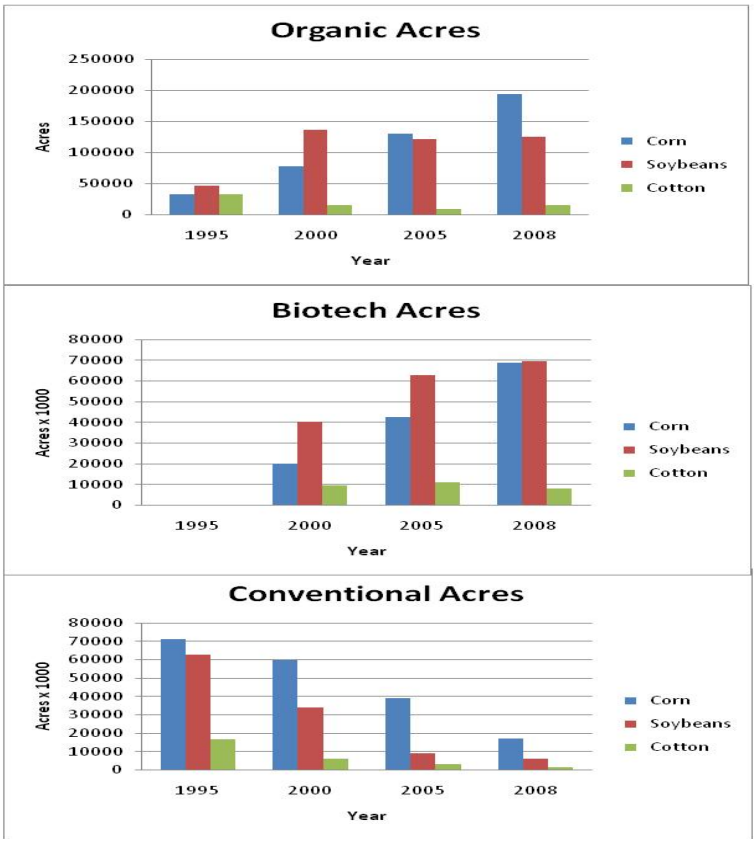
I.5.6. Potential Impacts to Organic Maize Production

I.5.6.1. Approval in Whole Alternative

Production systems designed prior to the introduction of MON 87427 or even prior to the introduction of biotechnology-derived maize have allowed for production of maize to meet varied customer demands. Organic maize producers use production practices designed to specifically avoid the presence of maize products that use herbicides or other pesticide treatments, as well as biotechnology-derived crops. These well established practices to avoid "excluded methods" will continue with the introduction of MON 87427. These practices include isolation zones, use of buffer rows surrounding the organic crop, adjusted planting dates and varietal selection (Born, 2005). Hence, organic or conventional maize producers can and have effectively implemented practices (e.g., isolation during the growing season, equipment cleaning during harvest, and post-harvest separation of harvested seed) that allow them to avoid the presence of biotechnology-derived maize and maintain organic production status. Also, recall that MON 87427 will be planted only on hybrid maize seed production acres (not exceeding 0.5M acres) or 0.6% of total maize acres in the U.S.

Despite the high adoption rate of biotechnology-derived glyphosate tolerant maize, organic crop production has been one of the fastest growing segments of U.S. agriculture for the past decade (Figure I-4). For example, from 2000 to 2006 the organic maize acreage increased by approximately 200% in the U.S. (USDA-ERS, 2010c) while during the same time frame the biotechnology-derived maize acreage increased from 25 to 61% of total U.S. maize acreage (USDA-ERS, 2010a). Currently approximately 0.25% of all maize grown in the U.S. is certified as organic (USDA-ERS, 2010c).

These national statistics suggest that the adoption of biotechnology-derived maize did not have a significant adverse effect on organic maize production. Nonbiotechnology-derived maize seed is currently available from numerous seed suppliers (Table I-2). Additional information on organic seed sources may be found at www.omri.org and http://attra.ncat.org/attra-pub/organic_seed/. Thus, growers have a choice in the maize variety they plant, and this is not expected to change with the introduction of MON 87427.



Source: (USDA-NASS, 2010b)

Figure I-4. Increase of Organic Acres Since the Introduction of Biotechnology-derived Product in 1995.

Table I-2. Organic and Conventional Maize Seed Sources

Organic Maize Seed Sources[1]	*Conventional Maize Seed Sources*
Albert Lea Seed House	Garst Seed[2]
Blue River Hybrids	Heirloom Seed[3]
Golden Grains	Kruger Seed[4]
Great Harvest Organics	Monsanto (DeKalb)[5]
Merit Seeds	Pioneer[6]

[1]http://www.organicgrains.ncsu.edu/
[2]http://www.garstseed.com/GarstClient/Products/Corn/
[3]http://www.heirloomseeds.com/corn.htm
[4]http://www.krugerseed.com/index.php
[5]http://www.asgrowanddekalb.com/seedresourceguide/search/seeds
[6]http://www.pioneer.com/web/site/portal/

I.5.6.2. No Action Alternative

Under the no action alternative MON 87427 would remain a regulated article and would not be available to seed producers. There would be no change in the current conditions from the introduction of MON 87427 and organic growers would continue to manage their production fields to avoid excluded methods including drift from pesticides. Therefore, the no action alternative and the approval in whole alternative would not differ in their impact to organic maize production.

I.5.7. Potential Impacts to Raw or Processed Agricultural Commodities

I.5.7.1. Approval in Whole Alternative

Within this petition, extensive data have been presented relating to plant growth parameters, disease susceptibility, insect susceptibility, and forage and grain composition of MON 87427 compared to conventional maize hybrids. These data indicate that there are no biologically relevant differences between MON 87427 and conventional maize, except for tissue-selective tolerance to glyphosate in vegetative and female reproductive tissues. Biotechnology-derived maize products like MON 87427 undergo a voluntary food and feed consultation process with the FDA prior to release on the market. Monsanto has already initiated this process and will complete the consultation prior to a commercial introduction of MON 87427.

Compositional assessments (Section VI) conducted on grain and forage support a conclusion that the composition of the forage and grain of MON 87427 is equivalent to that of the conventional control. The genetic modification in MON 87427, has no impact on the composition, and therefore on the food and feed safety or nutritional quality of this product compared to conventional maize.

Based on residue studies with the proposed late vegetative growth stage glyphosate use pattern for MON 87427, glyphosate residue tolerances on grain from MON 87427 do not need to change from the current residue tolerance of 5 ppm for commodity maize grain. Glyphosate residues on forage have increased from 6 to 13 ppm, due to the application of glyphosate at later plant growth stages. Monsanto has submitted an application to EPA to raise the glyphosate tolerance on forage accordingly. This is not a significant increase as there are higher tolerances for glyphosate on other animal feed crops such as the 400 ppm tolerance on non-grass animal feed and alfalfa hay. Also, recall that the new glyphosate use pattern will be utilized only on maize seed production acres (0.6% of total maize acres in the U.S.). Based on this information it is unlikely that the deregulation of MON 87427 would cause a significant impact on either raw or processed maize commodities.

I.5.7.2. No Action Alternative

Under the no action alternative MON 87427 would remain a regulated article and would not be available to seed producers or for use as food and feed. Hybrid maize seed

producers would continue to use other herbicides and other biotechnology-derived inbreds with glyphosate tolerance to produce glyphosate-tolerant seed on relatively small hybrid maize seed production acres. Therefore, the no action and approval in whole alternatives are similar regarding their impacts to raw and processed agricultural commodities.

I.5.8. Potential Impacts to Human Health and Worker Safety

Prior to the introduction of a biotechnology-derived crop product to the marketplace, Monsanto conducts tests to assure that the product is as safe as its conventional counterpart under the intended use conditions. Biotechnology-derived crops for food and feed use undergo a voluntary consultation process with the FDA prior to release onto the market. Although a voluntary process, Monsanto routinely completes a consultation with the FDA prior to placing a new biotechnology derived crop product on the market. Monsanto will complete the FDA consultation process prior to introduction of MON 87427. A list of completed consultations on biotechnology-derived crop products is available on the FDA website at: http://www.fda.gov/Food/Biotechnology/Submissions/default.htm

Under the Federal Food, Drug, and Cosmetic Act (FFDCA), pesticide residues in or on raw agricultural commodities or processed foods are allowed only after a tolerance or exemption from tolerance has been established. Residue tolerances and exemptions for pesticides are established by EPA under the FFDCA. The FDA enforces the tolerances set by the EPA. EPA also reviews the proposed use pattern for all herbicides and prior to approval and placement on herbicide labels determines that no unreasonable risk exists for the environment.

Currently, tolerances exist for glyphosate residues on maize grain at 5 parts per million (ppm) and maize forage at 6 ppm (40 CFR § 180.364) which are based on the approved post-emergence glyphosate use on glyphosate-tolerant maize for weed control. As previously mentioned, Monsanto has submitted an application to EPA to register a new use pattern for glyphosate on MON 87427 in seed corn production that will allow for post emergent applications of glyphosate just prior and/or during tassel development stages (approximate maize vegetative growth stages ranging from V8 to V13) resulting in the formation of a male sterile phenotype for hybrid maize seed production, through tissue-selective tolerance to glyphosate. Glyphosate residue data generated on MON 87427 to support this new use pattern show low levels of glyphosate residues on maize grain (less than 1 ppm) and confirm the current maize grain tolerance of 5 ppm is adequate for MON 87427. Monsanto has petitioned the EPA to raise the glyphosate tolerance for maize forage from 6 ppm to 13 ppm, based on the results of the residue study for the new use pattern of the herbicide on seed production acres.

Under full deregulation, MON 87427 inbreds could be grown on limited hybrid seed production acres, and MON 87427-containing hybrids could be grown across the U.S. Grain and forage produced from MON 87427 would enter the food and feed chain and would be consumed by humans and animals. The impacts associated with the

introduction of MON 87427 and later growth stage applications of glyphosate on limited hybrid seed production acres to human health are discussed below.

I.5.8.1. Human Health

I.5.8.1.1. Approval in Whole Alternative

MON 87427 expresses the CP4 EPSPS protein in vegetative and female reproductive tissues, conferring tolerance to glyphosate in those tissues. The CP4 EPSPS protein is structurally homologous to EPSPS proteins that are part of the amino acid synthesis pathway of all plants.

MON 87427 was developed through *Agrobacterium*-mediated transformation of maize meristem tissue using the binary transformation plasmid PV-ZMAP1043 (Section III; Figure III-1, and Table III-1). MON 87427 contains one copy of the insert at a single integration locus. No additional genetic elements from the transformation vector were detected in the genome of MON 87427, including backbone sequence from plasmid PV-ZMAP1043. On the basis of these data, it is concluded that only the expected CP4 EPSPS protein is produced from the inserted DNA.

The safety of CP4 EPSPS protein present in multiple biotechnology-derived crops has been extensively evaluated (Harrison et al., 1996). The EPA has also reviewed the safety of the CP4 EPSPS protein and has established a tolerance exemption for the protein and the genetic material necessary for its production in or on all raw agricultural commodities (40 CFR § 174.523). This exemption was based on a safety assessment that demonstrated rapid digestion in simulated gastric fluids, lack of homology to known toxins and allergens, and lack of toxicity in an acute oral mouse gavage study. A history of safe use is supported by the lack of any documented reports of adverse effects since the introduction of the first Roundup Ready crop in 1996.

Compositional equivalence between maize improved through biotechnology-derived traits and conventional hybrids provides an "approach to safety assessment based on substantial equivalence as being the most practical approach to addressing the safety of foods and food components derived through modern biotechnology" (OECD, 2002). Compositional analyses of forage and grain from MON 87427 were conducted to assess the levels of nutrients, anti-nutrients, and secondary metabolites for comparison to conventional maize. These results, based on evaluation of 78 different components confirmed that the forage and grain derived from MON 87427 is compositionally and nutritionally equivalent to those derived from conventional maize with a history of safe consumption (Section VI). As such, based on the safety of the CP4 EPSPS protein and the compositional equivalence of MON 87427 to conventional maize hybrids, it can be concluded with reasonable certainty that dietary exposure to MON 87427 poses no meaningful risk to humans.

Glyphosate has a complete and comprehensive regulatory data base (toxicity, environmental fate, and ecological toxicity) that has been evaluated by EPA to support all currently approved uses including glyphosate-tolerant maize. The EPA has stated that it

has a high level of confidence in the quality of the existing studies and the reliability of the toxicity endpoints that are the basis for risk assessment of glyphosate (U.S. EPA, 2006a; b). In establishing food and feed tolerances to support the use of glyphosate on crops used for animal feed and forage, the EPA noted that it had conducted "a complete and thorough review of the available data for glyphosate," and determined that "glyphosate will not pose unreasonable risks or adverse effects to humans or the environment" (U.S. EPA, 2002). A worst case risk assessment of food and food ingredients derived from crops treated with glyphosate concludes that human dietary exposure and risk are minimal and that glyphosate is not a carcinogen, nor does it cause mutations (U.S. EPA, 1993). Food and feed tolerances have been established in the U.S. for glyphosate residues since the early 1980s, and glyphosate has successfully completed the re-registration process, as required for all pesticides registered before 1984. Additionally, glyphosate has been approved by the EPA for food and feed uses associated with glyphosate-tolerant crops, including glyphosate-tolerant maize. Based on the thorough review by the EPA, the lack of toxicity associated with glyphosate and that glyphosate use on MON 87427 will not exceed the six lbs a.e./acre, the maximum over-season application to maize permitted currently by the EPA, it can be concluded that deregulation of MON 87427 and associated glyphosate use poses no meaningful risk to humans.

I.5.8.1.2. No Action Alternative

Under the no action alternative MON 87427 would remain a regulated article and would not be available to seed producers or for use as food and feed. Growers would continue to use previously deregulated glyphosate-tolerant maize products on millions of acres devoted to commercial grain production, and continue to treat these acres with glyphosate herbicides. Therefore, the no action alternative and the approval in whole alternative would not differ in their impact on human health.

I.5.8.2. Worker Safety

I.5.8.2.1. Approval in Whole Alternative

Prior to the development of MON 87427, glyphosate has not been used in hybrid maize seed production fields because not all inbreds were glyphosate-tolerant. With the introduction of MON 87427, female parent inbreds with tissue-selective glyphosate-tolerance in vegetative and female reproductive tissues and male parent inbreds with glyphosate tolerance from a previously deregulated product (e.g. Roundup Ready corn 2 event NK603) will be used for the production of hybrid seed. Therefore, it is anticipated that two glyphosate applications at early tassel development stages (approximately V8 through V13 growth stages) will be used to induce a male sterile phenotype through tissue-selective glyphosate tolerance in the MON 87427-containing inbred lines. Additionally, the use of glyphosate at earlier growth stages (prior to V8 stage) in hybrid maize seed production fields for weed control will be an option if the EPA approves proposed label changes, and has been considered in terms of total glyphosate application. Applications of glyphosate in hybrid seed production fields will not exceed the

established rate for season-long over the top applications of six lbs a.e./acre as proscribed by the EPA.

Currently, there is no farm worker exposure to glyphosate in hybrid maize seed production fields. With the introduction of MON 87427 there exists the potential for farm worker glyphosate exposure in hybrid seed production fields due to glyphosate applications for weed control applied at relatively early growth stages as directed on the Roundup agricultural product label followed by later growth stage (V8 through V13) applications of the herbicide to induce the male sterile phenotype through tissue-selective glyphosate tolerance. Glyphosate has low acute toxicity and an absence of other toxicological concerns, (U.S. EPA, 1993) and should not pose significant issues in terms of farm worker safety during application. Some glyphosate herbicide formulations do cause eye and/or skin irritation from splashes during mixing and loading or from spray applications that can be avoided by the use of personal protective equipment (i.e. long-sleeved shirts, pants, shoes, socks, gloves, etc.).

Depending on the glyphosate formulation, the reentry interval into a field that has been treated with glyphosate is four to twelve hours as proscribed by the EPA. Some farm workers will still be required to walk the fields containing MON 87427 to confirm the efficacy of glyphosate-induced male sterility and perform manual detasseling if needed. The number of farm workers scouting MON 87427 seed production fields would be many fewer than would be required for detasseling. The scouting would occur two to three weeks after the last glyphosate treatment. Therefore it is anticipated that farm worker exposure to glyphosate will be minimal, will occur beyond the glyphosate-treated field reentry intervals established by the EPA, and should not present a significant impact to farm worker safety.

I.5.8.2.2. No Action Alternative

Under the no action alternative MON 87427 would remain a regulated article and would not be available to seed producers. Maize hybrid seed producers would continue to produce seed using manual and mechanical detasseling methods with conventional and S-*cms* and C-*cms* inbreds, and potential for worker injury associated with these activities would remain. Manual detasseling can be labor intensive and pose safety issues for farm workers. From working in the field during the hot summer months varying degrees of dehydration can occur, dermatological reactions (maize rash) are not uncommon, and the detasseling activity itself is repetitive giving rise to concerns over ergonomic injury. Monsanto has been extremely proactive in providing seasonal farm workers with safety training and personal protective equipment to avoid serious injury, with the result that recordable injuries have been approximately 0.5% for the last several years and consist primarily of sprains and strains. However the potential for injury remains especially with such a large seasonal work force.

Also, in terms of worker safety, insecticides, fungicides and post emergent herbicides would continue to be used in hybrid seed fields prior to the VT stage when the last branch of the tassel is completely visible, and the silks have not yet emerged, which is desirable in seed production to improve inbred plant health and yield potential. Seed producers

train all employees, including seasonal workers, in the Worker Protection Standard (40 CFR § 170) that restricts field re-entry to specified intervals following chemical applications. However, under the no action alternative, large numbers of workers would continue to enter fields to perform manual detasseling with possible exposure to previously applied pesticides, although this would usually be beyond the reentry intervals established for those pesticides.

I.5.9. Associated Potential Economic Impacts

I.5.9.1. Approval in Whole Alternative

The use of MON 87427-containing inbred lines is the most recent technological improvement in an ongoing effort to minimize time constraints, improve the efficiency and reduce the costs of manual detasseling. The first commercial maize hybrids were produced in the U.S. in the 1920s and were quickly accepted in the 1930s and 1940s (Wych, 1988). The large-scale introduction of hybrid seed production required thousands of seasonal workers for detasseling which typically lasts from two to four weeks. However, it was recognized that manual detasseling posed logistical and cost concerns, and in the early 1950s CMS technology was introduced. CMS varieties did not always require detasseling, and became the dominant form of pollen control in U.S. hybrid maize seed production (Craig, 1977) until 1970 when a Southern maize leaf blight epidemic exposed the susceptibility of the CMS germplasm in widespread use to this disease. As a result, there was a resumption of some manual detasseling as a method of pollination control and the introduction of mechanical detasselers in the 1970s (Craig, 1977) to address cost concerns. Subsequent improvements to mechanical detasselers (i.e. wheel pullers) to improve efficiency have further reduced the need for manual detasseling of plants in the field.

In 2008 there were approximately 800,000 agricultural workers in the U.S. including migrant workers who move from job to job as required to support crop production and harvesting (USBLS, 2010). According to the U.S. Bureau of Labor Statistics, overall employment for agricultural workers is expected to show little or no change in the next decade (Table I-3). Some slight decline is anticipated because of continued consolidation of farms and technological advancements in farm equipment that is raising output per farm worker. However, job openings in the agricultural sector should be plentiful particularly for crop, greenhouse and nursery farm workers, many of whose jobs are seasonal, because of the relatively large numbers of workers who leave these jobs for other occupations (USBLS, 2010).

Table I-3. Anticipated Change in U.S. Agricultural Worker Employment for the Next Decade

Occupation	2008 Employment	2018 Projected Employment	2008-2018 Change (#)	2008-2018 Change (%)
Agricultural Workers	807,000	788,800	-18,200	-2%

Source: (USBLS, 2010).

During hybrid maize seed production, Monsanto contracts for the services of approximately 10,000 agricultural workers for roughly four weeks (or approximately 0.1% of the agricultural work force annualized) to detassel maize in a combined manual and mechanized detasseling operation. The Monsanto detasseling work force is comprised of 70% teenagers and 30% migrant farm workers (Patrick Geneser, Monsanto Migrant Seasonal Labor Manager, personal communication). Recent publications place detasseling costs anywhere from USD $130 per acre using a combination of mechanical and manual detasseling, to USD $200 per acre with manual detasseling (Koetters, 2007).

It is anticipated that introduction of MON 87427 will reduce Monsanto's detasseling work force from 10,000 to approximately 500 to 1,000 seasonal workers for four weeks (representing a decline from 0.1% to approximately 0.01% of the agricultural work force annualized), which is a very small percentage of the total agricultural workers in the U.S. Thus, the introduction of MON 87427 is unlikely to have a significant impact on overall agricultural worker employment.

I.5.9.2. No Action Alternative

Under the no action alternative MON 87427 would remain a regulated article and would not be available to seed producers who would continue to employ seasonal labor for hand and mechanical detasseling. Economic impacts to U.S. agricultural labor would not be significantly impacted from not having MON 87427 available for seed production. However, as detasseling costs continue to increase, the cost of hybrid maize seed production will continue to increase, and less costly alternatives will continue to be sought.

I.5.10. Potential Impacts to Plant, Animal and Microbial Communities Including Threatened or Endangered Species and Biodiversity

The following section addresses potential impacts due to deregulation of MON 87427 and the application of glyphosate in hybrid maze seed production fields as a related activity on plant and animal communities, including soil organisms.

I.5.10.1. Animals

I.5.10.1.1. Approval in Whole Alternative

MON 87427 expresses the CP4 EPSPS protein in vegetative and female reproductive tissues, conferring tolerance to glyphosate, which is the active ingredient in the Roundup® family of agricultural herbicides. CP4 EPSPS is structurally homologous to other EPSPS proteins that play an important role in the biosynthesis of amino acid plants (Devine and Preston, 2000). The safety of CP4 EPSPS protein present in biotechnology-derived crops has been extensively evaluated (Harrison et al., 1996) and reviewed by the EPA which established a tolerance exemption for the protein and the genetic material necessary for its production in or on all raw agricultural commodities (40 CFR §

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174.523). A history of safe use is supported by the lack of any documented reports of adverse effects since the introduction of the first Roundup Ready crop in 1996.

Even though CP4 EPSPS protein is not known to have adverse effects on pest or non-pest organisms, a number of studies have been conducted to examine the potential effects of Roundup Ready crops to various pest or non-pest organisms (Goldstein, 2003; Jamornmarn, et al., 2004; Jasinski, et al., 2003; McPherson, et al., 2003). Representative soil organisms, beneficial arthropods and pest species were exposed to seed and foliage tissues from Roundup Ready crops. These studies, although varying in design, all reported a lack of toxicity observed in various species exposed to Roundup Ready crops producing the CP4 EPSPS protein. These results are consistent with the data generated for MON 87427, and support the conclusion that MON 87427 is not likely to have a significant impact on animals interacting with MON 87427 compared to conventional maize.

Furthermore, the composition of the grain and forage produced by hybrids containing MON 87427 is comparable from conventional maize (Section VI). This information indicates that there would be no negative effects to mammals that forage on MON 87427. Similarly, it is expected that there would be no impact to birds or other animals that may consume MON 87427 forage or grain. During field trials no changes in insect feeding damage were observed (Section VII.D.) indicating similar insect susceptibility for MON 87427 compared to conventional maize. Additionally, in a quantitative assessment of pest and beneficial arthropod abundance, no statistically significant differences were detected between MON 87427 and the conventional control. As MON 87427-containing hybrids exhibit no toxic effects on animals or pollinators of other plants in or around fields cultivated with MON 87427, it is unlikely insects and animals will be significantly affected.

As noted above, the maximum individual and season-long application rates for glyphosate over the top of glyphosate tolerant maize as proscribed by the EPA are 1.25 lbs a.e./acre and six lbs a.e./acre, respectively. Applications of glyphosate in hybrid seed production fields with MON 87427 will not exceed these established rates. A comprehensive human safety evaluation and risk assessment concluded that glyphosate has low toxicity to mammals, is not a carcinogen, does not adversely affect reproduction and development, and does not bioaccumulate in mammals ((Williams et al., 2000); Appendix K - Impact of Glyphosate on Human Health and the Environment). An ecotoxicological risk assessment (Giesy et al., 2000) and the EPA (U.S. EPA, 1993) concluded that the use of glyphosate does not pose an unreasonable risk of adverse effects to non-target species, such as birds and fish, when used according to label directions, nor does it pose an unreasonable risk of adverse effects to insects outside of the application area. Therefore, deregulation of MON 87427 may have minimal indirect effects on animals, insects and plants that live near or in MON 87427 seed production fields due to the application of glyphosate. However, these effects would be no different from the effects on 80% of maize acres that are glyphosate-tolerant that are already treated with glyphosate in the U.S.

I.5.10.1.2. No Action Alternative

Under the no action alternative MON 87427 would remain a regulated article and would not be available to seed producers. Hybrid maize seed producers would continue to use other herbicides and other biotechnology-derived inbreds with glyphosate tolerance to produce glyphosate-tolerant seed on relatively small hybrid maize seed production acres. Therefore, the no action and approval in whole alternatives are similar regarding their impacts to animal communities.

I.5.10.2. Plants

I.5.10.2.1. Approval in Whole Alternative

The potential for MON 87427 to impact nearby vegetation is related to its weediness potential, which could result in the uncontrolled spread into surrounding environments, as well as its ability to interbreed with nearby sexually compatible plants. In addition, off-target movement from spray or vapor drift occurs with all herbicide applications, and effects on non-target plants do occasionally occur as a result of their use. The degree of injury to non-target plants that may occur from off-target movement is dependent on the sensitivity of the plant to the herbicide; however these impacts can be minimized through good management practices such as decreasing spray pressure, lowering boom height, increasing nozzle size, avoiding making applications during high winds, etc. (Jordan et al., 2009; University of Illinois, 2010).

Modern maize cannot survive as a weed due to intense selection during domestication, in which traits often associated with weediness such as seed dormancy, dispersal mechanisms, or the ability to form reproducing populations outside of cultivation have not been selected. Consequently maize is not capable of surviving without human assistance (Baker, 1965; Galinat, 1988; Keeler, 1989) and all maize hybrids, including those containing MON 87427, have extremely low potential for weediness. Even when individual kernels of maize are distributed within a field or along transportation routes from the fields to storage or processing facilities, sustainable, volunteer maize populations are not found growing in fence rows, ditches, or road sides.

In comparative studies conducted between MON 87427 and a conventional control, dormancy and germination, growth and development, and reproductive characteristics were evaluated for changes that would impact plant pest potential, and in particular, plant weediness potential. No meaningful differences were detected between MON 87427 and conventional control (Section VII). These data indicate that MON 87427 exhibits no characteristics that would improve the ability of this maize to survive without human intervention, and that its cultivation should not interfere with the cultivation of other maize hybrids or result in its uncontrolled spread into non-agricultural environments. Thus, the results support a conclusion of no increased weediness potential of MON 87427 compared to conventional maize.

In assessing the potential impact of MON 87427 on plant communities, the potential for gene movement and introgression from MON 87427 was evaluated because movement

and establishment of the gene and trait to related species could have indirect impacts to plant communities that extend beyond the original recipient organism. Two primary issues have been considered: 1) the potential for gene transfer and introgression: and 2) the potential impact of introgression.

The potential for gene transfer from MON 87427 is limited to sexually compatible relatives such as other *Zea* species or members of the *Tripsacum* genus. Monsanto is aware of no reports of the transfer of genetic material from maize to other species with which maize cannot sexually interbreed.

Maize and annual teosinte (*Zea mexicana*), perennial teosinte (*Zea perennis*) and *Zea mays* subsp. *parviglumis* are genetically compatible, wind-pollinated and may hybridize when in close proximity to each other (Ellstrand, et al., 2007; OECD, 2003; Wilkes, 1967). Small feral populations of annual teosinte (*Zea mexicana*) are found in Florida, Alabama and Maryland while perennial teosinte (*Zea perennis*) is found only in South Carolina and *Zea mays* ssp. *parviglumis* is found only in Florida (USDA-NRCS, 2010). Gene introgression from MON 87427 to wild *Zea* species is unlikely to occur in these regions as limited quantities of maize seed are produced in the southern states due to high temperatures during pollination, inadequate rainfall during the growing season, and a higher incidence of insects and diseases (C. Peters, Monsanto, Global Operations, personal communication, 2010).

Under field conditions teosinte subspecies normally flower later than cultivated maize (Wilkes, 1967), limiting the opportunity for gene transfer. Research (Kermicle and Allen, 1990) has shown that maize can introgress to teosinte; however, it was determined that gene transfer under natural conditions was largely from teosinte to maize (Baltazar, et al., 2005; Evans and Kermicle, 2001). Additionally, there is incompatibility between some maize populations and certain types of teosinte resulting in low fitness of hybrids that prevents a higher rate of introgression (Evans and Kermicle, 2001). In an experimental field study where maize and teosinte species were planted together (Ellstrand et al., 2007), low hybridization rates (less than 1%) were observed for maize and *Zea mexicana.* These first generation maize-teosinte hybrids are generally less fit for survival and dissemination, and they show significantly reduced reproductive capacity (Baltazar et al., 2005). Temporal and geographical separation present barriers to genetic transfer among *Zea* related species as well as lack of hybrid vigor. Thus, genetic transfer of MON 87427 into related *Zea* species is highly unlikely in the U.S.

It is only with extreme difficulty and special techniques that maize and *Tripsacum sp*. hybridize (Russell and Hallauer, 1980). Moreover, the offspring of these crosses show varying levels of sterility (Galinat, 1988; Russell and Hallauer, 1980). Given the level of difficulty for natural hybridization between species of *Tripsacum* and *Zea*, it is very unlikely there would be any genetic transfer to *Tripsacum* due to the introduction of MON 87427

Based on the data and information presented in this petition, (Section IX) it is demonstrated that MON 87427 is highly unlikely to be a plant pest or to have increased weediness potential compared to conventional maize. Nor would MON 87427 be

considered a "noxious weed" as it has no potential to cause direct injury or damage (physical harm) to any protected interest. Therefore, in the unlikely event that gene transfer from MON 87427 to a teosinte or *Tripsacum* species were to occur, no increases in the weediness potential of these species is anticipated.

Introduction of MON 87427 will result in the use of glyphosate in hybrid maize seed production fields (not to exceed 0.5 M total U.S. acres) where it has not been previously used in-crop. It will also result in a new use pattern for glyphosate, that is, applications at later plant growth stages. Glyphosate is a non-selective herbicide with activity on a large number of annual and perennial plants. As such, exposure to glyphosate could put emergent aquatic plants and terrestrial non-target plants as well as threatened and endangered plants at risk (U.S. EPA, 1993). Non-target plants may potentially be at risk from applications of glyphosate as a result of spray drift. Off-target movement can occur with all herbicide applications, and can affect non-target plants and seed growers in particular rely heavily on herbicides for effective weed control, since inbred maize lines do not compete effectively with weeds. The degree of injury to non-target plants can be managed through good management practices (Jordan et al., 2009; University of Illinois, 2010) and both growers and commercial herbicide applicators have over fifteen years experience in making glyphosate applications in maize.

However, at the maximum application rate proposed for MON 87427, ground applications of glyphosate are not expected to affect non-target or threatened or endangered plants (Appendix K). Monsanto's glyphosate label and the Pre-Serve web site (www.pre-serve.org), a web-based program designed by Monsanto that provides information on the location of TES plant species in relation to commercial and seed production acres provide information regarding appropriate conditions for application of Roundup agricultural herbicide that are designed to minimize damage to TES. Off-site movement of spray drift is further minimized in MON 87427 fields by the practice of planting additional male parent inbred border rows around the perimeter of the seed production plots to increase desirable pollen shed and reduce the potential for contamination from external pollen sources. Additionally, approximately 80% of all current maize acres have glyphosate-tolerance, and are routinely treated with glyphosate, and measures are already being taken to minimize impacts on non-target plants. Therefore, although glyphosate has the potential to have an adverse impact to non-tolerant plants, the additional applications of glyphosate to MON 87427 are not expected to have a significant impact on plant communities.

I.5.10.2.2. No Action Alternative

Under the no action alternative MON 87427 would remain a regulated article and would not be available to seed producers. Maize hybrid seed producers would likely continue to use other biotechnology-derived inbreds with glyphosate tolerance to produce glyphosate-tolerant seed and alternative weed control methods, including chemical control methods that have the potential to result in drift impacting surrounding plant communities. Therefore, the no action and approval in whole alternatives are similar regarding their impacts to plant communities.

I.5.10.3. Threatened and Endangered Species

Section 7 of the Endangered Species Act requires that each federal agency shall, "in consultation with and with the assistance of the Secretary [of the Interior], insure that any action authorized, funded, or carried out by such agency… ["agency action"] is not likely to jeopardize the continued existence of any endangered or threatened species or result in the destruction or adverse modification of" the critical habitat of any such species. In this appendix, the biology of MON 87427 and the agricultural practices associated with the cultivation of MON 87427 have been considered for potential adverse impact on TES and their critical habitats.

Several lines of evidence can be used to assess the potential for MON 87708 to have adverse effects on TES. The first line of evidence is based on the characteristics and evaluation of CP4 EPSPS. The second line of evidence is the potential for MON 87427 to interact with other maize plants including TES. The third line of evidence is based on the expectation that the introduction of MON 87427 will result in use of glyphosate in hybrid maize production fields.

I.5.10.3.1. Approval in Whole Alternative

The Environmental Protection Agency has established an exemption from the requirement of a tolerance for residues of CP4 EPSPS protein and the genetic material necessary for its production (40 CFR § 174.523). This exemption was based on a safety assessment that included rapid digestion in simulated mammalian gastrointestinal fluids, lack of homology to toxins and allergens, and lack of toxicity in an acute oral mouse gavage study (Harrison et al., 1996). Because the MON 87427-produced CP4 EPSPS protein is equivalent to the exempted CP4 EPSPS protein, a similar conclusion can be reached that the MON 87427-produced CP4 EPSPS protein is safe for human and animal consumption.

Given the lack of adverse effects of the CP4 EPSPS protein, it is unlikely that MON 87427 will have an effect on TES.

As stated in Section I.5.11.2., the potential for gene transfer from MON 87427 is limited to sexually compatible relatives such as other *Zea* species and *Tripsacum* species. No *Zea* or *Tripsacum* species are listed as threatened or endangered by the U.S. Fish and Wildlife Service (USFWS, 2010).

However, the species *Tripsacum floridanum* (Florida gamma grass) has been categorized as a threatened species by the state of Florida (USDA-NRCS, 2010). This species is found in extreme southern Florida, in both highly urbanized and non-agricultural, swampy areas of the state where maize is not typically grown, and hence is not expected to be impacted by the introduction of MON 87427.

Tripsacum dactyloides (Eastern gamma grass), found primarily throughout the eastern U.S., has been categorized as endangered in Massachusetts and Pennsylvania, and as threatened in New York (USDA-NRCS, 2010). *Tripsacum dactyloides* is best adapted to

wet habitats and remnant colonies are commonly found in flood plains and along stream banks. Although found extensively throughout the eastern U.S. at one time, Eastern gamma grass is rarely found now in large natural stands. Eastern gamma grass was regarded as a high-quality forage crop by early settlers, but native stands were destroyed to produce grain crops or grazed out by livestock (Roberts and Kallenbach, 1996). Given the preference of *Tripsacum dactyloides* for wet habitats it is highly unlikely that hybrid maize seed production would occur in the same localities and therefore the introduction of MON 87427 is not expected to significantly impact remaining stands of Eastern gamma grass.

Finally, it is generally recognized that only with extreme difficulty and special techniques will maize and gamma grass (*Tripsacum sp*.) hybridize (Russell and Hallauer, 1980). Moreover, the offspring of these crosses show varying levels of sterility (Galinat, 1988; Russell and Hallauer, 1980). Given the level of difficulty for natural hybridization between species of *Tripsacum* and *Zea*, it is very unlikely there would be any impact on *Tripsacum* due to the introduction of MON 87427. Additionally MON 87427 has no increased weediness potential, and is no more likely to displace threatened and endangered *Tripsacum sp.* from their habitats than any other maize variety.

Impacts to TES from off-target movement of glyphosate may occur. The degree of injury to TES plants is dependent on the sensitivity of the plant to the herbicide. In a TES risk assessment previously provided to USDA-APHIS in support of Roundup Ready alfalfa petition 04-110-01p, Monsanto identified some plant, but no animal species that may be at risk from the use of glyphosate-based herbicides in the Roundup Ready crop system (Honegger, et al., 2008). A similar assessment has been completed for the use of glyphosate in glyphosate-tolerant maize and submitted to the EPA.

Impacts to TES plants can be minimized through good management practices (Jordan et al., 2009; University of Illinois, 2010). Recall that 80% of commercial maize acres grown in the U.S. are already planted with glyphosate-tolerant maize, and are routinely treated with glyphosate. Commercial growers are required through agreements with Monsanto to use Pre-Serve (www.pre-serve.org), a web-based program designed by Monsanto that provides information on the location of TES plant species in relation to maize production acres and dictates mitigation measures to be taken as necessary. With this information growers can develop management practices that minimize potential impacts to TES resulting from the agricultural use of herbicides that contain glyphosate. Pre-Serve instructs growers to observe specific precautions when spraying glyphosate herbicides on Roundup Ready crops near TES plant species that may be at risk. Hybrid seed producers will also be required to employ these mitigation measures. Based on this analysis, the introduction of MON 87427 is not expected to impact TES.

I.5.10.3.2. No Action Alternative

Under the no action alternative MON 87427 would remain a regulated article and would not be available for planting. Maize hybrid seed producers would likely continue to use other biotechnology-derived inbreds with glyphosate tolerance to produce glyphosate-tolerant seed and alternative weed control methods, including chemical control methods

that have the potential to result in drift impacting surrounding plant communities. Therefore, the no action and approval in whole alternatives are similar regarding their impacts to TES.

I.5.10.4. Soil Microorganisms

I.5.10.4.1. Approval in Whole Alternative

Given the lack of adverse effects of the CP4 EPSPS protein, it is unlikely that MON 87427 will have an effect on soil microorganisms. The effects of glyphosate on soil microorganisms have been extensively investigated (Giesy et al., 2000; Sullivan and Sullivan, 2000). Long-term studies following repeated applications of glyphosate in the field for six (Olson and Lindwall, 1991) or over ten years (Biederbeck, et al., 1997; Hart and Brookes, 1996) have shown no detectable adverse effects on soil microbes. Investigations by Haney (2002; 2000) related to the increased use of glyphosate-tolerant crops demonstrated that glyphosate was degraded over time by soil microorganisms without adversely impacting soil microbial communities. Based on this body of evidence, to significant impacts on soil microorganisms are anticipated from the deregulation of MON 87427.

I.5.10.4.2. No Action Alternative

Under the no action alternative MON 87427 would remain a regulated article and would not be available for planting. Maize hybrid seed producers would likely continue to use biotechnology-derived maize with glyphosate tolerance and pesticides that have the potential impact soil microbial communities. . Therefore, the no action and approval in whole alternatives are similar regarding their impacts to soil microbial communities.

I.5.10.5. Biodiversity

I.5.10.5.1. Approval in Whole Alternative

Analysis of available information (Section IX) indicates that MON 87427 exhibits no traits that would cause increased weediness, or that its unconfined cultivation would not lead to increased weediness of other sexually compatible relatives, or that it is likely to have effects on non-target organisms common to agricultural ecosystems or TES recognized by the U.S. Fish and Wildlife Service. Therefore, MON 87427 is unlikely to have effects on non-target organisms common to agriculture ecosystems.

The use of herbicides in agricultural fields is likely to indirectly impact biodiversity by decreasing weed species present in the field. However, agricultural fields are purposefully managed to be weed-free resulting in greater economic benefit to the grower. Therefore, introduction of MON 87427 is unlikely to affect the animal or plant communities found in commercial maize production systems any more than other deregulated biotechnology-derived products containing the CP4 EPSPS protein. Based on this analysis, it is concluded that deregulation of maize products containing MON 87427 would have no significant impact on biodiversity.

I.5.10.5.2. No Action Alternative

Under the no action alternative MON 87427 would remain a regulated article and would not be available for planting. Maize hybrid seed producers would likely continue to use alternative physical and chemical weed control methods that have the potential to impact biodiversity. Therefore, the no action and approval in whole alternatives are similar regarding their impacts to biodiversity.

I.5.11. Cumulative Impacts - Conventional Breeding with Other Biotechnology-derived or Conventional Maize Products

I.5.11.1. Approval in Whole Alternative

As previously mentioned, several biotechnology-derived maize crop products have been deregulated or are under consideration for deregulation by APHIS (Table I-1). Following deregulation MON 87427 may be bred with these deregulated biotechnology-derived maize crop products as well as with conventional maize, creating new improved products. APHIS has determined that none of the biotechnology-derived individual maize products it has deregulated display increased plant pest characteristics relative to their conventional counterparts, and that any progeny derived from crosses of these maize crop products with other conventional or biotechnology-derived maize are unlikely to exhibit new plant pest properties.

An assessment of the stability of the genetic insert in MON 87427 was conducted, and data have been presented in this petition demonstrating that MON 87427 is stable in progeny. Having established that the genetic material is stable and that MON 87427 is inherited in a Mendelian fashion, and based on experience with MON 87427 in Monsanto's plant breeding program, it can be concluded that the phenotype of MON 87427 is likewise stable. Furthermore, the process of conventional breeding to combine biotechnology-derived traits or biotechnology-derived and conventional products to produce combined trait products would likely identify and remove off-types during development of new products. Breeders use standard testing and assessment procedures to further examine and confirm the equivalence of the combined trait products, compared to the single event products, in terms of phenotypes, agronomic characteristics, and the efficacy of the traits. Given that there have been no plant pest characteristics associated with MON 87427, or with any of the previously deregulated events, no significant impacts are expected to other maize products through the use of MON 87427 in breeding programs, and in combination with any of the previously deregulated maize crop products.

All biotechnology-derived maize products on the market today have satisfactorily completed the FDA consultation process established to review the safety of foods and feeds derived from biotechnology-derived crops for human and animal consumption. Given its broad applicability in the production of hybrid maize, MON 87427 is expected to be a base trait to be bred with numerous maize events previously deregulated or under review by APHIS for deregulated status. No impacts to public health (e.g., food or feed

safety) are expected due to combination of these events through conventional breeding because the deregulated events have a history of safe use, and, on the basis of knowledge of the type of modifications made to each of the deregulated events, and to the events under review, the biochemical pathways are not likely to unexpectedly interact or result in the production of novel constituents.

The decision to deregulate MON 87427 would also allow breeding of this product with conventional maize products of diverse genetic backgrounds and previously deregulated products. These combined trait products would include commercial traits introduced through either one or both of the male or female parent inbred lines. A homozygous MON 87427 inbred would be the female parent inbred in hybrid maize seed production. The male parent inbred would typically include a homozygous glyphosate-tolerance trait to facilitate the production of hybrid maize seed. No impacts to public health (e.g., food or feed safety) or environmental safety are expected due to the breeding of MON 87427 with these other maize products, because these products have an established history of safe use.

I.5.11.2. No Action Alternative

Under the no action alternative MON 87427 would remain a regulated article and would not be available for breeding. There are no effects that have been identified from combining MON 87427 with other biotechnology-derived maize products that have been deregulated by the USDA. Therefore, the no action and approval in whole alternatives are similar regarding their effects to public health and environmental safety.

I.6. Highly Uncertain, Unique or Unknown Risks

MON 87427 has been thoroughly characterized and data submitted in this petition demonstrate that it poses no increased plant pest risk compared to conventional maize. Monsanto Company has developed MON 87427 to promote efficiency in maize hybrid seed manufacturing. USDA-APHIS has previously deregulated 23 biotechnology-derived maize crop products (http://www.aphis.usda.gov/brs/not_reg.html accessed 05/05/10) that have resulted in no unexpected effects on the quality of the human environment as defined under NEPA, and have provided benefits to growers, consumers and the environment. In this respect, a decision to deregulate a new biotechnology-derived maize product is not precedent setting, nor are the effects to the quality of the human environment highly uncertain or unpredictable.

References for Appendix I

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.

Alibhai, M.F. and W.C. Stallings. 2001. Closing down on glyphosate inhibition - with a new structure for drug discovery. Proceedings of the National Academy of Sciences of the United States of America 98: 2944-2946.

AOSCA. 2009. Seed certification handbook: including genetic and crop standards, procedures, and AOSCA service programs. Association of Official Seed Certifying Agencies, Moline, Illinois.

Atlas, R.M. and R. Bartha. 1997. Development of microbial communities. Pages 179-209 in Microbial Ecology: Fundamentals and Applications. Addison Wesley Longman, Inc, Menlo Park, California.

Baker, H.G. 1965. Characteristics and modes of origin of weeds. Pages 147-172 in The Genetics of Colonizing Species. H.G. Baker and G.L. Stebbins (eds.). Academic Press, New York, New York.

Baltazar, B.M., J. de Jesús Sánchez-Gonzalez, L. de la Cruz-Larios and J.B. Schoper. 2005. Pollination between maize and teosinte: an important determinant of gene flow in Mexico. Theoretical and Applied Genetics 110: 519-526.

Biederbeck, V.O., C.A. Campbell and J.H. Hunter. 1997. Tillage effects on soil microbial and biochemical characteristics in a fallow-wheat rotation in a dark brown soil. Canadian Journal of Soil Science 77: 309-316.

Born, H. 2005. Marketing organic grains. National Sustainable Agricultural Information Service, Fayetteville, Arkansas.

Bradford, K.J. 2006. Methods to maintain genetic purity of seed stocks. Publication 8189. University of California, Oakland, California.

Buckley, D.H. and T.M. Schmidt. 2001. The structure of microbial communities in soil and the lasting impact of cultivation. Microbial Ecology 42: 11-21.

CaJacob, C.A., P. Feng, G.R. Heck, M.F. Alibhai, D. Sammons and S.R. Padgette. 2004. Enigeering resistance to herbicides. Pages 353-372 in Handbook of Plant Biotechnology. P. Christou and H. Klee (eds.). John Wiely & Sons, Ltd., Hoboken, New Jersey.

Carpenter, J.E., A. Felsot, T. Goode, M. Hammig, D. Onstad and S. Sankula. 2002. Pages 1-189 in Comparative Environmental Impacts of Biotechnology-Derived and Traditional

Soybean, Corn, and Cotton Crops. Council for Agricultural Science and Technology, Ames, Iowa.

Cerdeira, A.L. and S.O. Duke. 2006. The current status and environmental impacts of glyphosate-resistant crops: A review. Journal of Environmental Quality 35: 1633-1658.

Craig, W.F. 1977. Production of hybrid corn seed. Pages 671-719 in Corn and Corn Improvement. Second Edition. G.F. Sprange (ed.). American Society of Agronomy, Madison, Wisconsin.

Devine, M.D. and C. Preston. 2000. The molecular basis of herbicide resistance. Pages 72-104 in Herbicides and Their Mechanisms of Action. A.H. Cobb and R.C. Kirkwood (eds.). CRC Press, Boca Raton, Florida.

Duvick, D.N. 1972. Potential usefulness of new cytoplasmic male steriles and sterility systems. Pages 197-201 in Proceedings of the 27th Annual Corn Sorghum Conference, ASTA, Washington, D.C.

Duvick, D.N. 2001. Biotechnology in the 1930s: The development of hybrid maize. Nature Reviews in Genetics 2: 69-74.

Ellstrand, N.C., L.C. Garner, S. Hegde, R. Guadagnuolo and L. Blancas. 2007. Spontaneous hybridization between maize and teosinte. Journal of Heredity 98: 183-187.

Evans, M.M.S. and J.L. Kermicle. 2001. *Teosinte crossing barrier1*, a locus governing hybridization of teosinte with maize. Theoretical and Applied Genetics 103: 259-265.

FAOSTAT. 2009. Food and Agricultural Organization statistical database. Food and Agricultural Organization of the United Nations, New York, New York. http://faostat.fao.org/site/567/default.aspx#ancor [Accessed August 16, 2010].

Feng, P.C.C., O. Heredia and A.A. Rosielle. 2009. Method to enhance yield and purity of hybrid crops. Patent Application 2009/0165166 A1, U. S. Patent Office, Washington, D.C.

Galinat, W.C. 1988. The origin of corn. Pages 1-31 in Corn and Corn Improvement. Volume 18. G.F. Sprague and J.W. Dudley (eds.). American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, Wisconsin.

Giesy, J.P., S. Dobson and K.R. Solomon. 2000. Ecotoxicological risk assessment for Roundup® Herbicide. Pages 35-120 in Reviews of Environmental Contamination and Toxicology. Volume 167. Springer-Verlag, New York, New York.

Goldstein, S.M. 2003. Life history observations on three generations of *Folsomia candida* (Willem) (*Collembola: Isotomidae*) fed yeast and Roundup Ready soybeans and corn. Michigan State University, East Lansing, Michigan.

Haney, R.L., S.A. Senseman and F.M. Hons. 2002. Effect of Roundup Ultra on microbial activity and biomass from selected soils. Journal of Environmental Quality 31: 730-735.

Haney, R.L., S.A. Senseman, F.M. Hons and D.A. Zuberer. 2000. Effect of glyphosate on soil microbial activity and biomass. Weed Science 48: 89-93.

Harrison, L.A., M.R. Bailey, M.W. Naylor, J.E. Ream, B.G. Hammond, D.L. Nida, B.L. Burnette, T.E. Nickson, T.A. Mitsky, M.L. Taylor, R.L. Fuchs and S.R. Padgette. 1996. The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phosphate synthase from *Agrobacterium* sp. strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. Journal of Nutrition 126: 728-740.

Hart, M.R. and P.C. Brookes. 1996. Soil microbial biomass and mineralisation of soil organic matter after 19 years of cumulative field applications of pesticides. Soil Biology and Biochemistry 28: 1641-1649.

Hoeft, R.G., E.D. Nafziger, R.R. Johnson and S.R. Aldrich. 2000a. Corn as a crop. Pages 1-29 in Modern Corn and Soybean Production. MCSP Publications, Champaign, Illinois.

Hoeft, R.G., E.D. Nafziger, R.R. Johnson and S.R. Aldrich. 2000b. Planting decisions and operations. Pages 81-105 in Modern Corn and Soybean Production. MCSP Publications, Champaign, Illinois.

Hoeft, R.G., E.D. Nafziger, R.R. Johnson and S.R. Aldrich. 2000c. Nutrient management for top profit. Pages 107-171 in Modern Corn and Soybean Production. MCSP Publications, Champaign, Illinois.

Honegger, J.L., S.R. Mortensen and K.H. Carr. 2008. Overview of the analysis of possible risk to threatened and endangered species associated with use of glyphosate-containing herbicides in alfalfa production. Study RPN-2007-228, Monsanto Company, St. Louis, Missouri.

Jamornmarn, S., S. Sopa, S. Kumsri, T. Anantachaiyong and S. Rattithumkul. 2004. Effect of Roundup Ready corn NK603 on green lacewing - *Mallada basalis* (Walker) under laboratory condition. 15th Annual Meeting of the Thai Society for Biotehnology Sustainable Development of sMEs through Biotechnology, Chiang Mai, Thailand.

Jasinski, J.R., J.B. Eisley, C.E. Young, J. Kovach and H. Willson. 2003. Select nontarget arthropod abundance in transgenic and nontransgenic field crops in Ohio. Environmental Entomology 32: 407-413.

Jordan, T., G. Nice, B. Johnson and T. Bauman. 2009. Reducing spray drift from glyphosate and growth regulator herbicide drift caution. Purdue Extension, Purdue University, West Lafayette, Indiana. http://www.ag.purdue.edu/btny/weedscience/Documents/ReducingDrift09.pdf [Accessed December 18, 2009].

Keeler, K.H. 1989. Can genetically engineered crop become weeds? Bio-Technology 7: 1134-1139.

Kermicle, J.L. and J.O. Allen. 1990. Cross-incompatibility between maize and teosinte. Maydica 35: 399-408.

Koetters, M. 2007. Teens drawn to cash rewards of detasseling corn. Pantagraph, Bloomington, Illinois. July 28, 2007.

Levings, C.S., III and J.N. Siedow. 1992. Molecular basis of disease susceptibility in Texas cytoplasm of maize. Plant Molecular Biology 19: 135-147.

Loussaert, D. 2004. Trihalogenated methylsulfonamides as specific male gametocides. Sexual Plant Reproduction 16: 299-307.

McDonald, M.B. and L.O. Copeland. 1997. Cereal seeds. Corn (*Zea mays* L.). Pages 193-205 in Seed Production: Principles and Practices. Chapman & Hall, New York, New York.

McPherson, R.M., W.C. Johnson, B.G. Mullinix, W.A. Mills and F.S. Peebles. 2003. Influeince of herbicide tolerant soybean production systems on insect pest populations and pest-induced crop damage. Journal of Economic Entomology 96: 690-698.

Monsanto Company. 2009. Monsanto Biotechnology Trait Acreage: Fiscal Years 1996-2009. http://www.monsanto.com/pdf/investors/2009/q4_biotech_acres.pdf [Accessed May 12, 2010].

OECD. 2002. Consensus document on compositional considerations for new varieties of maize (*Zea mays*): key food and feed nutrients, anti-nutrients and secondary plant metabolites. OECD ENV/JM/MONO (2002)4. Organization of Economic Co-Operation and Development, Paris, France.

OECD. 2003. Consensus document on the biology of *Zea mays* subsp. *mays* (maize). ENV/JM/MONO(2003)11. Organization for Economic Co-Operation and Development, Paris, France.

Olson, B.M. and C.W. Lindwall. 1991. Soil microbial activity under chemical fallow conditions: Effects of 2,4-D and glyphosate. Soil Biology and Biochemistry 23: 1071-1075.

Pring, D.R. and D.M. Lonsdale. 1989. Cytoplasmic male sterility and maternal inheritance of disease susceptibility in maize
Annual Review of Phytopathology 27: 483-502.

Riebsame, W.E. 1990. The United States great plains. Pages 561-575 in The Earth as Transformed by Human Action. B.L. Turner (ed.). Cambridge University Press, New York, New York.

Roberts, C. and R. Kallenbach. 1996. Eastern gamagrass. University of Missouri Extension, Columbia, Missouri.

Roundup PowerMAX Herbicide. 2007. Safety Data Sheet Commerical Product. Monsanto Company, St. Louis, Missouri.

Roundup PowerMAX Herbicide. 2008. Supplemental Label. Monsanto Company, St. Louis, Missouri.

Roundup WeatherMAX Herbicide. 2002. Material Safety Data Sheet Monsanto Company, St. Louis, Missouri.

Roundup WeatherMAX Herbicide. 2009. Roundup Ready corn 2: weed control recommendations. Monsanto Company, St. Louis, Missouri.

Russell, W.A. and A.R. Hallauer. 1980. Corn. Pages 299-312 in Hybridization of Crop Plants. W.R. Fehr and H.H. Hadley (eds.). American Society of Agronomy, Inc, and Crop Science Society of America, Inc, Madison, Wisconsin.

Smith, D.R. and D.G. White. 1988. Diseases of corn. Pages 687-766 in Corn and corn improvement. G.G. Sprague and J.W. Dudley (eds.). American Society of Agronomy, Inc, Crop Science Society of America, Inc, and Soil Science Society of America, Inc, Madison, Wisconsin.

Sullivan, D.S. and T.P. Sullivan. 2000. Non-target impacts of the herbicide glyphosate: A compendium of references and abstracts. Applied Mammal Research Institute, Summerland, British Columbia.

Tiedje, J.M., S. Asuming-Brempong, K. Nusslein, T.L. Marsh and S.J. Flynn. 1999. Opening the black box of soil microbial diversity. Applied Soil Ecology 13: 109-122.

Troyer, A.F. 1999. Review and interpretation: Background of US hybrid corn. Crop Science 39: 601-626.

U.S. EPA. 1993. RED facts: Glyphosate. U.S. Environmental Protection Agency Office of Prevention, Pesticides, and Toxic Substances, Washington, D.C. http://www.epa.gov/oppfead1/endanger/litstatus/effects/glyphosate-red.pdf [Accessed August 16, 2010].

U.S. EPA. 2002. Glyphosate; pesticide tolerances 67 FR 60934. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 2006a. Glyphosate human health risk assessment for proposed use on Indian mulberry and amended use on pea, dry. Petition No. 5E6987 DP 321992 Decision No. 360557. U.S. Environmental Protection Agency, Washington, D.C. http://www.regulations.gov/search/Regs/home.html#documentDetail?R=09000064801dce5f [Accessed September 9, 2010].

U.S. EPA. 2006b. Glyphosate human health risk assessment for proposed uses on safflower and sunflower. Petition 4E6878 DP 314476. U.S. Environmental Protection Agency, Washington, D.C. http://www.regulations.gov/search/Regs/home.html#documentDetail?R=09000064801dce6b [Accessed September 9, 2010].

U.S. EPA. 2010. Agricultural practices that sequester carbon and/or reduce emissions of other greenhouse gases. U.S. Environmental Protection Agency, Washington, D.C. http://www.epa.gov/sequestration/ag.html [Accessed May 19, 2010].

Ullstrup, A.J. 1972. The impacts of the southern corn leaf blight epidemics of 1970-1971. Annual Review of Phytopathology 10: 37-50.

University of Illinois. 2010. Reducing pesticide drift: Specialty crops and conventional crops as good neighbours. University of Illinois Extension, Urbana-Champaign, Illinois. http://www.specialtygrowers.org/releases/ReducingPesticideDrift.pdf [Accessed June 30, 2010].

USBLS. 2010. Occupational Outlook Handbook, 2010-11 Edition. U.S. Bureau of Labor and Statistics, Washington, D.C. http://data.bls.gov/cgi-bin/print.pl/oco/ocos349.htm [Accessed June 21, 2010].

USDA-AMS. 2010. National organic program. Washington, D.C. http://www.ams.usda.gov/AMSv1.0/nop [Accessed May 14, 2010].

USDA-ERS. 2009a. Planted acres-corn-2008-all states. U.S. Department of Agriculture, Economic Research Service, Washington, D.C. http://maps.ers.usda.gov/BaseAcres/index.aspx [Accessed July 14, 2010].

USDA-ERS. 2009b. Briefing rooms/Corn. U.S. Department of Agriculture, Economic Research Services, Washington D.C. http://www.ers.usda.gov/Briefing/Corn/ [Accessed July 13, 2010].

USDA-ERS. 2010a. Adoption of genetically engineered crops in the U.S.: corn varieties. U.S. Department of Agriculture, Economic Research Services, Washington D.C. http://www.ers.usda.gov/Data/BiotechCrops/ExtentofAdoptionTable1.htm [Accessed July 31, 2010].

USDA-ERS. 2010b. Feed grains database: custom queries. U.S. Department of Agriculture, Economic Research Service, Washington, D.C. http://www.ers.usda.gov/Data/FeedGrains/CustomQuery/Default.aspx [Accessed August 17, 2010].

USDA-ERS. 2010c. Organic production. U.S. Department of Agriculture, Economic Research Services, Washington D.C. . http://www.ers.usda.gov/Data/organic/ [Accessed May 5, 2010].

USDA-NASS. 2010a. Data and statistics - Quick stats. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C.

USDA-NASS. 2010b. Acreage. U.S. Department of Agriculture, National Agricultural Statistics Service, Washington, D.C. http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1000 [Accessed July 31, 2010].

USDA-NRCS. 2010. The PLANTS database. U.S. Department of Agriculture, Natural Resources Conservation Service, Washington, D.C. http://www.plants.usda.gov/index.html [Accessed August 11, 2010].

USFWS. 2010. Endangered species program. U.S. Fish and Wildlife Service. http://www.fws.gov/endangered [Accessed September 28, 2010].

Wilkes, H.G. 1967. Wild teosinte and its genetic isolation from maize. Pages 1 and 121-127 in Teosinte: the Closest Relative of Maize. The Bussey Institution of Harvard University, Cambridge, Massachusetts.

Williams, G.M., R. Kroes and I.C. Munro. 2000. Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. Regulatory Toxicology and Pharmacology 31: 117-165.

Wych, R.D. 1988. Production of hybrid seed corn. Pages 565-607 in Corn and Corn Improvement. Third Edition. G.F. Sprague and J.W. Dudley (eds.). American Society of Agronomy Inc., Crop Science Society of America Inc., and Soil Science Society of America Inc., Madison, Wisconsin.

Appendix J: Herbicide Resistance

J.1. Introduction

Herbicides are pesticides intended to prevent or kill weeds that can compete with a crop for nutrients, water and in some cases, sunlight. By killing weeds, herbicides allow planted crops to grow and thrive, thereby increasing crop yield that allows these crops to be grown on fewer acres and protect habitat and its wildlife from unnecessary expansion of cropland production wherever possible.

Plant populations can develop resistance to a herbicide due to the selection of individuals that carry specific genetic code(s) that can render those individuals tolerant to the lethal effects of a herbicide. The application of a herbicide to the plant does not, itself, cause a mutation in subsequent generations. Rather, over time, those few plant biotypes that are not susceptible to a herbicide become dominant in the population with repeated use of the herbicide in the absence of other control methods, such as use of other herbicides and/or use of cultural control methods. The development of resistant populations is common to all herbicides. The probability for resistance is a function of: frequency of resistant allele(s), mechanism of resistance, dominance or recessive nature of the resistant allele(s), relative fitness of the resistant biotype, and frequency of herbicide use in the absence of other control methods (Beckie, 2006; Jasieniuk, et al., 1996; Sammons, et al., 2007). The probability of resistance is not the same for all herbicides with some herbicides (e.g., ALS and ACCase classes) exhibiting resistance more quickly that other herbicides (e.g. glyphosate, auxins (dicamba), dinitroanilines).

Herbicide resistance could become a limiting factor in crop production if the resistant weed population cannot be controlled with other herbicides or cultural practices. This generally has not been the case for any herbicide. For most crops, there are multiple herbicide options for growers to use. However, good management practices to retard the development of herbicide resistance have been identified, are being actively promoted by the public and private sectors, and are being implemented by growers.

Monsanto considers product stewardship to be a fundamental component of customer service and business practices. Stewardship of the glyphosate molecule to preserve its usefulness for growers is an important aspect of Monsanto's stewardship commitment. Although herbicide resistance may eventually occur in weed species when a herbicide is widely used, resistance can be postponed, contained and managed through research, education and good management practices. These are the key elements of Monsanto's approach to providing stewardship of glyphosate relative to all uses including use in the MON 87427 maize seed production system. Monsanto will invest in research and in contract-grower education and training programs to provide information on best practices to manage glyphosate weed resistance in maize seed production. This document provides an overview of Monsanto's approach to the development of best management practices to mitigate glyphosate resistance.

J.2. The Herbicide Glyphosate

Glyphosate (N-phosphonomethyl-glycine) (CAS Registry #: 1071-83-6), the active ingredient in the Roundup® family of nonselective, foliar-applied, postemergent agricultural herbicides, is among the world's most widely used herbicidal active ingredients. Glyphosate is highly effective against the majority of economically significant annual and perennial grasses and broadleaf weeds. Currently glyphosate is labeled for control of more than 300 weed species world-wide. Glyphosate kills plant cells by inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme involved in the shikimic acid pathway for aromatic amino acid biosynthesis in plants and microorganisms (Franz, et al., 1997). Glyphosate is the only known herbicide with this mode of action (Franz et al., 1997). The relevant aromatic amino acid pathway is not present in mammalian metabolic systems (Cole, 1985). A comprehensive human safety evaluation and risk assessment concluded that glyphosate has low toxicity to mammals, is not a carcinogen, does not adversely affect reproduction and development, and does not bioaccumulate in mammals (Williams, et al., 2000). An ecotoxicological risk assessment concluded that the use of glyphosate does not pose an unreasonable risk of adverse effects to nontarget species, such as birds and fish, when used according to label directions (Giesy, et al., 2000). Glyphosate has favorable environmental characteristics, including a low potential to move through the soil to reach ground water and is degraded over time by soil microbes. Because it binds tightly to soil, glyphosate's bioavailability is reduced immediately after application, which is why glyphosate has no residual soil activity.

J.3. Herbicide Use in Maize Seed Production Systems and Herbicide-resistant Weeds

Weed control in maize seed production fields is critical for obtaining optimized yields, as it is in any other maize cultivation. Because failure to control weeds within the crop can result in decreased yields and reduced crop quality, an intensive program for weed control is essential to ensure a grower can meet the terms of a maize-seed production contract. In using MON 87427 to facilitate the production of hybrid maize seed, weed control will be obtained through the use of glyphosate plus other herbicides, in particular, herbicides applied at planting that will provide residual control of grass and broadleaf species. Control of weeds in a crop is essential because weeds compete with the crop for the same limited resources in the field, including sunlight, water and nutrients (Ross and Lembi, 1985a; b). Lack of effective weed control in maize fields can result in significant yield losses.

With any herbicide use, however, comes the potential for the selection of weeds resistant to that herbicide. Within a weed species individuals may possess an inherent ability to withstand the effects of a particular herbicide. Repeated use of that herbicide will expose the weed population to a "selection pressure," which may lead to an increase in the number of surviving resistant individuals in the population (WSSA, 2000). In other

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words, plants susceptible to the applied herbicide will die, while those few having some type of natural resistance may survive and reproduce.

A resistant weed must demonstrate two criteria as defined by the Weed Science Society of America website at www.wssa.net: (1) the ability to survive application rates of herbicide product that once were effective in controlling it and, (2) resistance is heritable. Herbicide-resistant weeds are neither a new phenomena nor is resistance unique to glyphosate. Growers have been managing herbicide-resistant weeds for decades with the use of alternative herbicides and/or cultural methods such as tillage or crop rotation that are combined to provide a diverse weed management program.

J.4. Characteristics of Herbicides and Herbicide Use Influencing Resistance

While the incidence of weed resistance is often associated with repeated applications of a herbicide product, the actual onset of resistance within a population depends very much on the specific herbicide chemistry in question, as well as the inherent presence of gene(s) that confer the ability of a plant to be resistant to a particular chemical within a specific weed species and even a specific population of that species (Sammons et al., 2007). Some herbicide products are much more prone to develop herbicide resistance than others (Heap, 2010). Glyphosate has been used extensively for over three decades with relatively few cases of resistance development, particularly when compared to many other herbicides (e.g., ALS inhibitors, triazines, and ACCase inhibitors), and considering the substantial worldwide glyphosate-treated acreage and the total number of weeds that glyphosate can control. The graph in Figure J-1 illustrates the instances of weed resistance to various herbicide groups. The different slopes observed are largely due to the factors described above, which relate to chemistry and function, in addition to levels of exposure in the field. The summary below describes herbicide-specific factors determined to be important in the process of selecting for individuals that are inherently resistant to a herbicide.

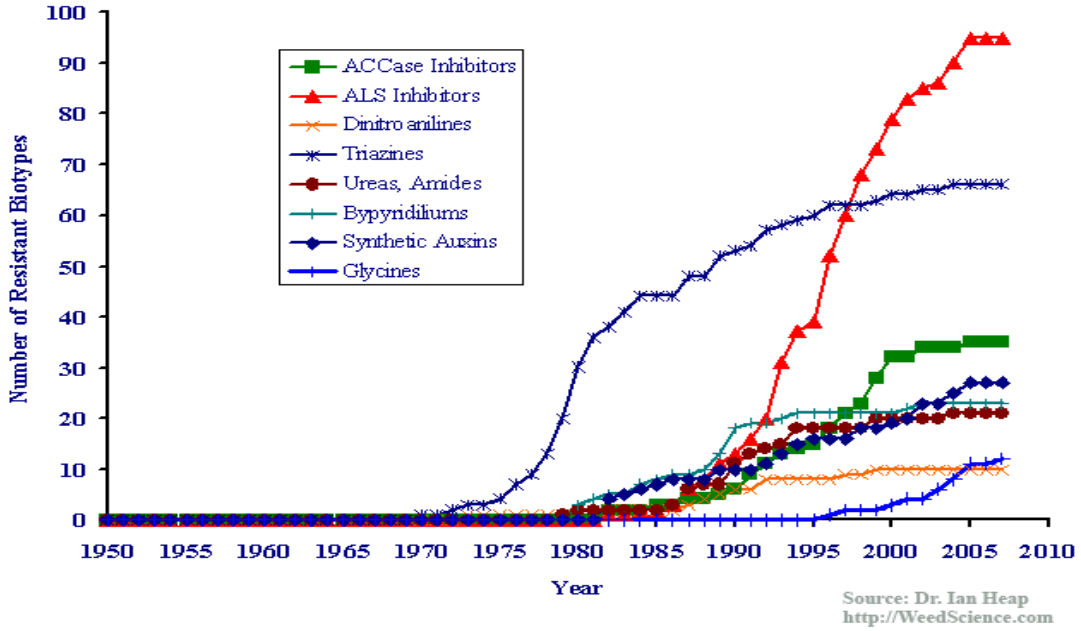

Figure J-1. Weed Resistance to Various Herbicide Chemical Families

J.5.1. Mechanisms of Resistance

The application of herbicide to a weed does not, itself, cause a mutation in later generations of the plant. Rather, over time, the repeated application of a herbicide selects for those few biotypes that are less susceptible to the herbicide and become dominant within a population. To date, the three known mechanisms by which a weed species develops resistant to a herbicide have been identified as target site alteration (target site), enhanced metabolism of the herbicides (metabolism), and reduced absorption and/or translocation of the herbicide such that the herbicide does not get to the site of action within the plant cell (exclusion) (Sammons et al., 2007).

Herbicide resistance via target site is the most common resistance mechanism among the various herbicide classes. It has been found that a target site mechanism is the most common mechanism for ALS inhibitors, ACCase inhibitors, and triazines, but is less common for glyphosate. One type of target site alteration involves amino acid substitution(s) in the enzyme that prevents the binding of the herbicide without impacting enzyme activity and the plant is able to grow normally without any fitness penalty. For ALS inhibitors, the level of resistance conferred by a target site mechanism has been found to be as high as 3,400 X (Ferguson, et al., 2001). (Note: X is the labeled or recommended rate for a herbicide on a particular weed species.) For glyphosate, species found to exhibit a target site mechanism often show low levels of resistance (2-3X) due to the fact that glyphosate is a true transition state inhibitor (Sammons et al., 2007; Schonbrunn, et al., 2001) and this differentiates glyphosate from ALS inhibitors and ACCase inhibitors. The transition state is an unstable state during an enzyme reaction half-way between the substrate and product. Transition state inhibitors are very effective enzyme inhibitors. In addition, multiple alterations of the same enzyme have been found for ALS and ACCase inhibitors (Tranel and Wright, 2002). This may explain the apparent high frequency of resistance and the short time in which resistance developed to herbicides in these two classes of chemistries. Only one altered site in the targeted plant EPSPS enzyme has been found for glyphosate (Baerson, et al., 2002). Another type of a target site resistance mechanism, recently discovered for glyphosate, is an over amplification of the EPSPS gene which results in an overproduction of the EPSPS enzyme (Gaines, et al., 2010). This mechanism was discovered in palmer pigweed.

The second general type of herbicide resistance mechanism, metabolism, has not been found to be a resistance mechanism associated with glyphosate in any of the weed species studied thus far. However, legumes have been shown to degrade glyphosate and therefore this type of resistance mechanism may be active in some species (Reddy, et al., 2008).

Herbicide resistance as a result of exclusion mechanisms is the glyphosate resistant mechanism among the majority of the weed species studied to date. This resistance mechanism has also been found to be associated with 2,4 D and paraquat. Within this category, there are two types of translocation alterations that have been observed for glyphosate; (a) restricted movement of glyphosate from leaf cells into the meristematic cells of the plant and (b) restricted movement of glyphosate within a cell into the chloroplast due to accumulation within the vacuole (Shaner, 2009). The level of

glyphosate resistance conferred with this mechanism is higher (6-8X) than for species exhibiting amino acid substitution type target site mutations (2-3X).

In some species, the experimental evidence suggests that multiple mechanisms of glyphosate resistance may occur within the same plant to protect the plant from the phytotoxic effects of glyphosate (Yu, et al., 2007). This implies that multiple genes (polygenic resistance) are necessary and thus the selection of plants with multiple genes needed to confer resistance would be expected to occur at a low frequency.

In summary, the overall low occurrence of glyphosate resistance may be in part explained by: (1) the nature of the target site inhibition by glyphosate relative to other herbicides, (2) the lack of metabolism as a mechanism of selectivity for weed resistance, and (3) evidence of multiple mechanisms being necessary for resistance; thus, resistance is polygenic and difficult to assemble and maintain. Recommendations to manage glyphosate resistance are not dependent upon the type of resistant mechanism operating within a species or population of a species.

J.5.2. Use of Recommended Rate

The interaction between herbicide application rate and resistance for postemergence herbicides, such as glyphosate, is dependent upon the nature of the plant gene(s) conferring resistance to the chemical. In general, herbicide rate has more effect on selecting for resistant individuals in a population if the resistant gene is semi-dominant or recessive as compared to the resistant gene being dominant. Likewise, herbicide rates would have more of an effect on the onset of resistance if commercially significant resistance required the additive effect of multiple genes (i.e. quantitative or polygenic resistance). Low rates would tend to allow certain biotypes to survive and mate with other biotypes of the same or an alternate resistant gene. The offspring of this mating may then be able to survive a full rate.

Less-than-recommended or suboptimal rates have been implicated or speculated as the causal factor in herbicide resistance for several different weed species, including chlortoluron-resistant blackgrass, diclofop-resistant ryegrass and dicamba-resistant kochia (Beckie, 2006). Busi et al. (2009) demonstrated that, in three generations of a ryegrass biotype sprayed at sublethal rates of diclofop-methyl or glyphosate, a high level of resistance evolved to diclofop-methyl and a moderate level to glyphosate. The conclusion of this research was that growers should avoid lowering the application rate of herbicides, especially where major cross-pollinating weed species, such as lolium, are present.

J.6. Weeds Resistant to Glyphosate

As with any other herbicide, the use of glyphosate may lead to the development of glyphosate-resistant weed species. A list of glyphosate resistant weeds is provided below in Table J-1. However, the potential for the development of a glyphosate-resistant weed needs to be considered in the following context: (1) if a glyphosate-based weed control system were not available, other herbicide(s) with equal or greater potential for resistance

would be used to control weeds and (2) other herbicides and cultural practices can be used to manage the glyphosate resistant species (Gustafson, 2008; Neve, 2008).

Through August 2010, biotypes of nineteen weed species resistant to glyphosate have been identified and confirmed worldwide. Ten species resistant to glyphosate have been confirmed in the U.S., two of which were identified outside of Roundup Ready® cropping systems. The speed of spread and geographical distribution of the resistant species has varied. Some species with resistant biotypes, such as common ragweed (*Ambrosia artemisfolia*), have been found in a limited number of sites across the mid-west, whereas marestail (*Conyza canadensis*) has been found in many states in the northeast, mid-west and the south. The reproductive biology of the particular weed species involved appears to be a factor contributing to the spread of resistant biotypes. In the above examples, marestail produces a large number of wind-dispersed seeds, which contributes to rapid spread, while ragweed seeds do not have features that allow for such easy distribution by the wind (Weaver, 2001).

Table J-1. U.S. Glyphosate Resistant Weeds through August 2010

Weeds identified outside of Roundup Ready Systems	Rigid ryegrass (*Lolium rigidum*)
	Hairy fleabane (*Conyza bonariensis*)
Weeds identified in Roundup Ready Systems	Horseweed (*Conyza canadensis*) Common ragweed (*Ambrosia artemisiifolia*) Giant ragweed (*Ambrosia trifida*) Palmer amaranth (*Amaranthus palmeri*) Common waterhemp (*Amaranthus rudis*) Italian ryegrass (*Lolium multiflorum*) Johnson grass (*Sorghum halepense*) Kochia (*Kochia scoparia*)

Some weed species, such as *Equisetum arvensis* (field horseweed), are tolerant, as opposed to resistant, to glyphosate. Further, some species are more difficult to control with glyphosate than others (e.g. lambsquarters (*Chenopodium album*) and morninglory (*Ipomea sp.*)) and require more care to make sure the correct amount of glyphosate is applied at the right growth stage. For these difficult-to-control weeds, environmental conditions can affect herbicide performance more than for weeds that are easier to control, and therefore it is more critical that the correct rate be applied at the right growth stage when making applications to weeds in the difficult-to-control category. Weed control situations involving tolerant or difficult-to-control species are often confused with resistance.

J.7. Use of Glyphosate for In-crop Weed Management

Monsanto has developed plants through biotechnology to be tolerant to glyphosate. The development, approval and cultivation of these Roundup Ready crops have facilitated

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additional uses of glyphosate in crops where such uses were not previously possible given the non-selective nature of glyphosate. This development has provided growers with an additional weed management option and benefits relative to existing weed management options. The glyphosate tolerance in Roundup Ready crops has no effect per se on the control of weeds. From a weed resistance standpoint, the use of glyphosate with glyphosate-tolerant maize is no different than the use of a selective herbicide in a conventional maize crop.

The most often cited benefits of glyphosate, as an in-crop weed management option, are simplicity, flexibility of application timing, weed spectrum, crop safety, and environmental safety (Dill, 2005). The ability to use glyphosate in-crop has allowed farmers to change their farming practices in some cases. For example, Roundup Ready cotton and Roundup Ready soybean have often been cited as a major reason for an increase in conservation-tillage practices (Dill, et al., 2008).

Since Monsanto commercialized the first Roundup Ready maize hybrids in 1998, growers have enthusiastically adopted the technology. The Roundup Ready maize system, (i.e., planting Roundup Ready maize and applying glyphosate in-crop), has become the standard weed control program in U.S. maize production. In addition, weed control in a Roundup Ready maize system likely will involve not only glyphosate-based herbicides but also other herbicides and weed management practices to effectively manage weeds, thus increasing crop yield and reducing development of resistant weed populations. State Universities/Cooperative Extension Services (CES) publish information on best weed management practices in Roundup Ready crops to address both of these objectives (see Table J-2). In addition Monsanto and other companies selling glyphosate products provide information on these same best management practices as detailed later in this Appendix.

J.8. Weed Resistance Management Strategies for Glyphosate

As part of Monsanto's stewardship of Roundup® agricultural herbicides and Roundup Ready crop systems, the company has conducted investigations and worked extensively with academics and other herbicide manufacturers to understand the best practices to manage resistance. These investigations have demonstrated that one of the major factors that can contribute to the development of resistant weed populations is weed control management practices such as the application of herbicides at rates below those indicated on the EPA-approved label for the weed species, and sole reliance on a particular herbicide for weed control without the use of other herbicides or cultural control methods (i.e. pre-plant and in-crop tillage) (Beckie, 2006; Peterson, et al., 2007).

As detailed in the Petition and Appendix I, the purpose of MON 87427 is to facilitate hybrid maize seed production. Its presence in the resulting hybrids does not impact commercial grain production practices. Monsanto will communicate to all seed-maize growers recommended weed resistance management practices in the seed-maize production contracts and through dissemination of information as part of Monsanto's

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general weed resistance stewardship programs. Monsanto will provide instructions to seed maize growers regarding reporting any incidence of repeated non-performance of Roundup agricultural herbicides on a particular weed, and Monsanto will investigate cases of unsatisfactory weed control to determine the cause as defined in Section J-10 of this appendix. In cases where resistance is confirmed, Monsanto will provide recommendations for alternative control methods for farmers (see Table J-2). These recommendations are made available through Monsanto supplemental labels, the Monsanto Technology Use Guide (TUG), Monsanto and University publications and internet sites for growers, consultants, retailers and distributors. In all cases of glyphosate-resistant weeds in the U.S. and globally, there are alternative herbicides and cultural methods available to farmers to effectively control these species. Some examples of these recommendations from University/CES personnel are found in Table J-2. It is important to note that there are many alternative options in each situation.

The weed resistance management recommendations that will be made for the use of glyphosate in conjunction with hybrids containing MON 87427 will not differ from recommendations currently being made for commercial hybrids containing event NK603 (Roundup Ready2 maize hybrids). These recommendations are consistent with the Herbicide Resistance Action Committee's guidelines for prevention and management of herbicide resistance (HRAC, 2009). These guidelines recommend an integrated approach to weed resistance management including crop management (i.e. row spacings, etc), cultural techniques and herbicides. Specific requirements regarding weed management of seed production fields will be provided in the seed production contracts executed with each grower.

EPA is the U.S. federal regulatory agency that administers the federal law governing pesticide sale and use (FIFRA). EPA encourages pesticide manufacturers to provide growers with information regarding a herbicide's mode of action to aid growers in planning herbicide use practices and to foster the adoption of effective weed-resistance management practices as specified by EPA in PR Notice 2001-5. In that document EPA states that "this approach to resistance management is sound and would be highly beneficial to pesticide manufacturers and pesticide users" (EPA PR Notice 2001-5 at http://www.epa.gov/opppmsd1/PR_Notices/pr2001-5.pdf). EPA approves all pesticide label use instructions based on the agency's evaluation of supporting data supplied by the pesticide registrant or manufacturer. After EPA approves a pesticide label, it is a violation of federal law to use the pesticide for a use or in a manner not in accordance with the label directions.

Monsanto incorporates EPA's guidelines for pesticide resistance management labeling on its glyphosate-based agricultural herbicide labels, and will do so on the label for products to be applied over the top of hybrids developed from MON 87427 (An example of current Roundup WeatherMAX product label is available at www.cdms.net/ldat/ld5UJ029.pdf). EPA-approved labels for Roundup branded herbicide weed-resistant management recommendations are designed to minimize the potential for the development of glyphosate-resistant weeds. By approving a label for a glyphosate-based agricultural herbicide, EPA has concluded that the product will not cause

unreasonable adverse effects to the environment or human health when used in accordance with the label's directions.

The weed resistance management guidelines on the labels of Roundup agricultural herbicides include recommendations that are well-documented in the scientific literature as being appropriate and effective for weed control and to mitigate weed resistance. Significant research has been conducted to identify the appropriate application rate of glyphosate required to control a particular weed at various growth stages under various agronomic and environmental conditions. These rates are based on over 35 years of ongoing research at Monsanto to evaluate the efficacy of Roundup agricultural herbicides. Studies have included efficacy of weed control for a broad spectrum of weeds and under a wide range of conditions. A key element of effective weed control and weed resistance management, therefore, is using the correct rate of glyphosate at the right time for the weed species and the size of the weed (i.e., using a lethal dose which avoids the need for subsequent applications). This important strategy is well-supported by field research studies at several universities (Jeschke and Stoltenberg, 2006; Stoltenberg, 2002; Wilson, et al., 2006). Additionally, it is accepted in the weed science community that the use of multiple herbicide modes of action via tank mixtures, use of herbicides with different modes of action in a rotational crop, or using multiple herbicides in sequence within a crop will reduce the risk of developing weed resistance (Beckie, 2006; Gressel and Segel, 1990). Tank-mixing involves mixing two or more herbicides in the spray tank immediately prior to application. To provide growers with the tools needed to minimize resistant weed development, Monsanto will continue to investigate and recommend appropriate residual and postemergence herbicide products that have a different mode of action from glyphosate. As an example, the herbicide metolachlor (tradename DUAL II MAGNUM™) is a residual herbicide that will help reduce flushes of annual grasses and pigweed which could slow the selection and potential spread of glyphosate-resistant weeds in Roundup Ready maize systems. The general concept that Monsanto promotes for management of resistance has been referred to by several authors as applying "diversity" across cropping/fallow seasons to manage weed resistance (Beckie, 2006; Powles, 2008). Crop rotation and management of the fallow period and cover crops, can be important considerations in managing resistance.

Table J-2. Management Recommendations for Control of Glyphosate Resistant Weeds

Glyphosate Resistant Weed	**State**	**Crop**	**Recommendations for alternative herbicides to manage glyphosate resistant weeds**	**Reference** (Bulletin No.)
Palmer amaranth	AR	Soybean	Burndown: flumioxazin and Pre: flumioxazin or metolachlor, and/or Post: fomesafin	U of AR (FSA2152) www.uaex.edu
	AR	Cotton	PPI: triflualin or pendimethalin and/or Pre: diuron or fluometuron and/or E. Post: metolachlor and/or Post directed: diuron or prometryn or Layby: flumioxazin	U of AR (FSA2152) www.uaex.edu
Waterhemp	MO	Maize	Pre: metolachlor or acetachlor or isoxaflutole or mesotrione or atrazine, and/or Post: atrazine or dicamba or 2,4D	U of MO (IPM1030) www.extension.missouri.edu
	MO	Soybean	Pre: metribuzine or sulfentrazone or metolachlor or flumioxazin and/or Post: lactofen or fomesafen or aciflorfen	U of MO (IPM1030) www.extension.missouri.edu
Common ragweed	OH / IN	Maize	Pre: atrazine or dicamba or acetochlor and/or Post: dicamba or tembotrione or mesotrione, or troprmezone	2010 OH/IN Weed Control Guide (789) www.btny.purdue.edu/weedscience
	OH / IN	Soybean	Burndown: 2,4D and Pre: metribuzine or flumioxazin or cloransulam and/or Post: cloransulam or fomesafen or lactofen	2010 OH/IN Weed Control Guide (789) www.btny.purdue.edu/weedscience
Giant ragweed	OH / IN	Maize	Burndown: 2,4D +atrazine and Pre: Lumax or atrazine+isoxaflutole and/or Post: atrazine or dicamba or tembotrione or mesotrione, or troprmezone	2010 OH/IN Weed Control Guide (789) www.btny.purdue.edu/weedscience

Table J-2 (cont.). Management Recommendations for Control of Glyphosate Resistant Weeds

Glyphosate Resistant Weed	**State**	**Crop**	**Recommendations for alternative herbicides to manage glyphosate resistant weeds**	**Reference** (Bulletin No.)
Giant ragweed	OH / IN	Soybean	Burndown: 2,4D and Pre: Canopy or Envive or imazaquin or Authority or flumioxazin or cloransulam and/or Post: cloransulam or fomesafen or lactofen+bentazon	2010 OH/IN Weed Control Guide (789) www.btny.purdue.edu/weedscience
Marestail	TN	Maize	Burndown: 2,4D or dicamba or Pre: atrazine and/or Post: dicamba	2010 TN Weed Control Guide (PB1580) www.weeds.utk.edu
		Soybean	Burndown: 2,4D or dicamba or flumioxizin or Pre: metribuzin or fluioxazin and/or Post:cloransulam	2010 TN Weed Control Guide (PB1580) www.weeds.utk.edu
		Cotton	Burndown: dicamba or flumioxazin or trifloxysulfuron or Pre: fluometuron or diron or prometryn and/or Post:trifloxysulfuron and/or Post-directed: flometuron+MSMA or diuron+MSMA or prometryn+trifloxysulfuron	2010 TN Weed Control Guide (PB1580) www.weeds.utk.edu

(Burndown=before planting; Pre= preemergence; Post= postemergence; Post-directed= applied postemergence directed at the base of the crop; PPI=Pre Plant Incorporated)

J.9. Monsanto Weed Performance Evaluation and Weed Resistance Management Plan

Monsanto and/or Monsanto seed company Licensees are directly in a position to be aware of the performance of glyphosate in all seed maize production fields and will be in a position to directly work with the growers to manage poor-performance situations and, if appropriate, perform follow up testing to determine if the poor performance was related to resistance. In addition, Monsanto is in a position to be aware of the performance of glyphosate at the end-user level through its extensive presence in the markets where Roundup Ready maize is grown, through its relationship with farm advisors, and its relationship with key University/CES personnel. This will allow the timely recognition of performance issues that could arise related to weed resistance or other means. Monsanto field employees and hired consultants are trained and provided processes for responding to product performance inquiries. As warranted individual performance issues that could be related to potential resistance are promptly handled. In addition performance inquires are periodically reviewed for trends that could indicate the need for follow up action on a broad scale. If broad scale actions in the areas where seed maize is produced, Monsanto and/or Licensees will alert the seed maize growers of the need for any prescribed action.

In general, when resistance is confirmed, the scientific and grower communities are notified and a weed resistance mitigation plan is implemented by Monsanto in cooperation with the University/CES. The mitigation plan is designed to manage the resistant biotype through effective and economical weed management recommendations implemented by the grower. The scope and level of intensity of the mitigation plan may vary depending on a combination of the following factors: (a) biology and field characteristics of the weed (seed shed, seed dormancy, etc.), (b) importance of the weed in the agricultural system, (c) resistance status of the weed to other herbicides with alternate modes of action, and (d) availability of alternative control options. These factors are analyzed by Monsanto and University/CES personnel in combination with economic and practical management considerations to develop a tailored mitigation strategy. The plan considers what is technically appropriate for the particular weed and incorporates practical management strategies that can be implemented by the grower.

After a mitigation plan is developed, Monsanto communicates the plan to the grower community through the use of supplemental labeling (labeling which includes newly approved uses, use directions, or other instructions which have been added since the last EPA-approved Master label), informational fact sheets, retailer training programs, agriculture media and/or other means, as appropriate.

In addition to the grower inquiry initiated process, Monsanto, alone and in cooperation with University/CES, conducts field studies to understand the potential for weed resistance and weed shifts as the result of various weed management programs implemented in a Roundup Ready maize system. These studies allow researchers to better track specific factors that can influence the development of resistance to specific weeds.

J.10. Summary

Development of weed resistance is a complex process. No single agronomic practice will mitigate resistance for all herbicides or all weeds. As a result, weed resistance needs to be managed on a case-by-case basis and tailored for the particular herbicide and weed in order to meet grower needs. Using good weed management principles, built upon achieving high levels of control through proper application rate, choice of cultural practices, and appropriate companion weed control tools will allow Roundup agricultural herbicides to continue to be used effectively. In cases where weed populations have developed resistance to glyphosate, effective management options are available and experience has shown that growers continue to find value in using glyphosate in their weed control programs.

The key principles for effective stewardship of glyphosate use, including Roundup Ready crops, include: (1) basing weed management and weed resistance management practices on local needs and using the tools necessary to optimize crop yield, (2) using proper rate and timing of application, (3) not relying solely on one herbicide weed control option across a cropping system, (4) responding rapidly to instances of unsatisfactory weed control, and (5) providing up to date weed management and weed resistance management training.

References for Appendix J

Baerson, S.R., D.J. Rodriguez, M. Tran, Y. Feng, N.A. Biest and G.M. Dill. 2002. Glyphosate-resistant goosegrass. Identification of a mutation in the target enzyme 5-enolpyruvylshikimate-3-phosphate synthase. Plant Physiology 129: 1265-1275.

Beckie, H.J. 2006. Herbicide-resistant weeds: Management tactics and practices. Weed Technology 20: 793-814.

Busi, R., S.M. Velayudhan and S.B. Powles. 2009. Use of below label herbicide rates can lead to evolution of herbicide resistant weeds. IBG Workshop, Weed Science Society of America, Lawrence, Kansas. http://wssa.net/Meetings/WSSAAbstracts/abstractsearch.php [Accessed June 24, 2009].

Cole, D.J. 1985. Mode of action of glyphosate-a literature analysis. Pages 48-74 in The Herbicide Glyphosate. E. Grossbard and D. Atkinson (eds.). Butterworth's & Co., Boston, Massachusetts.

Dill, G.M. 2005. Glyphosate-resistant crops: History, status and future. Pest Management Science 61: 219-224.

Dill, G.M., C.A. CaJacob and S.R. Padgette. 2008. Glyphosate-resistant crops: Adoption, use and future considerations. Pest Management Science 64: 326-331.

Ferguson, G.M., A.S. Hamill and F.J. Tardif. 2001. ALS inhibitor resistance in populations of Powell amaranth and redroot pigweed. Weed Science 49: 448-453.

Franz, J.E., M.K. Mao and J.A. Sikorski. 1997. Uptake, transport, and metabolism of glyphosate in plants. Pages 143-186 in Glyphosate: A Unique Global Herbicide. American Chemical Society, Washington, D.C.

Gaines, T.A., W. Zhang, D. Wang, B. Bukun, S.T. Chisholm, D.L. Shaner, S.J. Nissen, W.L. Patzoldt, P.J. Tranel, A.S. Culpepper, T.L. Grey, T.M. Webster, W.K. .Vencill, R.D. Sammons, J. Jiang, C. Preston, J.E. Leach and P. Westra. 2010. Gene amplification confers glyphosate resistance in *Amaranthus palmeri*. Proceedings of the National Academy of Sciences of the United States of America 107: 1029-1034.

Giesy, J.P., S. Dobson and K.R. Solomon. 2000. Ecotoxicological risk assessment for Roundup® Herbicide. Pages 35-120 in Reviews of Environmental Contamination and Toxicology. Volume 167. Springer-Verlag, New York, New York.

Gressel, J. and L.A. Segel. 1990. Modeling the effectiveness of herbicide rotations and mixtures as strategies to delay or preclude resistance. Weed Technology 4: 186-198.

Gustafson, D.I. 2008. Sustainable use of glyphosate in North American cropping systems. Pest Management Science 64: 409-416.

Heap, I. 2010. International survey of herbicide resistant weeds. Weed Science, Corvallis, Oregon. www.weedscience.org/in.asp [Accessed June 23, 2010].

HRAC. 2009. Classification of herbicides according to mode of action. Herbicide Resistance Action Committee, Corvallis, Oregon. http://www.hracglobal.com/Publications/ClassificationofHerbicideModeofAction/tabid/222/Default.aspx [Accessed June 29, 2009].

Jasieniuk, M., A.L. BruleBabel and I.N. Morrison. 1996. The evolution and genetics of herbicide resistance in weeds. Weed Science 44: 176-193.

Jeschke, M.R. and D.E. Stoltenberg. 2006. Weed community composition over eight years of continuous glyphosate use in a corn-soybean rotation. Pages 33-34, Weed Science Society of America, Lawrence, Kansas.

Neve, P. 2008. Simulation modelling to understand the evolution and management of glyphosate resistance in weeds. Pest Management Science 64: 392-401.

Peterson, D., B. Olson, K. Al-Khatib, R. Currie, J.A. Dille, J. Falk, P. Geier, D. Regehr, P. Stahlman and C. Thompson. 2007. Glyphosate stewardship. MF-2767, Kansas State University Agricultural Experiment Station and Cooperative Extension Service, Manhattan, Kansas.

Powles, S.B. 2008. Evolved glyphosate-resistant weeds around the world: lessons to be learnt. Pest Management Science 64: 360-365.

Reddy, K.N., A.M. Rimando, S.O. Duke and V.K. Nandula. 2008. Aminomethylphhosphonic acid accumulation in plant species treated with glyphosate. Journal of Agricultural and Food Chemistry 56: 2125-2130.

Ross, M.A. and C.A. Lembi. 1985a. Weed life cycles and control. Pages 216-217 in Applied Weed Science. Purdue University, West Lafayette, Indiana.

Ross, M.A. and C.A. Lembi. 1985b. Characteristics and importance of weeds. Pages 10-11 in Applied Weed Science. Purdue University, West Lafayette, Indiana.

Sammons, R.D., D.C. Heering, N. Dinicola, H. Glick and G.A. Elmore. 2007. Sustainability and stewardship of glyphosate and glyphosate-resistant crops. Weed Technology 21: 347-354.

Schonbrunn, E., S. Eschenburg, W.A. Shuttleworth, J.V. Schloss, N. Amrhein, J.N.S. Evans and W. Kabsch. 2001. Interaction of the herbicide glyphosate with its target enzyme 5-enolpyvuvylshikimate 3-phosphate synthase in atomic detail. Proceedings of the National Academy of Sciences of the United States of America 98: 1376-1380.

Shaner, D.L. 2009. Role of translocation as a mechanism of resistance to glyphosate. Weed Science 57: 118-123.

Stoltenberg, D.E. 2002. Weed management and agronomic risks associated with glyphosate-resistant corn and soybean cropping systems. Proceedings of the Fertilizer Aglime and Pest Management Conference, University of Wisconsin Cooperative Extension Service, Madison, Wisconsin.

Tranel, P.J. and T.R. Wright. 2002. Resistance of weeds to ALS-inhibiting herbicides: What have we learned? Weed Science 50: 700-712.

Weaver, S.E. 2001. The biology of Canadian weeds. 115. *Conyza canadensis*. Canadian Journal of Plant Science 81: 867-875.

Williams, G.M., R. Kroes and I.C. Munro. 2000. Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. Regulatory Toxicology and Pharmacology 31: 117-165.

Wilson, R.G., S. Miller, P. Westra and P. Stahlman. 2006. Sustainability of glyphosate resistant cropping systems. Page 60 in Weed Science Society of America Proceedings, Lawrence, Kansas.

WSSA. 2000. Classification of herbicides according to mode of action. Weed Science Society of America, Herbicide Resistance Action Committee, Champaign, Illinois. http://www.plantprotection.org/HRAC/Bindex.cfm?doc=MOA.html [Accessed August 11, 2010].

Yu, Q., A. Cairns and S. Powles. 2007. Glyphosate, paraquat and ACCase multiple herbicide resistance evolved in a *Lolium rigidum* biotype. Planta 225: 499-513.

Appendix K: Potential Impact of Glyphosate on Human Health and the Environment

K.1. Overview

Glyphosate is a herbicide approved for use (registered) by the U.S. Environmental Protection Agency (EPA or the agency) for the control of weeds that would interfere with the growth of many food and non-food crops, including biotechnology-derived crops, as well as for control of weeds growing in non-crop areas. It has been registered, and food and feed tolerances have been established in the U.S. for its residues, since 1979. In 2001, EPA identified glyphosate as the most widely used conventional agricultural herbicide in the U.S. (Kiely et al., 2004).

Glyphosate has a comprehensive regulatory data base that has been evaluated by EPA to support all currently approved uses. EPA has repeatedly stated that it has a high level of confidence in the quality of the existing studies and the reliability of the toxicity endpoints that are the basis for human health and environmental risk assessments (U.S. EPA, 2006a; U.S. EPA, 2006b). In establishing food and feed tolerances to support the use of glyphosate on animal feed and forage crops, EPA concluded, "that there is a reasonable certainty that no harm will result to the general population, and to infants and children from aggregate exposure to glyphosate residues" (U.S. EPA, 2006d).

The following discussion provides an overview of the regulatory and risk assessment processes applicable to glyphosate and all other agricultural use pesticides. Glyphosate has been approved by the EPA for a large number of food and feed uses, including uses associated with glyphosate-tolerant crops. Over 180 food and feed tolerances (40 CFR § 180.364) have been established for glyphosate in support of these uses. A complete listing of all U.S. glyphosate tolerances is provided in Attachment 1.

K.1.1. Pesticide Registration and Tolerance Setting

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) requires that before sale or distribution of a pesticide in the U.S., a person or company must obtain a registration, or license, from EPA. Before registering a new pesticide or a new use for a previously registered pesticide, EPA must first ensure that the pesticide, when used according to its label directions, will not cause unreasonable adverse effects on the environment. In order to address this standard, EPA must evaluate potential risks to humans and the environment, and may require applicants to submit more than 100 different scientific studies and tests conducted according to EPA guidelines. According to EPA, glyphosate is one of more than 1055 active ingredients currently registered as pesticides, which are formulated into many thousands of pesticide products that are available in the marketplace.

The process of registering a pesticide is a scientific, legal, and administrative procedure through which EPA examines the ingredients of the pesticide; the particular site or crop on which it is to be used; the amount, frequency, method and timing of application, and other conditions of its use; and storage and disposal practices. In evaluating a pesticide

registration application, EPA assesses a wide variety of potential human health and environmental effects associated with use of the product.

The data required by EPA are used to evaluate whether a pesticide has the potential to cause adverse effects on humans, wildlife, fish, and plants (including endangered species and non-target organisms that the pesticide is not intended to act against). This includes potential human health and safety risks range from short-term toxicity to long-term effects such as cancer and reproductive system disorders. The registration applicant must also supply data addressing the pesticide's potential impact on surface water or ground water (which might result from leaching or runoff, for example).

EPA also must approve the language that appears on each pesticide label. A pesticide product can only be used legally according to the directions for use on the labeling accompanying it at the time of sale. Following these directions carefully and precisely is necessary to ensure safe use as defined by FIFRA.

A pesticide's initial registration is not the only opportunity an agency like the EPA has to evaluate that product's safety. For example the 1988 amendments to the FIFRA authorized EPA to conduct a re-registration program of pesticides first registered before November 1, 1984. The goal of the re-registration program was to ensure that these pesticides met current scientific and regulatory standards and may be declared "eligible" for re-registration. The results of EPA's reviews are summarized in Re-registration Eligibility Decision (RED) documents. In 1993 the EPA produced a 291-page RED on glyphosate (EPA, 1993), setting forth the data on which it made a decision to reregister all then-existing uses of the pesticide, based on the pesticide having met the no unreasonable adverse effects standard found in FIFRA. As mandated by the Food Quality Protection Act of 1996, EPA initiated the Registration Review program to periodically re-evaluate all registered pesticides to ensure that as changes in science, public policy, and pesticide use practices occur, products in the marketplace can still be used safely. The Registration Review process for glyphosate started in 2009 and is expected to be completed in 2015. During the Registration Review process, EPA will be requesting generation of additional data for glyphosate due to recent changes in FIFRA data requirements and updating the risk assessments for all currently registered glyphosate uses.

EPA also sets tolerances (maximum pesticide residue levels), where pesticides may be used on food or feed crops, for the amount of the pesticide that can legally remain in or on foods. EPA undertakes this analysis under the authority of the Federal Food, Drug, and Cosmetic Act (FFDCA). Under the FFDCA, EPA must find that such tolerances will be safe, meaning that there is a reasonable certainty that no harm will result from aggregate exposure to the pesticide chemical residue and other potential exposure routes. This finding must be made and the appropriate tolerance established before a pesticide can be registered for use on the particular food or feed crop in question. Several factors must be addressed before a tolerance can be established, including:

- the aggregate exposure from the pesticide (now including occupational exposure,[10] exposure through diet, from using pesticides in and around the home, and from drinking water);

- the cumulative effects from exposure to different pesticides that produce similar effects in the human body;

- whether there is increased exposure to infants and children, or other potentially high exposure subpopulations; and

- whether the pesticide produces an effect in humans similar to an effect produced by a naturally occurring estrogen or produces other endocrine-disruption effects.

K.1.2. Pesticide Risk Assessment

The process EPA uses for evaluating the health impacts of a pesticide, under either FIFRA or the FFDCA, is called risk assessment. EPA uses the National Research Council's four-step process for human health risk assessment, which involves hazard identification, dose-response assessment, exposure assessment and risk characterization. Each of these steps is discussed below:

The first step in the risk assessment process is to identify potential health effects, or hazards that may occur from different types of pesticide exposure. EPA considers the full spectrum of a pesticide's potential health effects. Hazards are identified through a battery of studies that examine the potential toxicity of the pesticide in various tests including, where appropriate, tests with laboratory animals.

Generally, for human health risk assessments, many toxicity studies are conducted, based on EPA guidelines, by pesticide companies in independent laboratories following the Good Laboratory Practice (GLP) standards, and evaluated for acceptability by EPA scientists. EPA evaluates pesticides for a wide range of effects, from eye and skin irritation to cancer and birth defects. EPA may also consult the public literature or other sources of information on any aspect of the chemical.

The next step of the risk assessment considers the levels at which the pesticide produces adverse effects. Dose-response assessment involves considering the dose levels at which adverse effects were observed in test animals, and using these dose levels to calculate an equal dose in humans.

Step three of the process involves an exposure assessment. People can be exposed to pesticides in three ways:

[10] Historically, issues associated with potential occupational exposure for each new use are were considered seapartely under FIFRA's unreasonable risk standard; however, under a recently announced revised policy, EPA has stated that it intends to begin to include occupational exposure into the aggregate assessment (74 FR 65121, EPA–HQ–OPP–2009-0889; December 9, 2009).

1. Inhaling pesticides (inhalation exposure),
2. Absorbing pesticides through the skin (dermal exposure), and
3. Ingesting pesticides (oral exposure).

Depending on the situation, pesticides could enter the body by any one or all of these routes. Typical sources of pesticide exposure include agricultural (food); home and personal use pesticides; pesticides applied to lands that make their way into the drinking water; or occupational exposure for agricultural workers or pesticide applicators.

Risk characterization is the final step in assessing human health risks from pesticides. It is the process of combining the hazard, dose-response and exposure assessments to describe the overall risk from the use of a pesticide. It explains the assumptions used in assessing exposure as well as the uncertainties that are built into the dose-response assessment. The strength of the overall database is considered, and broad conclusions are made. EPA's role is to evaluate both toxicity and exposure and to determine the risk associated with use of the pesticide.

The risk to human health from pesticide exposure depends on both the toxicity of the pesticide and the likelihood of people coming into contact with it (exposure). At least *some* exposure and *some* toxicity are required to result in a risk. For example, if the pesticide is found to have a high level of toxicity, but people are not exposed to the pesticide, there is no risk. Likewise, if there is ample exposure but the pesticide is essentially nontoxic, there is no risk. However, usually when pesticides are used, there is some toxicity and exposure, which results in a potential risk.

EPA recognizes that effects of exposure to all pesticides including glyphosate vary between animals of different species (interspecies extrapolation) and from person to person (intraspecies variability). To account for this variability, a 100-fold *uncertainty factor* is built into the risk assessment (10X for interspecies extrapolation and 10X for intraspecies variability). This uncertainty factor creates an additional margin of safety for protecting people who may be exposed to the pesticides. FQPA requires EPA to use an extra 10-fold safety factor, if necessary; this additional factor is meant to afford additional protection to infants and children from the potential effects of the pesticide.

Once EPA completes the risk assessment process for a pesticide, the Agency uses this information to determine if (when used according to label directions), there is a reasonable certainty that the pesticide will not harm a person's health and to not cause unreasonable adverse effects on the environment.

Using the conclusions of a risk assessment, EPA can then make a more informed decision regarding whether to approve a pesticide chemical or use, as proposed, or whether additional protective measures are necessary to limit occupational or non-occupational exposure to a pesticide. For example, EPA may prohibit a pesticide from being used on certain crops because consuming that commodity treated with the pesticide may result in an unacceptable risk to consumers. Another example of protective measures is requiring workers to wear personal protective equipment (PPE) such as a respirator or chemical

resistant gloves, or not allowing workers to enter treated crop fields until a specific period of time has elapsed.

If, after considering all appropriate risk reduction measures, the pesticide still does not meet EPA's safety standard, the Agency is legally mandated not to approve the proposed chemical registration or allow its use. Regardless of the specific measures enforced, EPA's primary goal is to ensure that legal uses of the pesticide are protective of human health, especially the health of children, and the environment.

K.2. Potential Impact of Glyphosate on Human Health

Glyphosate presently has 186 established food and feed tolerances in the U.S (see Attachment 1). Each time EPA reviews (U.S. EPA, 2006a; U.S. EPA, 2006b) an application to add a new food or feed use to the glyphosate label the Agency is required by FFDCA to conduct an aggregate risk assessment, considering all sources of human exposure to the pesticide, and find that aggregate exposure to the pesticide will be safe as defined by the statute and regulations. As noted above, historically, issues associated with potential occupational exposure were considered separately under FIFRA's unreasonable risk standard; however, under a recently announced revised policy, EPA has stated that it intends to begin to include occupational exposure into the aggregate assessment (74 FR 65121, EPA–HQ–OPP–2009-0889; December 9, 2009).

Over the course of these numerous reviews (U.S. EPA, 2006a; U.S. EPA, 2006b), the toxicology of glyphosate has been extensively studied. Multiple comprehensive toxicological studies in animals have demonstrated that glyphosate does not cause cancer, birth defects, mutagenic effects, nervous system effects or reproductive problems (U.S. EPA, 1993; EC, 2002; WHO/FAO 2004). In fact, after a thorough review of all available toxicology data, the EPA concluded that glyphosate should be classified in Group E - Evidence of Non-carcinogenicity in Humans, the most favorable category possible (U.S. EPA, 1993).

K.2.1. Glyphosate Safety Evaluations

Despite this extensive safety data, glyphosate safety is reviewed with every new use for which registration is sought, including, where necessary, uses associated with glyphosate-tolerant crops developed through biotechnology. As discussed above, prior to the approval of any new use of an existing registered pesticide, EPA must consider the potential human health effects from the aggregate (total combined) human exposure to that pesticide, combining the potential exposure from the proposed new use with all other existing exposures to the pesticide. Dietary exposure is considered, which addresses pesticide residues that may remain on food from crops on which the pesticide is applied (pre- or postemergence), as well as any residue that could be found in drinking water as a result of pesticide use. Non-dietary exposure is also included in this assessment, which includes exposure to the pesticide through residential use, such as on lawns or in flower beds, as well as exposure in a recreational context, such as from a golf course or sports field. Based on these data, EPA must be able to make a determination of reasonable certainty of no harm to human health as required by the FFDCA.

EPA does not conduct an acute dietary risk assessment for glyphosate because no acute human health concerns have ever been determined from toxicological studies conducted with glyphosate. Accordingly, EPA does not expect glyphosate to pose an acute risk (U.S. EPA, 2006a; U.S. EPA, 2006b; U.S. EPA, 2006c). EPA does conduct a chronic dietary (food and water) risk assessment for glyphosate based on a theoretical worst case exposure estimate. For food, this estimate assumes that glyphosate is used on 100 percent of all the crops on which the pesticide is currently approved for use. It further assumes that the resulting pesticide residues found on all harvested food crops are at the level of the legally established tolerance (i.e., the maximum allowable pesticide residue level). For water, EPA assumes that glyphosate is used to control weeds in water bodies by direct application to the water at the maximum application rate, without taking into account degradation in the water body or partitioning to sediment within the water column (U.S. EPA, 2006a; U.S. EPA, 2006b).

Applying this unrealistic, theoretical maximum exposure estimate, EPA determines how much of the established Reference Dose (RfD) would be utilized by all currently approved product uses. The chronic RfD (cRfD) is an estimate of the amount of daily pesticide exposure to the human population that can occur over a lifetime with a reasonable certainty of no harm to human health.[11] For glyphosate, the RfD is 1.75 mg per kg body weight per day (mg/kg/day) (U.S. EPA, 2006a; U.S. EPA, 2006b). EPA will utilize the Chronic Population Adjusted Dose (cPAD, the cRfD with any FQPA uncertainty factors applied) from aggregated exposures and from the exposure assessment determine if these exposures not exceed the EPA level of concern (i.e., 100 percent of the cPAD).

If the aggregate risk assessment shows that utilization of the cPAD does not exceed the EPA level of concern, then EPA will conclude that the new use does not pose an unreasonable risk to human health. EPA will then establish or revise, as needed, any food or animal feed crop tolerances to allow for the presence of glyphosate residue on that crop. However, under a recently announced policy aimed at increasing transparency to the general and regulated public, EPA may choose to publish the risk assessment and proposed regulatory decision on the Office of Pesticides website and ask for public comment; this posting occurs prior to the establishment of the tolerances (http://www.epa.gov/pesticides/regulating/registration-status.html). EPA publishes the new tolerances in the Federal Register, along with a final summary of the risk assessment and approves pesticide labelling for the new use. In issuing the final tolerance rule, EPA considers and discusses any comments received in response to the original notice regarding EPA's intention to establish tolerances that was published in the Federal Register and any comments received in the transparency policy notice.

Despite the large number of approved food and feed uses of glyphosate, including uses associated with glyphosate-tolerant crops, a large margin of safety exists for glyphosate. While use of glyphosate has increased in the decade since the introduction of glyphosate-tolerant crops, the associated risk to human health as a result of the increased human

[11] RfD is the current terminology used by EPA; however earlier EPA risk assessment terminology used the term Allowable Daily Intake (ADI). RfD and ADI are synonymous.

exposure to glyphosate remains low, due to the low mammalian toxicity of glyphosate and the relatively low dietary exposure associated with the herbicide's approved uses.

Prior to the first approval of a glyphosate-tolerant crop (soybean) in 1996, theoretical dietary exposure for all registered conventional uses of glyphosate utilized approximately 2.9% of the glyphosate RfD for the most highly exposed subpopulation of non-nursing infants less than one year old (U.S. EPA, 1993). A more recent EPA risk estimate determined chronic dietary exposure estimates for food and drinking water to glyphosate to be below the EPA level of concern for both the general U.S. population and population subgroups; 2% and 9% of the chronic population adjusted dose (cPAD) for the general population and non-nursing infants (most exposed subpopulation) respectively (U.S. EPA, 2006b). The combined short/intermediate estimated exposure for dietary and non-dietary exposures with all current registered uses of glyphosate utilizes only 11% of the glyphosate cPAD for the most sensitive subpopulation of non-nursing infants less than one year old (U.S. EPA, 2006b). The utilization of the glyphosate cPAD, which is well below 100 percent, has allowed EPA to continue to make the conclusion of reasonable certainty of no harm to human health for each glyphosate use, including new glyphosate-tolerant crop uses.

These figures are supported by the data provided in the tables below. Table K-1 summarizes the established food and feed tolerances supporting the use of glyphosate in the conventional crops of alfalfa, cotton, sugar beet and soybean prior to the first glyphosate-tolerant crop in 1996. A summary of the regulatory approvals, including new or modified food and feed tolerances, and associated dietary exposure assessments for approved glyphosate-tolerant crops is provided in Table K-2. Table K-3 summarizes the most recent chronic and short/intermediate-term aggregate risk assessments for glyphosate.

Table K-1. Established Glyphosate Tolerances Prior to Glyphosate-tolerant Crops (1993)

Crop	Established Food/Feed Tolerances	Publication	% of Reference Dose (RfD)
Soybean	• Seed – 20 ppm • forage & hay – 15 ppm • hulls – 100 ppm	Glyphosate Re-registration Eligibility Decision Document Sept. 1993 (U.S. EPA, 1993)	General Population - 1.2 Non-nursing infants <1 year old - 2.9
Alfalfa	200 ppm		
Cotton	forage, hay, & seed – 15 ppm		
Sugar beet	Roots – 0.2 ppm		
Maize	0.1 ppm seed 0.2 ppm forage/fodder		
Canola	none		

Table K-2. Summary of EPA Approvals for Glyphosate Use in Glyphosate-tolerant Crops

	Commercial Introduction Year	Required Changes in Food/Feed Tolerances	Federal Register Publication Establishing New or Modified Tolerance	Dietary Exposure Only (Food + Water)
Roundup Ready® soybean	1996	• Increase soybean forage to 100 ppm. • Increase soybean hay to 200 ppm. • Establish new tolerance for aspirated grain fractions at 50 ppm.	61 FR 15192 Petition No. 4F4369 Apr. 1996 (U.S. EPA, 1996b)	General Population – 1% of RfD Non-nursing infants- 2.5% of RfD
Roundup Ready cotton	1997	Establish new tolerance for gin byproduct at 100 ppm.	61 FR 7729 Petition No. 5F4493 Feb. 1996 (U.S. EPA, 1996a)	General Population – 1% of RfD Non-nursing infants - 2.4% of RfD
Roundup Ready maize	1998	Establish new tolerance for maize forage at 1 ppm.	62 FR 17723 Petition No. 5F4555 Apr. 1997 (U.S. EPA, 1997)	General Population – 1% of RfD Non-nursing infants < 1 year old – 3% of RfD
Roundup Ready canola	1999	Establish new tolerances for canola. • seed at 10 ppm • meal at 15 ppm	64 FR 18360 Petition No. 2E4118 Apr. 1999 (EPA, 1999)	General Population - 1.5% of RfD Non-nursing infants <1 year old - 3.3 % of RfD
Roundup Ready sugar beet	2008	Establish new tolerances for sugar beet. • roots at 10 ppm • tops at 10 ppm • pulp (dried) at 25 ppm		
Roundup Ready maize 2	2004	Increased tolerance for maize forage to 6 ppm.	68 FR 36472 Jun. 2003 (U.S. EPA, 2003)	Change in forage tolerance did not affect estimated dietary exposure from animal products; therefore no dietary risk assessment was conducted.
Roundup Ready Flex cotton	2006	• Increase tolerance for gin byproduct to 175 ppm. • Increase tolerance for cottonseed to 35 ppm.	69 FR 65081 Petition No. 3F6570 Nov. 2004 (U.S. EPA, 2004c)	General Population - 2.2% of cPAD All infants < 1 year old - - 3.9% of cPAD Children 1-2 years - 5.4% of cPAD

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Table K-2 (Continued) Summary of EPA Approvals for Glyphosate Use in Glyphosate-tolerant Crops

	Commercial Introduction Year	Required Changes in Food/Feed Tolerances	Federal Register Publication Establishing New or Modified Tolerance	Dietary Exposure Only (Food + Water)
Roundup Ready alfalfa	2006	Establish new tolerances for alfalfa seed at 0.5 ppm.	70 FR 7861 Petition No. 2F6487 Feb. 2005 (U.S. EPA, 2005)	Dietary exposure insignificant, did not conduct new risk assessment. Deferred to assessment conducted for flex cotton as published in 69 FR 65081.
Roundup Ready maize 2	2004	Increased tolerance for maize grain to 5 ppm.	73 FR 52607 Sept. 2008 (U.S. EPA, 2008a)	Tolerance adjusted to harmonize with CODEX.

Table K-3. Aggregate Exposure Assessment for Glyphosate

Population Subgroup	Acute Aggregate[2]	RfD (mg/kg/day) [2]	Chronic Aggregate[1,2]		Short/Intermediate Term Aggregate[2,3]	
			Exposure (mg/kg/day)	% cPAD	Exposure (mg/kg/day)	% RfD
General U.S. population	Not applicable	1.75	0.041	2	-	-
All infants (<1 year)			0.127	7	0.157	9
Non-nursing infants (<1 year)			**0.158**	**9**	**0.188**	**11**
Children 1-2 years			0.095	5	0.125	7
Children 3-5 years			0.088	5	0.118	7
Children 6-12 years			0.059	3	0.089	5
Youth 13-19 years			0.037	2	-	-
Adults 20-49 years			0.033	2	0.063	4
Adults 50+ years			0.028	2	-	-
Females 13-49 years			0.031	2	-	-

[1] These aggregate exposure assessments were performed by U.S. EPA prior to the issuance of latest guidance (December 2009), and thus do not include occupational exposure. As such, the chronic aggregate exposure estimated in this fashion was the same as chronic dietary exposure because chronic non-dietary exposure was not expected based upon the registered non-crop uses of glyphosate.

[2] U.S.EPA (2006b) OPPTS. Glyphosate Human Health Risk Assessment for Proposed Uses on Safflower and Sunflower. Petition No. 4E6878. Sept. 5, 2006.

3 Calculated from values given in U.S.EPA (2006b) OPPTS. Glyphosate Human Health Risk Assessment for Proposed Uses on Safflower and Sunflower. Petition No. 4E6878. Sept. 5, 2006.

K.2.2. Glyphosate Safety Evaluation for Applicator and Bystander Exposure

Another potential impact of the use of glyphosate on human health that EPA considers in its human health analysis is applicator and bystander exposure resulting from increased glyphosate use. Based on the toxicity of glyphosate and its registered uses, including use on glyphosate-tolerant crops, EPA has concluded that occupational exposures (short-term dermal and inhalation) to glyphosate are not of concern because no short-term dermal or inhalation toxicity endpoints have been identified for glyphosate (U.S. EPA, 2006a; U.S. EPA, 2006b).

Additional evidence to support the EPA conclusion can be found in the Farm Family Exposure Study (Acquavella et al., 2004), a biomonitoring study of pesticide applicators conducted by independent investigators. This biomonitoring study determined that the highest estimated systemic dose of glyphosate for applicators as the result of routine labeled applications of registered glyphosate-based agricultural herbicides to crops, including glyphosate-tolerant crops, was approximately 400 times lower than the RfD established for glyphosate. Furthermore, investigators determined that 40% of applicators did not have detectable exposure on the day of application, and 90% of the applicators had an estimated systemic dose of glyphosate more than 1000 times lower than the RfD (Acquavella et al., 2004).

The biomonitoring study also found little evidence of detectable exposure to individuals on the farm who were not actively involved in or located in the immediate vicinity of labeled applications of glyphosate-based agricultural herbicides to crops. Considering the similarity of the use pattern and application rates of the glyphosate products in this study compared to those registered for use on glyphosate-tolerant crops, bystander exposure attributed to the use of glyphosate on glyphosate-tolerant crops is expected to be negligible.

K.3. Potential Impact of Glyphosate on the Environment

Potential environmental effects are carefully considered as a part of the FIFRA pesticide registration process. Prior to the approval of a new pesticide or a new use (including a change in pesticide application rates and/or timing) and before reregistering an existing pesticide, EPA must consider the potential for environmental effects and make a determination that no unreasonable adverse effects to the environment will be caused by the new pesticide, new use or continued use.

To make this determination, EPA requires a comprehensive set of environmental fate and ecotoxicology data on the pesticide's active ingredient (40 CFR § 158). EPA uses these data to assess the pesticide's potential environmental risk (exposure/hazard). The required data include both short and long-term hazard data on representative organisms that are used to predict hazards to terrestrial animals (birds, nontarget insects, and mammals), aquatic animals (freshwater fish and invertebrates, estuarine and marine organisms), and nontarget plants (terrestrial and aquatic).

EPA re-evaluated the environmental safety of glyphosate in 1993 as part of the FIFRA-required re-registration of all pesticides. At the end of this evaluation, EPA concluded that all registered uses of glyphosate were eligible for re-registration, including terrestrial (i.e., land-based) applications up to 6 pounds glyphosate acid equivalents (a.e.) per acre on crops and 8 pounds glyphosate a.e per acre for certain limited uses.

Since the re-registration evaluation in 1993, EPA has reviewed and approved a significant number of new glyphosate uses: conventional crops such as legume vegetables and sunflower/safflower seed, glyphosate-tolerant crops such as alfalfa, maize, cotton, canola, sugar beet and soybean, and non-crop areas. In each case, EPA concluded that the new use, including any incremental environmental exposure to glyphosate caused by that new use, did not pose an unreasonable risk to the environment, and approved pesticide labeling for the new use.

The studies and data collected by Monsanto, both for the initial EPA registration and re-registration of glyphosate, as well as data developed by independent academics, present a well-established safety profile for glyphosate. The following sections provide greater detail regarding some of the key findings from these studies.

K.3.1. Persistence of Glyphosate in the Soil

Persistence of agricultural chemicals in the soil is widely regarded as an undesirable environmental characteristic. Glyphosate has been shown to degrade over time from most agricultural ecosystems across a wide range of soil and climatic conditions, with a median soil half-life (the time it takes for half of the glyphosate to dissipate in the soil) of 13.9 days (U.S. EPA, 1993). The potential for glyphosate to accumulate in soil following repeated applications has been studied both in the laboratory and the field.

A laboratory study was conducted on two soil samples, with each sample receiving up to three sequential applications of 5 pounds glyphosate a.e. per acre over a 6-week period, at two-week intervals. The concentration of glyphosate in soil 24 weeks following application had declined to 1-5% of the concentration immediately after application, regardless of whether it was the first, second or third application.

Glyphosate degradation in the soil following multiple glyphosate applications was also shown under field conditions. Soil was collected from pesticide efficacy and tolerance trials in orchards and vineyards that received repeated applications of glyphosate over a one- to six-year period, at cumulative rates of 6 to 120 pounds glyphosate a.e. per acre. These soil samples did not show any accumulation of glyphosate residues, even at the exaggerated rate of three sequential applications of eight pounds glyphosate a.e. per acre within a three-month interval for five out of six sequential years. Glyphosate degradation continued after multiple applications, and less than 10 percent of the total applied glyphosate remained in the soil one year after the last glyphosate application.

Just as in all other maize and other crops, a typical agronomic (annual) use pattern for glyphosate on MON 87427 could include a preemergence burn down application of up to 1.125 pounds of glyphosate acid equivalent (a.e.) per acre. This could be followed by

one to two early (up to V8) postemergence applications for weed control of up to 1.125 pounds glyphosate a.e. per acre. The unique feature of glyphosate use in MON 87427 is the two applications at early tassel development timings to produce a male sterile phenotype through tissue-selective glyphosate tolerance. Submitted labeling allows the two applications to be made at early tassel development timings are made between the V8 to V13 growth stages at up to 1.125 pounds glyphosate a.e. per acre. The total amount of glyphosate that could be applied per season (preemergence through preharvest) can not exceed 6 pounds glyphosate a.e. per acre, as in other crops. Thus, the maximum labeled rates and typical use patterns of glyphosate on MON 87427 are well within the rates and frequencies used in the soil persistence studies described above, and are within the current labeled rates of glyphosate applications to Roundup Ready maize 2. As a result, glyphosate is not expected to accumulate in soil due to labeled uses in MON 87427.

K.3.2. Persistence of Surfactant in the Soil

Herbicide products approved for application to emerged weeds normally are applied with surfactants. Glyphosate products are formulated with surfactants to increase the permeability of the cuticle wax of the weed foliage, resulting in increased foliar uptake of glyphosate. In other words, the surfactant acts to break down the plant's natural protective wax coating, allowing the plant to better absorb the glyphosate, thereby improving the efficacy of the herbicide.

One common surfactant used in formulated glyphosate products is polyethoxylated alkyl amine (POEA). When degradation of POEA was investigated in three types of soil (silt loam, silty clay loam, and sandy loam), microbial degradation was determined to be the primary degradation route, with minimal degradation occurring under sterile conditions. Approximately 25-30% of applied ^{14}C-POEA was mineralized to $^{14}CO_2$ within seven weeks. The estimated degradation half-life for parent POEA was less than one week and possibly as short as one to two days. Because limited data are available for POEA dissipation, a conservative estimate of half-life values for POEA in soil would be 7-14 days (Giesy et al., 2000). Glyphosate and the POEA surfactant have similar soil dissipation rates and the same primary route of dissipation, i.e., microbial degradation. Therefore, it is reasonable to assume that the POEA surfactant will behave similarly to glyphosate in field soil, and an increase in residual soil concentrations (accumulation) of the POEA surfactant is not anticipated as a result of increased use of glyphosate associated with the planting of MON 87427.

K.3.3. Surface Water and Groundwater

Glyphosate binds strongly to agricultural soils and has a low potential to move offsite to surface water or leach to groundwater (U.S. EPA, 1993). The EPA has used computer models to estimate worst-case glyphosate levels in surface water based on presently approved use patterns. Relying on toxicological data from acute and chronic tests on fish and other aquatic organisms, EPA has determined that "the potential for environmental effects of glyphosate in surface water is minimal" (U.S. EPA, 2002).

K.3.4. Wildlife

1. Animals: As a part of the re-registration evaluation under FIFRA, EPA conducted an ecological assessment for glyphosate. This assessment compared the results from toxicity tests with glyphosate conducted with various plant and animal species to a conservative estimate of the concentration of glyphosate to which an organism might be exposed in the environment. This estimate, called the Estimated Exposure Concentration (EEC), is a point estimate for exposure that does not take into account normal environmental dilution or dissipation, or the frequency of exposure to the pesticide by wildlife. In the Re-registration Eligibility Decision (RED) for glyphosate (U.S. EPA, 1993), the exposure estimates were determined assuming an application rate of 5.0625 lb a.e./A, which exceeds the maximum labeled use rate for a single application for agricultural purposes. When the EECs were calculated for aquatic plants and animals, the direct application of this rate to water was assumed. Based on this assessment, EPA concluded that effects to birds, mammals, fish and invertebrates are minimal based on available data (U.S. EPA, 1993).

Glyphosate is practically nontoxic to honey bees (which are used to assess effects on nontarget insects in general) and practically nontoxic to slightly toxic to birds, freshwater fish, marine and estuarine species, aquatic invertebrates and mammals (U.S. EPA, 1993). Glyphosate has a low octanol-water coefficient, indicating that it has a tendency to remain in the water phase rather than move from the water phase into fatty substances; therefore, it is not expected to accumulate in fish or other animal tissues.

The glyphosate end-use products used in agriculture contain a surfactant to facilitate the uptake of glyphosate into the plant (Ashton and Crafts, 1981). Depending on the surfactant used, the toxicity of the end-use product may range from practically nontoxic to moderately toxic to fish and aquatic invertebrates (U.S. EPA, 1993). For this reason, the 1993 Glyphosate RED stated that some formulated end-use products of glyphosate needed to be labeled as “Toxic to fish” if they were labeled for direct application to water bodies. Due to the associated hazard to fish and other aquatic organisms, glyphosate end-use products that are labeled for applications to water bodies generally do not contain surfactant, or contain a surfactant approved for direct application to water bodies.

2. Plants: Glyphosate is a non-selective herbicide with postemergence activity on essentially all annual and perennial plants. As such, exposure to glyphosate could put aquatic and terrestrial nontarget plants as well as threatened or endangered plants at risk (U.S. EPA, 1993). Nontarget plants may potentially be at risk from applications of glyphosate as a result of spray drift. As discussed earlier, glyphosate binds tightly to agricultural soils and is not likely to move offsite. Moreover, glyphosate is not taken up from agricultural soils by plants. Therefore, risks to nontarget plants are only attributed to the spray drift of the pesticide. Pesticide labels include specific risk management measures to manage spray drift, including mandatory requirements for aerial applications.

During the re-registration process in 1993, additional data on terrestrial nontarget plants were requested by the EPA. These additional data have been utilized in conjunction with an exposure assessment to further understand the potential risk to threatened and

endangered plants from the use of glyphosate herbicides in agriculture (Mortensen SR et al., 2008).

K.3.5. Endangered and Threatened Species

The EPA Endangered Species Protection Program web site, http://www.epa.gov/espp/, describes the EPA assessment process for endangered species. The essential elements of that process, generally taken from the web site, are summarized below.

The Endangered Species Act (ESA) was intended to protect and promote the recovery of animals and plants that are in danger of becoming extinct. All federal agencies are required under the ESA to ensure that their regulatory actions, including EPA's registration of pesticides in the U.S., are not likely to jeopardize the continued existence of threatened or endangered species ("listed" species) or destroy or adversely modify their critical habitat.

EPA's Endangered Species Protection Program (ESPP) helps promote the recovery of listed species. The ESPP is a program designed to determine whether pesticide use in a certain geographic area may affect any listed species.

When registering a pesticide or reassessing the potential ecological risks from use of a currently registered pesticide, EPA evaluates extensive toxicity and ecological effects data to determine how a pesticide will move through and break down in the environment. Risks to birds, fish, invertebrates, mammals and plants are routinely assessed and used in EPA's determinations of whether a pesticide may be licensed for use in the U.S.

EPA's core pesticide risk assessment and regulatory processes ensure that protections are in place for all populations of nontarget species. Because endangered species may need specific protection, EPA has developed risk assessment procedures described in the Overview of the Ecological Risk Assessment Process (U.S. EPA, 2004d) to determine whether individuals of a listed species have the potential to be harmed by a pesticide, and if so, what specific protections may be appropriate. EPA's conclusion regarding the potential risks a pesticide may pose to a listed species and any designated critical habitat for the species, after conducting a thorough ecological risk assessment, results in an "effects determination."

An assessment of the effects of glyphosate use on all types of threatened and endangered species was conducted by Monsanto. This assessment generally followed the procedures described in the Overview of the Ecological Risk Assessment Process (U.S. EPA, 2004d), as summarized in Figure K-1.

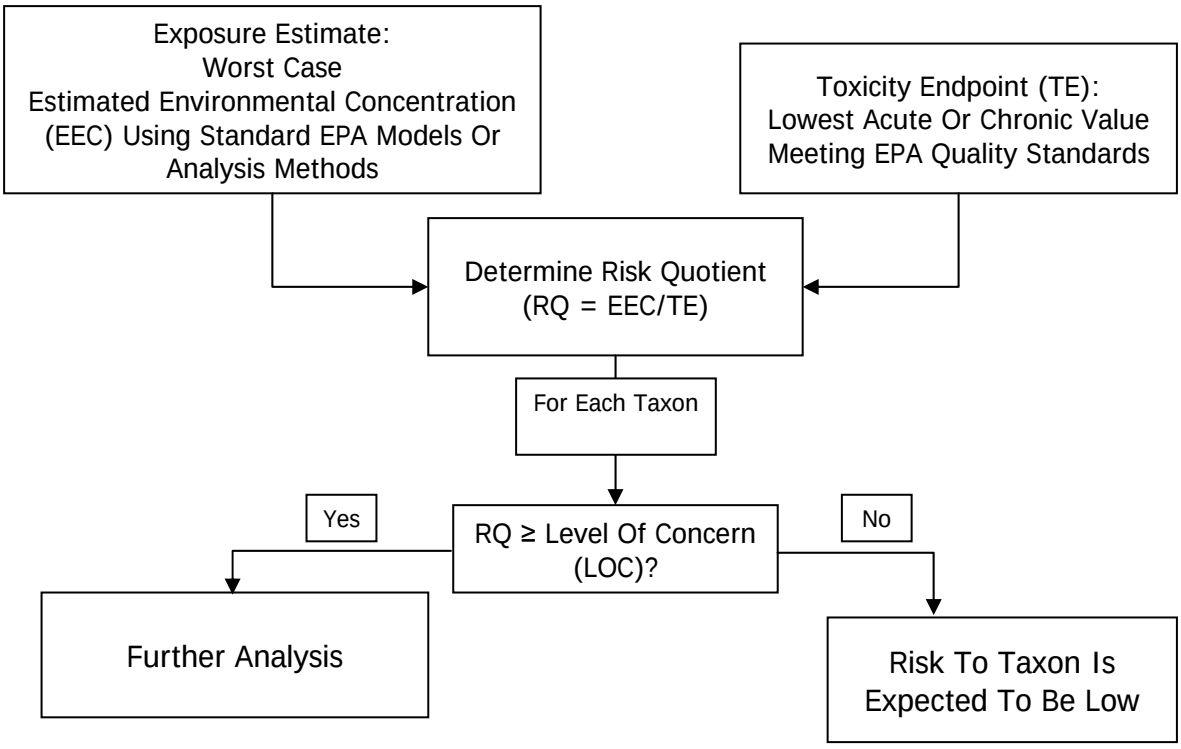

Figure K-1. Tier I Endangered Species Assessment

Risk quotients (RQ's) were calculated as the quotient of the Estimated Environmental Concentration (EEC) and the relevant toxicity endpoint for the most sensitive species for a given taxon (class of species). For acute studies of a few days duration, the concentration calculated to result in 50% mortality (LC_{50}) or 50% designated effect (EC_{50}) on the test species was utilized in the RQ calculation. For chronic studies, representing a significant portion of the species life-cycle, the highest concentration at which no effects were observed (No Observed Effect Concentration, NOEC) was used in the RQ calculation.

Toxicity values (effects endpoints) for most categories of species were taken from the EPA assessment for new glyphosate uses on bentgrass (U.S. EPA, 2006c), or from EPA guideline studies conducted by Monsanto if these endpoints were lower. Studies from the literature were considered when the study design was appropriate for the assessment being made and where sufficient information regarding glyphosate or formulation test concentrations was available. Exposure estimates were based on standard EPA methods for calculating exposure (U.S. EPA, 2004d). For aquatic organisms, the model GENEEC2 (U.S. EPA, 2004d), which calculates high-end estimates of surface water concentrations of pesticides in a generic farm pond, was utilized. When formulation toxicity was considered, default drift values and the EPA standard pond[12] were utilized for estimation of aquatic exposure. For terrestrial animals, the T-REX model (U.S. EPA, 2008c) was utilized to calculate estimated dietary exposure and risk. For terrestrial and

[12] A water body with a depth of 2 m and a volume of 20,000 liters.

semi-aquatic plants, only the drift component of the TerrPlant model (U.S. EPA, 2004d) was used to determine exposure levels (the runoff component was disregarded). Runoff was not considered to contribute to exposure, since glyphosate binds very tightly to agricultural soils and does not have herbicidal properties when bound to soil (U.S. EPA, 2006c).

The conclusion from this assessment, submitted to USDA and EPA, is that threatened or endangered terrestrial or semi-aquatic plant species are not at risk[13] from ground applications of glyphosate at rates less than 3.5 lb glyphosate (a.e.) per acre, or from aerial applications at rates less than 0.70 lb a.e. per acre. However, potential risk to these species cannot be excluded when rates exceed these levels. Since the maximum single in-crop application rate for MON 87427 is 1.125 lb a.e. per acre by ground application only, listed plant species outside of maize fields are not predicted to be at risk from in-crop glyphosate application to MON 87427. For other glyphosate-tolerant crops, in-crop application rates are typically less than 3.5 lb a.e. per acre, resulting in a prediction of no risk to listed plant species from in-crop applications. Rates that exceed 3.5 lb a.e. per acre, if used by growers, are generally for control of perennial species prior to crop emergence or prior to harvest.

The same assessment determined that other taxa (including birds, mammals, insects, fish, amphibians, aquatic invertebrates, and non-vascular aquatic plants) were not at risk from the use of glyphosate herbicides in crop production. Furthermore, this assessment determined that these other taxa were not at risk from indirect effects resulting from habitat alteration from the use of glyphosate, since non-endangered terrestrial or semi-aquatic plants were not considered to be at risk of direct effects.

Based on Monsanto's determination that threatened and endangered plant species may be at risk from certain uses of glyphosate in crop production (e.g., aerial applications at the maximum aerial rate), a more detailed evaluation of the locations of threatened and endangered plant species relative to areas of crop production has been undertaken. The first crop to be assessed was alfalfa (Honegger et al. 2008), but canola, maize, cotton, soybeans and sugar beets have now also been evaluated. The assessment process was divided into three phases, as outlined below.

- First, the co-occurrence of observations of threatened or endangered plant species and the presence of alfalfa, canola, maize, cotton, soybeans or sugar beet production was determined at the county level. This assessment (Phase 1) considered the 3028 counties in which at least one of these six crops are grown, which comprise 96% of the 3141 counties and equivalent areas[14] in the 50 states of the U.S. Species were reviewed for applicable exclusions at the county-level, which indicated, for some species, that glyphosate use in these crops posed no risk of adverse effects to these species.

[13] Risk to threatened or endangered plant species is only assessed outside of agricultural production areas.
[14] Equivalent areas include independent cities that are not within the boundaries of a county.

- Next, for threatened or endangered plant species where risks could not be excluded at the county level, the possible exposure to glyphosate was assessed at the sub-county level (Phase 2) in the same counties considered in Phase 1. This assessment used information available at the sub-county level for threatened and endangered plant species locations and for land use. Land uses considered in this assessment are those classified as Pasture/Hay and Cultivated Crops.[15]
- Finally, in sub-county areas where, under certain application conditions, the potential for threatened and endangered plant species to be at risk from exposure to glyphosate could not be excluded, areas have been defined so that grower practices can be implemented to limit glyphosate exposure (Phase 3). Measures to limit glyphosate exposure in these areas have been proposed. These measures include (1) limiting ground application rates to less than 3.5 lb glyphosate a.e. per acre in areas identified for potential use limitation when the potential habitat for the threatened or endangered species is present; and (2) for aerial applications greater than 0.7 lb a.e./acre, implementing an unsprayed buffer between the potential habitat for the listed species and the application area. Proposed buffer distances are based on application rate, droplet size and wind direction.

This analysis was initially completed for the 3028 U.S. counties in which alfalfa, canola, maize, cotton, soybeans and sugar beets were grown based on the 2002 Ag Census (USDA, 2002) and listed species information available through early 2008.

Of the 3028 U.S. counties where alfalfa, canola, maize, cotton, soybeans and sugar beets were produced, 11% of counties (334 counties) required the definition of potential areas for use limitations. In the other 2694 counties, either there were no threatened or endangered plant species present, or the species present were either excluded from concern (based on habitat or proximity information), had existing protections, or were not in proximity to potential areas of production of the six crops evaluated. This analysis is being updated based on the 2007 Ag Census and updated listed species information.

The Roundup Ready crop assessment considered the land classifications where agricultural crop production may occur (in counties with reported farms producing any of the six crops) in the assessment of proximity to observations of threatened or endangered species. Thus, the identification of potential use limitation areas also applies to other crops in those counties.

[15] Land use was based on the National land Cover Database (2001) for the continental U.S. and on the NOAA (National Oceanic and Atmospheric Administration) Coastal Services Center land cover data for Hawaii.

K.3.6. Potential Effects on Endangered Animal Species Identified by EPA or in Litigation

As previously discussed, no indirect effects on threatened or endangered animal species are predicted, since no significant direct effects due to pesticide drift onto non-endangered plant species are predicted. In the Glyphosate Re-registration Eligibility Decision (RED) (EPA, 1993), EPA suggested that glyphosate may have effects on the habitat of the Houston Toad. After the issuance of the 1993 Glyphosate RED, Monsanto conducted a vegetative vigor study. When relevant effects data from that study are considered, it can be determined that the amount of glyphosate per unit area predicted to drift away from the site of an agricultural application is less than the amount per unit area observed to have a 25% effect on plant dry weight or growth of the most sensitive of ten species tested in the study. Thus, the habitat of the toad is not likely to be significantly affected by glyphosate drift, and hence the toad is not likely to be at risk from the agricultural use of glyphosate.

The EPA evaluated the effect of glyphosate on the California Red-legged Frog in response to a consent agreement reached in a lawsuit filed by the Center for Biological Diversity.[16] In the California red-legged frog effects determination (U.S. EPA, 2008b), EPA considered glyphosate rates up to 7.95 lb a.e./A. Even at this high rate, EPA concluded that there would be no direct effects to the aquatic phase of the California red-legged frog (CRLF) from application of glyphosate or any glyphosate formulation or salts.[17] EPA also concluded that there are no direct effects to the terrestrial-phase of the CRLF at rates of 3.85 lb a.e./A and below (with the exception of one formulation that is not registered for use in MON 87427)[18]. Since the maximum in-crop application rate for MON 87427 is 1.125 lb a.e./A, glyphosate formulations and rates used in MON 87427 do not pose any potential risk of direct effects to the aquatic or terrestrial-phase of the CRLF.

With respect to indirect effects on prey, EPA concluded that no effect on the following prey items: algae, aquatic invertebrates, aquatic-phase frogs or fish, or terrestrial-phase frogs would occur at glyphosate rates of 3.84 lb a.e./A .[19] The potential for some effects on terrestrial invertebrate and small mammal prey items were identified but only at rates above 7.5 lb a.e./A and 3.75 lb a.e./A, respectively.[20] No chronic effects on mammals

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[16] *Center For Biological Diversity v. Leavitt*, 2005 WL 2277030 (N.D.Cal., September 19, 2005).

[17] U.S. EPA. 2008a. Risks of Glyphosate Use to Federally Threatened California Red-legged Frog (*Rana aurora draytonii*). http://www.epa.gov/espp/litstatus/effects/redleg-frog/glyphosate/determination.pdf., Page 11, "The acute and chronic LOC's for freshwater fish and aquatic-phase amphibians are not exceeded for either glyphosate, its salts or its formulations".

[18] Ibid., Page 14, Table 1.3.

[19] Ibid., Page 15, Table 1.4.

[20] Ibid., Page 156, ibid. For terrestrial invertebrate prey items that were not listed (i.e. not threatened or endangered), no effects were predicted at any rate up to 7.95 lb a.e./A for large invertebrates, and the Level of Concern was only exceeded for small invertebrates at application rates of 7.5 lb a.e./A and above. Levels of Concern were exceeded at lower rates for listed (threatened or endangered) terrestrial invertebrates, but listed species would not be anticipated to be a significant portion of the CRLF diet. Listed small invertebrates in areas adjacent to a MON 87427 field, would not be at risk from spray drift exposure from glyphosate applications to MON 87427.

were predicted at rates of 3.75 lb a.e./A and below.[21] Therefore, no effects on CRLF prey were identified at the maximum single application rate for MON 87427. Similarly, no effects on terrestrial plant habitat are identified for ground applications at 1.125 lb a.e./A.[22]

Based on the CRLF effects determination conducted by EPA, it is not expected that the glyphosate rates applied to MON 87427 would have any direct or indirect impact on the CRLF.

The EPA also has evaluated the potential effect of glyphosate on salmon in eleven areas in California and Southern Oregon[23] in response to the consent agreement reached in another lawsuit[24]. The conclusion of the EPA's risk assessment is as follows:

"For all uses with application rates of 5 lb a.i. per A or below, the Agency has determined that glyphosate will have No Effect on the subject listed species." (U.S. EPA, 2004a; U.S. EPA, 2004b). All glyphosate use rates for agricultural uses are 5 lb a.i. per acre (3.75 lb glyphosate a.e. per acre) or below, so no risk to salmon is anticipated from these uses.

K.3.7. Other Potential Environmental Impacts Associated with Glyphosate Use in Glyphosate-tolerant Crops

As discussed more fully below, the potential impacts to soil attributable to the change in production (cultivation) practices associated with the deregulation of glyphosate-tolerant crops have been assessed. The adoption of glyphosate-tolerant crops and the ability to use glyphosate-based agricultural herbicides is not expected to significantly change agricultural practices, except to enable the adoption of no-till seeding practices.

1. No-Till Practices: No-till production is the practice of establishing an agricultural seed bed and controlling weeds without mechanically tilling the soil. Instead, the only tillage of the soil is done at the time of planting, with the crop being seeded directly into the previous year's crop residue. Among other environmental benefits, no-till production reduces soil erosion and the use of petroleum-based fuels for tractors. The practice has been shown to minimize surface water runoff and soil erosion and to improve soil quality by increasing the soil organic matter that helps bind soil nutrients and prevent their loss to runoff, erosion and leaching (Leep et.al., 2003). Less soil erosion into surface waters would positively impact stream dynamics (McVay et al., 2005).

No-till agriculture can provide benefits to water bodies, as well. No-till practices reduce soil erosion to surface water bodies, decreasing the amount of sediment in rivers and

[21] Ibid., Page 12. The potential for chronic effects on small mammals were only identified at rates of 3.84 lb a.e./A and above (Risk Quotient (RQ) greater than the LOC). Since the next lower rate considered in the CRLF evaluation is 3.75 lb a.e./A, it can be concluded that the RQ did not exceed the LOC for these rates, and, therefore, no effects would be anticipated from an application rate of 1.13 lb a.e./A)

[22] Ibid., Page 136-138. Risk quotients < 1 are below the Level of Concern. Table 5.6 and 5.7 indicate RQ's for ground applications of 1.54 lb a.e./A are well below the LOC of 1, Since the maximum in-crop application rate for MON 87427 is 1.125 lb a.e./A no risk of indirect effects to CRLF because of effects on plants in the habitat is predicted from glyphosate use on MON 87427.

[23] These areas are call Evolutionarily Significant Units based on the salmonid populations present in these areas.

[24] *Washington Toxics Coalition v. Environmental Protection Agency*, 413 F.3d 1024 (9th Cir. 2005).

streams. Sedimentation increases the turbidity (cloudiness) of surface water bodies, reducing light penetration, impairing photosynthesis and altering oxygen levels, which cause a reduction of food sources for some aquatic organisms. Sediment can also cover spawning beds and impact fish populations. Phosphorus (a major component of fertilizer) bound to soil particles can be transferred to rivers and lakes via soil erosion, giving rise to high levels of phosphorus in surface waters, which may lead to algae blooms that can impact desirable fish populations (Hill and Mannering, 1995).

2. Soil Microorganisms: Results of standardized tests with glyphosate formulations performed for submission to regulatory agencies indicate no long-term effects on microorganisms in soil even at rates that exceed maximum use rates (up to five times the labeled rate). In addition, independent researchers have reviewed numerous laboratory and field studies, investigating the effects of glyphosate on soil bacteria and fungi (Felsot, 2001; Giesy et al., 2000). Although some laboratory tests have shown effects on nitrogen-fixing bacteria (Moorman et al., 1992; Santos and Flores, 1995) and soil fungi (Busse et al., 2001; Estok et al., 1989), effects are typically observed only under artificial laboratory conditions and at glyphosate concentrations well above normal field application rates. Several researchers have concluded that it is difficult to extrapolate results from the laboratory to the natural soil environment (Busse et al., 2001; Estok et al., 1989; Wan et al., 1998).

In studying microorganisms from soil in pine plantations, (Busse et al., 2001) note: "Our findings suggest that artificial media assays are of limited relevance in predicting glyphosate toxicity to soil organisms and that field rate applications of glyphosate should have little or no affect on soil microbial communities in ponderosa pine plantations." Long-term studies following repeated applications of Roundup agricultural herbicides in the field at labeled use rates for multiple applications in one year (Olson and Lindwall, 1991) or over 15 years (Biederbeck et al., 1997; Hart and Brookes, 1996) have shown no long-term adverse effects on soil microbes. Investigations by Haney et al. (Haney et al., 2002; Haney et al., 2000) related to the increased use of glyphosate-tolerant crops indicate that glyphosate was degraded over time by soil microbes, even at high application rates, without adversely impacting the soil microbial community. In addition, results from field studies that have evaluated the fungal component of the soil microbial community indicate that glyphosate treatment had no deleterious effects on beneficial soil fungi (Araujo et al., 2003; Biederbeck et al., 1997; Busse et al., 2001; Wardle and Parkinson, 1990a; Wardle and Parkinson, 1990b). Moreover, the history of safe use and yield data obtained for nearly 10 years of glyphosate-tolerant crop production, combined with in-crop applications of glyphosate-based agricultural herbicides, reinforce the findings that soil microbes and microbially mediated processes are not adversely impacted by field-rate applications of glyphosate.

3. The Potential for Glyphosate Metal Chelation to Affect Soil Fertility: Plants are dependent on the uptake of a number of different metal cations from the soil for optimal growth. Glyphosate is known to chelate, or tightly bind, to several di- and trivalent metal cations such as Fe3+, Cu2+, Mn2+, Al3+, and Ca2+ that are needed by plants (Glass, 1984; Madsen et al., 1978). Cations that chelate glyphosate have been shown to reduce the efficacy of glyphosate when present in sufficient amounts in the tank mix spray

solution (Bernards et al., 2005). In the spray solution, there is a simple interaction between glyphosate and metal cations, which reduces the herbicidal activity of glyphosate. However, in the soil environment, the interactions between metals and chelators are much more complex (Parker et al., 2005). Glyphosate can interact with metals that are present on the surface of soil particles, as well as with dissolved metal ions in the water soil solution. In addition to glyphosate, many other potential ligands or chelators are present in soil that can also interact with metals. As a result, there is a complex multi-component equilibrium between glyphosate, other ligands or chelators, and numerous metals present in soil. Glyphosate is only one factor in this system. Numerous compositional analysis studies have demonstrated a lack of any significant immobilization of mineral nutrients by glyphosate in soil that results in reduced uptake by plants. These studies have shown that glyphosate-tolerant crops that have been sprayed with glyphosate do not have decreased micronutrient levels compared to untreated controls (McCann et al., 2006; Obert et al., 2004; Ridley et al., 2002).

4. Transport through the Soil – Surfactant: Available data also suggest that the POEA surfactant used in Roundup agricultural herbicides binds strongly to soil (estimated soil organic carbon-water partition coefficient (Koc) values range from 2500 to 9600[25]) and undergoes microbial degradation with an estimated half-life of less than 14 days (Marvel, et al., 1974). POEA is rapidly partitioned (half-life of 13 to 18 hours) from water to sediment in a water / sediment study (Wang et al., 2005). The rapid partitioning of the POEA surfactant to soil sediment combined with the high Koc values indicates that the surfactant will be tightly bound to the soil. The Groundwater Ubiquity Score, GUS, is an index that indicates the potential for compounds to leach from soil into groundwater, based on their half-life and Koc (Gustafson, 1989). Using an estimated half-life of 14 days and a Koc of 2500 as conservative estimates of the rate of degradation and binding to soil, the GUS index for the POEA surfactant is 0.69. According to the GUS movement ranking, this GUS index indicates that POEA has a very low potential to leach to groundwater.

[25] Estimated from the partition ratio between water and sterile soil as reported in the POEA soil degradation study, Marvel et al., 1974.

Attachment 1. Appendix K. U.S. Glyphosate Tolerances for Food & Feed Commodities (40 CFR § 180.364)

Commodity	Parts/million	Commodity	Parts/million
Acerola	0.2	Durian	0.2
Alfalfa, seed	0.5	Egg	0.05
Almond, hulls	25	Epazote	1.3
Aloe vera	0.5	Feijoa	0.2
Ambarella	0.2	Fig	0.2
Animal feed, nongrass, group 18	400	Fish	0.25
Artichoke, globe	0.2	Flax, meal	8.0
Asparagus	0.5	Flax, seed	4.0
Atemoya	0.2	Fruit, citrus, group 10	0.5
Avocado	0.2	Fruit, pome, group 11	0.2
Bamboo, shoots	0.2	Fruit, stone, group 12	0.2
Banana	0.2	Galangal, roots	0.2
Barley, bran	30	Ginger, white, flower	0.2
Barley, grain	20	Goat, kidney	4.0
Beet, sugar, dried pulp	25	Goat, liver	0.5
Beet, sugar, roots	10	Gourd, buffalo, seed	0.1
Beet, sugar, tops	10	Governor's plum	0.2
Berry group 13	0.2	Gow kee, leaves	0.2
Betelnut	1.0	Grain, aspirated fractions	100
Biriba	0.2	Grain, cereal, forage, fodder and straw, group 16, except maize forage	100
Blimbe	0.2	Grain, cereal, group 15, except barley, commercial maize, grain sorghum, oat and wheat	0.1
Borage, seed	0.1	Grape	0.2
Breadfruit	0.2	Grass, forage, fodder and hay, group 17	300
Cacao bean	0.2	Guava	0.2
Cactus, fruit	0.5	Herbs subgroup 19A	0.2
Cactus, pads	0.5	Hog, kidney	4.0
Canistel	0.2	Hog, liver	0.5
Canola, meal	15	Hop, dried cones	7.0
Canola, seed	10	Horse, kidney	4.0
Cattle, kidney	4.0	Horse, liver	0.5
Cattle, liver	0.5	Ilama	0.2
Chaya	1.0	Imbe	0.2
Cherimoya	0.2	Imbu	0.2
Citrus, dried pulp	1.5	Jackfruit	0.2
Coconut	0.1	Jaboticaba	0.2
Coffee, bean	1.0	Jojoba, seed	0.1
Maize, field, forage	6.0	Juneberry	0.2
Maize, field, grain	1.0	Kava, roots	0.2
Cotton, gin byproducts	175	Kenaf, forage	200
Cotton, undelinted seed	35	Kiwifruit	0.2
Cranberry	0.2	Lesquerella, seed	0.1
Crambe, seed	0.1	Leucaena, forage	200
Custard apple	0.2	Lingonberry	0.2

Commodity	Parts/million	Commodity	Parts/million
Date	0.2	Longan	0.2
Dokudami	2.0	Lychee	0.2
Mamey apple	0.2	Sapote, black	0.2
Mango	0.2	Sapote, mamey	0.2
Mangosteen	0.2	Sapote, white	0.2
Marmaladebox	0.2	Sesame, seed	0.1
Meadowfoam, seed	0.1	Sheep, kidney	4.0
Mioga, flower	0.2	Sheep, liver	0.5
Mustard, seed	0.1	Shellfish	3.0
Noni	0.20	Sorghum, grain, grain	15
Nut, pine	1.0	Soursop	0.2
Nut, tree, group 14	1.0	Soybean, forage	100
Oat, grain	20	Soybean, hay	200
Okra	0.5	Soybean, hulls	100
Olive	0.2	Soybean, seed	20
Oregano, Mexican, leaves	2.0	Spanish lime	0.2
Palm heart	0.2	Spearmint, tops	200
Palm heart, leaves	0.2	Spice subgroup 19B	7.0
Palm, oil	0.1	Star apple	0.2
Papaya	0.2	Starfruit	0.2
Papaya, mountain	0.2	Stevia, dried leaves	1.0
Passionfruit	0.2	Strawberry	0.2
Pawpaw	0.2	Sugar apple	0.2
Pea, dry	8.0	Sugarcane, cane	2.0
Peanut	0.1	Sugarcane, molasses	30
Peanut, hay	0.5	Sunflower	85
Pepper leaf, fresh leaves	0.2	Sunflower, seed	0.1
Peppermint, tops	200	Surinam cherry	0.2
Perilla, tops	1.8	Tamarind	0.2
Persimmon	0.2	Tea, dried	1.0
Pineapple	0.1	Tea, instant	7.0
Pistachio	1.0	Teff, grain	5.0
Pomegranate	0.2	Ti, leaves	0.2
Poultry, meat	0.1	Ti, roots	0.2
Poultry, meat byproducts	1.0	Ugli fruit	0.5
Pulasan	0.2	Vegetable, leafy, brassica, group 5	0.2
Quinoa, grain	5.0	Vegetable, bulb, group 3	0.2
Rambutan	0.2	Vegetable, cucurbit, group 9	0.5
Rapeseed, meal	15	Vegetable, foliage of legume, except soybean, subgroup 7A	0.2
Rapeseed, seed	10	Vegetable, fruiting, group 8	0.1
Rose apple	0.2	Vegetable, leafy, except brassica, group 4	0.2
Safflower	85	Vegetable, leaves of root and tuber, group 2, except sugar beet	0.2
Safflower, seed	0.1	Vegetable, legume, group 6, except soybean	5.0
Salal	0.2	Vegetable, legume, group 6 except soybean and pea,dry	5.0

Commodity	Parts/million	Commodity	Parts/million
Sapodilla	0.2	Vegetable, root and tuber, group 1, except sugar beet	0.2
Wasabi, roots	0.2	Wheat, grain	5.0
Water spinach, tops	0.2	Wheat, middlings	20
Watercress, upland	0.2	Wheat, shorts	20
Wax jambu	0.2	Yacon, tuber	0.2
Wheat, bran	20		

References to Appendix K

Acquavella, J.F., B.H. Alexander, J.S. Mandel, C. Gustin, B. Baker, P. Chapman and M. Bleeke. 2004. Glyphosate biomonitoring for farmers and their families: Results from the farm family exposure study. Environmental Health Perspectives 112: 321-326.

Araujo, A.S.F., R.T.R. Monteiro and R.B. Abarkeli. 2003. Effect of glyphosate on the microbial activity of two Brazilian soils. Chemosphere 52: 799-804.

Ashton, F.M. and A.S. Crafts. 1981. Mode of action of herbicides. Second Edition, John Wiley and Sons., New York, New York.

Bernards, M.L., K.D. Thelen, D. Penner, R.B. Muthukumaran and J.L. McCracken. 2005. Glyphosate interaction with manganese in tank mixtures and its effect on glyphosate absorption and translocation. Weed Science 53: 787-794.

Biederbeck, V.O., C.A. Campbell and J.H. Hunter. 1997. Tillage effects on soil microbial and biochemical characteristics in a fallow-wheat rotation in a dark brown soil. Canadian Journal of Soil Science 77: 309-316.

Busse, M.D., A.W. Ratcliff, C.J. Shestak and R.F. Powers. 2001. Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities. Soil Biology & Biochemistry 33: 1777-1789.

EC. 2002. Report for the active substance glyphosate. Directive 6511/V1/99. European Commission, Paris, France. http://ec.europa.eu/food/fs/ph_ps/pro/eva/existing/list1_glyphosate_en.pdf [Accessed May 12, 2010].

Estok, D., B. Freedman and D. Boyle. 1989. Effects of the herbicides 2 4-D, glyphosate, hexazinone and triclopyr on the growth of three species of ectomycorrhizal fungi. Bulletin of Environmental Contamination and Toxicology 42: 835-839.

Felsot, A. 2000. Withering wildlife? Pages 1-8 in Herbicide Tolerant Genes. Volume 178. Washington State University, Richland, Washington.

Giesy, J.P., S. Dobson and K.R. Solomon. 2000. Ecotoxicological risk assessment for Roundup® Herbicide. Pages 35-120 in Reviews of Environmental Contamination and Toxicology. Volume 167. Springer-Verlag, New York, New York.

Glass, R.L. 1984. Metal complex formation by glyphosate. Journal of Agricultural and Food Chemistry 32: 1249-1253.

Gustafson, D.I. 1989. Groundwater ubiquity score: A simple method for assessing pesticide leachability. Environmental Toxicology and Chemistry 8: 339-357.

Haney, R.L., S.A. Senseman and F.M. Hons. 2002. Effect of Roundup Ultra on microbial activity and biomass from selected soils. Journal of Environmental Quality 31: 730-735.

Haney, R.L., S.A. Senseman, F.M. Hons and D.A. Zuberer. 2000. Effect of glyphosate on soil microbial activity and biomass. Weed Science 48: 89-93.

Hart, M.R. and P.C. Brookes. 1996. Soil microbial biomass and mineralisation of soil organic matter after 19 years of cumulative field applications of pesticides. Soil Biology and Biochemistry 28: 1641-1649.

Hill, P.R. and J.V. Mannering. 1995. Conservation tillage and water quality. Purdue University Cooperative Extension Service, West Lafayette, Indiana. http://www.ces.purdue.edu/extmedia/WQ/WQ-20.html [Accessed July 2, 2009].

Honegger, J.L., S.R. Mortensen and K.H. Carr. 2008. Overview of the analysis of possible risk to threatened and endangered species associated with use of glyphosate-containing herbicides in alfalfa production. Study RPN-2007-228, Monsanto Company, St. Louis, Missouri.

Kiely, T., D. Donaldson and A. Grube. 2004. Pesticide industry sales and usage: 2000 and 2001 market estimates. U.S. Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, Washington, D.C.

Leep, R., D. Undersander, P. Peterson, D.-H. Min, T. Harrigan and J. Grigar. 2003. Steps to successful no-till establishment of forages. Extension Bulletin E-2880. Michigan State University, University of Wisconsin, University of Minnesota, and Natural Resources Conservation Service, East Lansing, Michigan.

Madsen, H.E.L., H.H. Christensen and C. Gottlieb-Petersen. 1978. Stability constants of copper(II), zinc, manganese(II), calcium, and magnesium complexes of *N*-(phosphonomethyl)*glycine* (Glyphosate). Acta Chemica Scandinavica A 32: 79-83.

Marvel, J.T., B.B. Brightwell and L.A. Suba. 1974. G3780A surfactant: Biodegradation, plant update and 14C distribution. Monsanto Technical Report No. 321, Monsanto Company, St. Louis, Missouri.

McCann, M.C., G.J. Rogan, S. Fitzpatrick, W.A. Trujillo, R. Sorbet, G.F. Hartnell, S.G. Riodan and M.A. Nemeth. 2006. Glyphosate-tolerant alfalfa is compositionally equivalent to conventional alfalfa (*Medicago sativa* L.). Journal of Agricultural and Food Chemistry 54: 7187-7192.

McVay, K.A., D.L. Levlin and J. Neel. 2005. Effects of conservation practices on water quality: Sediment. MF2682, Kansas State University Agricultural Experiment Station and Cooperative Extension Service, Manhattan, Kansas.

Moorman, T.B., J.M. Becerril, J. Lydon and S.O. Duke. 1992. Production of hydroxybenzoic acids by *Bradyrhizobium japonicum* strains after treatment with glyphosate. Journal of Agricultural and Food Chemistry 40: 289-293.

Mortensen, S.R., K.H. Carr and J.L. Honegger. 2008. Tier I endangered species assessment for agricultural uses of glyphosate and glyphosate-containing herbicides. Study RPN-2007-227, Monsanto Company, St. Louis, Missouri.

Obert, J.C., W.P. Ridley, R.W. Schneider, S.G. Riordan, M.A. Nemeth, W.A. Trujillo, M.L. Breeze, R. Sorbet and J.D. Astwood. 2004. The composition of grain and forage from glyphosate tolerant wheat MON 71800 is equivalent to that of conventional wheat (*Triticum aestivum* L.). Journal of Agricultural and Food Chemistry 52: 1375-1384.

Olson, B.M. and C.W. Lindwall. 1991. Soil microbial activity under chemical fallow conditions: Effects of 2,4-D and glyphosate. Soil Biology and Biochemistry 23: 1071-1075.

Parker, D.R., S.M. Reichman and D.E. Crowley. 2005. Metal chelation in the rhizosphere. Pages 57-93 in Roots and Soil Management: Interactions Between Roots and the Soil. R.W. Zobel and S.F. Wright (eds.). American Society of Agronomy, Madison, Wisconsin.

Ridley, W.P., R.S. Sidhu, P.D. Pyla, M.A. Nemeth, M.L. Breeze and J.D. Astwood. 2002. Comparison of the nutritional profile of glyphosate-tolerant corn Event NK603 with that of conventional corn (*Zea mays* L.). Journal of Agricultural and Food Chemistry 50: 7235-7243.

Santos, A. and M. Flores. 1995. Effects of glyphosate on nitrogen-fixation of free-living heterotrophic bacteria. Letters in Applied Microbiology 20: 349-352.

U.S. EPA. 1993. RED facts: Glyphosate. U.S. Environmental Protection Agency Office of Prevention, Pesticides, and Toxic Substances, Washington, D.C. http://www.epa.gov/oppfead1/endanger/litstatus/effects/glyphosate-red.pdf [Accessed August 16, 2010].

U.S. EPA. 1996a. Pesticide tolerance for glyphosate. 61 FR 7729. U.S. Environmental Protection Agency, Washington, D.C. http://www.epa.gov/EPA-PEST/1996/February/Day-29/pr-567.html [Accessed September 9, 2010].

U.S. EPA. 1996b. Pesticide tolerances for glyphosate. U.S. Environmental Protection Agency, Washington, D.C. . http://www.epa.gov/EPA-PEST/1996/April/Day-05/pr-636.html [Accessed Sep 9, 2010].

U.S. EPA. 1997. Glyphosate; Pesticide tolerances. 62 FR 17723. U.S. Environmental Protection Agency, Washington, D.C. http://www.epa.gov/EPA-PEST/1997/April/Day-11/p9231.htm [Accessed September 9, 2010].

U.S. EPA. 1999. Glyphosate; Pesticide tolerances. 62 FR 18360. U.S. Environmental Protection Agency, Washington, D.C. http://www.epa.gov/fedrgstr/EPA-PEST/1999/April/Day-14/p9317.htm [Accessed September 9, 2010].

U.S. EPA. 2002. Glyphosate; pesticide tolerances 67 FR 60934. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 2003. Glyphosate; Pesticide tolerance. 68 FR 36472. U.S. Environmental Protection Agency, Washington, D.C. http://www.epa.gov/EPA-PEST/2003/June/Day-18/p15128.htm [Accessed September 9, 2010].

U.S. EPA. 2004a. Effects determination for glyphosate on Pacific salmonid ESUs. Transmittal letter from Williams, A.-J. B., Chief, Environmental Field Branch to L. Allen, Acting Director, Office of Protected Resources, National Marine Fisheries Service. . U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 2004b. Glyphosate: Analysis of risks to endangered and threatened salmon and steelhead. U.S. Environmental Protection Agency, Washington, D.C. http://epa.gov/espp/litstatus/effects/glyphosate-analysis.pdf [Accessed July 2, 2009].

U.S. EPA. 2004c. Glyphosate; Pesticide tolerance. 69 FR 65081. U.S. Environmental Protection Agency, Washington, D.C. . http://www.epa.gov/EPA-PEST/2004/November/Day-10/p25098.htm [Accessed September 17, 2010].

U.S. EPA. 2004d. Overview of the ecological risk assessment process in the Office of Pesticide Programs U.S. Environmental Protection Agency, Washington, D.C. http://epa.gov/espp/consultation/ecorisk-overview.pdf [Accessed August 17, 2010].

U.S. EPA. 2005. Glyphosate; Pesticide tolerance. 70 FR 7861. U. S. Environmental Protection Agency, Washington, D.C. . http://www.epa.gov/fedrgstr/EPA-PEST/2005/February/Day-16/p2983.htm [Accessed August 17, 2010].

U.S. EPA. 2006a. Glyphosate human health risk assessment for proposed use on Indian mulberry and amended use on pea, dry. Petition No. 5E6987 DP 321992 Decision No. 360557. U.S. Environmental Protection Agency, Washington, D.C. http://www.regulations.gov/search/Regs/home.html#documentDetail?R=09000064801dce5f [Accessed September 9, 2010].

U.S. EPA. 2006b. Glyphosate human health risk assessment for proposed uses on safflower and sunflower. Petition 4E6878 DP 314476. U.S. Environmental Protection Agency, Washington, D.C. http://www.regulations.gov/search/Regs/home.html#documentDetail?R=09000064801dce6b [Accessed September 9, 2010].

U.S. EPA. 2006c. Glyphosate new use (bent-grass). U.S. Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances, Washington, D.C.

U.S. EPA. 2006d. Glyphosate; Pesticide tolerance 71 FR 76180. U.S. Environmental Protection Agency, Washington, D.C. http://www.epa.gov/EPA-PEST/2006/December/Day-20/p21490.htm [Accessed September 9, 2010].

U.S. EPA. 2008a. Benfluralin, carbaryl, diazinon, dicrotophos, fluometuron, formetanate hydrochloride, glyphosate, metolachlor, napropamide, norflurazon, pyrazon, and tau-fluvalinate; tolerance actions. Federal Register: 52607-52616.

U.S. EPA. 2008b. Risks of glyphosate use to federally threatened California red-legged frog (*Rana aurora draytonii*). U.S. Environmental Protection Agency, Washington, D.C. http://www.epa.gov/espp/litstatus/effects/redleg-frog/glyphosate/determination.pdf. [Accessed September 9, 2010].

U.S. EPA. 2008c. T-REX (Terrestrial Residue EXposure Model). Version 1.4.1., U.S. Environmental Protection Agency, Washington, D.C. . http://www.epa.gov/oppefed1/models/terrestrial/trex/t_rex_user_guide.htm [Accessed April 1, 2010].

Wan, M.T., J.E. Rahe and R.G. Watts. 1998. A new technique for determining the sublethal toxicity of pesticides to the vesicular-arbuscular mycorrhizal fungus *Glomus intraradices*. Environmental Toxicology and Chemistry 17: 1421-1428.

Wang, N., J.M. Besser, D.R. Buckler, J.L. Honegger, C.G. Ingersoll, B.T. Johnson, M.L. Kurtzweil, J. MacGregor and M.J. McKee. 2005. Influence of sediment on the fate and toxicity of a polyethoxylated tallowamine surfactant system (MON 0818) in aquatic microcosms. Chemosphere 59: 545-551.

Wardle, D. and D. Parkinson. 1990a. Effects of three herbicides on soil microbial biomass and activity. Plant and Soil 122: 21-28.

Wardle, D. and D. Parkinson. 1990b. Influence of the herbicide glyphosate on soil microbial community structure. Plant and Soil 122: 29-37.

WHO/FAO. 2004. Pesticide residues in food - 2004. World Health Organization, Food and Agriculture Organization, Rome, Italy.

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Riverdale, MD 20737

Subject: Technical completeness of Monsanto Company Petition Number 10-281-01p for a Determination of Nonregulated Status for Tissue-Selective Glyphosate Tolerant Corn MON 87427

Dear Ms. Eck:

This letter summarizes the clarifications and corrections to Petition # 10-281-01p for the Determination of Non-Regulated Status for MON 87427, as outlined in the June 13, 2011 email from Mr. John M. Cordts of APHIS and discussed during a conference call between APHIS and Monsanto on June 27, 2011. This information has been requested by USDA APHIS/BRS to make a determination of technical completeness for Petition # 10-281-01p. The table, figure, section, and page numbers below refer to those in the original petition, submitted to USDA APHIS/BRS on October 8, 2010.

None of the clarifications or corrections described in this letter alters the conclusion in the petition that MON 87427 is unlikely to be a plant pest. Should you have any questions concerning this letter, or wish to set up a meeting for further discussion, please contact Mr. Daniel J. Jenkins, U.S. Agency Regulatory Affairs Lead, Washington D.C., at 202-383-2851; or me at 314-694-9879.

Sincerely,

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cc: Dr. Carlos A. Blanco, USDA APHIS/BRS
Mr. Daniel J. Jenkins, Monsanto, Washington, D.C.
Regulatory files/10-CR-214U

1. p41. As stated in the first complete paragraph, two insertions and one deletion were observed in MON 87427. Do these modifications have a phenotypic effect on the product? Please clarify.

The 140 bp deletion was evaluated by bioinformatic analysis of the MON 87427 insertion site and flanking sequences, which indicated that no endogenous protein coding sequence was disrupted by the insertion of T-DNA, and no protein coding sequence was found in the ~1 kb region flanking the T-DNA in MON 87427. Based on these analyses, there is no reason to expect that either the insertion of the T-DNA or the deletion of 140 bp of endogenous nucleotide sequence would impact the phenotype of MON 87427.

In order to assess the impact of the insertion of the T-DNA and concomitant loss of 140 bp of endogenous DNA in MON 87427, a bioinformatic evaluation was performed using the MON 87427 insertion site and flanking sequence (total length 2170 bp which includes the 140 bp deletion) as a query for BLASTn and BLASTx analyses. BLASTn is a search algorithm that compares a nucleotide query sequence against a DNA sequence database, while the BLASTx algorithm compares a conceptual six-frame translation of a nucleotide sequence against a protein sequence database. Searches of the following databases were conducted using BLAST version 2.2.21+ algorithms:

- GenBank expressed sequencing tag database (EST database), 67,857,743 sequences posted December 30, 2010
- GenBank non-redundant nucleotide database (NT database), 14,564,296 sequences posted January 7, 2011
- GenBank non redundant amino acid database, 12,603,350 sequences posted January 3, 2011

All databases and software were downloaded from the National Center of Biotechnology Information (NCBI) and were used as provided.

Inspection of the top 50 alignments obtained using a BLASTn search of the EST database did not provide any evidence that the MON 87427 insertion site and flanking sequence query contained a protein coding region. Even the best alignments, those with database sequences obtained from corn, displayed nucleotide mismatches and required the insertion of gaps to optimize the alignment. Likewise, inspection of the top 50 BLASTn alignments obtained by searching the NT database yielded no evidence of the identification of a gene or the presence of protein coding sequence. The top alignment was found near the middle of a ~140 kb segment of the corn genome contained in a BAC clone, spanned only ~1.2kb of the 2.17 kb query sequence, and was characterized by numerous mismatches and gaps.

Inspection of the search results obtained using BLASTx revealed two alignments with putative corn sequences. The alignments contained gaps and mismatched amino acids and the annotation of the putative corn sequences showed that they were translated from sequence found in the ~1.2 kb segment of BAC clone that was found to be the top alignment in the BLASTn search of the NT database.

When viewed individually or in total, the results of the BLAST searches did not provide any indication that endogenous protein coding sequence was disrupted by the insertion of T-DNA, or that protein coding sequence is found in the ~1 kb region flanking the T-DNA in MON 87427 that was evaluated.

The 41 bp and 24 bp insertions were evaluated by bioinformatic analyses on the flanking genomic DNA sequences. Based on this analysis of putative polypeptides encoded by all six reading frames present in the 5' and 3' inserted DNA-5' and 3' flanking sequence junctions, there is no reason to expect that either the 41 bp insertion that occurred in the 5' flanking sequence DNA-inserted DNA junction, or the 24 bp insertion that occurred in the 3' flanking sequence DNA-inserted DNA junction would impact the phenotype of MON 87427.

In this analysis, ORFs spanning the 5' flanking sequence DNA-inserted DNA junctions, and 3' flanking sequence DNA-inserted DNA junctions were translated from stop codon to stop codon in all six reading frames (three forward reading frames and three reading frames in reverse complement orientation). The putative peptides/polypeptides from each reading frame were then compared to protein databases using bioinformatic tools. Based on these comparisons, there was no reason to expect that any of the putative peptides would be produced.

The conclusions from these bioinformatic analyses are supported by the agronomic and phenotypic observations that were obtained from field studies, where 14 phenotypic and agronomic characteristics were evaluated and provided a detailed description of MON 87427 compared to the conventional control. The results support a determination that MON 87427 is not fundamentally different than conventional maize.

2. p59. In the second paragraph it is stated that '*one surviving LH198 BC3F2 plant was identified.*' Does it mean that only one plant was used?

LH198 BC3F2 corn plants were sprayed with glyphosate and demonstrated the expected 3:1 (positive:negative) segregation ratio for glyphosate tolerance. The LH198 BC3F3 seed from one self-pollinated glyphosate-tolerant LH198 BC3F2 plant was genotyped. Seed that was homozygous for the MON 87427 trait was planted and the resulting LH198 BC3F3 plants were self-pollinated to produce LH198 BC3F4 seed that was also homozygous for the MON 87427 trait.

3. p61. In Figure IV-8., the recurrent parent (RP) described in lines 12, 13, and 15 is not totally clear. Is it LH298? Please clarify.

LH198 BC3F4 seed was used in trait integration and further commercial line development and was crossed with a recurrent parent that is a proprietary inbred line with a different genetic background than LH198. The RP can be designated by its manufacturing code HCL301.

4. p67. In Table V-1 sixth row (pollen) describes concentration of it in the fifth column. According to these values, pollen is still produced in low quantities. Is this going to affect hybrid seed production?

Previous proprietary research has demonstrated that MON 87427 produces CP4 EPSPS protein in anthers. As a result, the small amount of CP4 EPSPS protein found in some of the pollen samples could be due to the presence of anther tissue that can be mistakenly collected with the pollen from MON 87427. Alternatively, a low amount of CP4 EPSPS in MON 87427 may be inherent to this product due to the use of the e35S promoter (CaJacob, et al., 2004).

A separate proprietary study demonstrated that MON 87427 does not produce viable pollen when sprayed with glyphosate at appropriate developmental stages. The study evaluated the viability of pollen produced in MON 87427 corn that was sprayed with glyphosate at three growth stages: V3, V8, and V10. MON 87427 plants that received no glyphosate treatment produced 96.9% viable pollen and extruded anthers. Plants that received a single application of glyphosate at the V3 growth stage gave a similar response to untreated plants, indicating that glyphosate application did not result in a male sterile phenotype when plants were sprayed at this developmental stage only. In contrast, plants that received sequential applications of glyphosate at the V3, V8, and V10 growth stages produced 0% viable pollen and resulted in the expected male sterile phenotype. Based on results from this study, the use of the RHS system is expected to support the production of quality hybrid seed when glyphosate applications are made in agreement with technical guidelines.

REFERENCES:

CaJacob, C.A., P.C.C. Feng, G.R. Heck, M.F. Alibhai, R.D. Sammons and S.R. Padgette. 2004. Enigeering resistance to herbicides. Pages 353-372 in Handbook of Plant Biotechnology. P. Christou and H. Klee (eds.). John Wiley & Sons, Inc., New York, New York.

5. p78. Please provide sample size for Table VI-1.

Table VI-1 has been modified below to include the sample size and S.E. The sample size for the combined site analyses of MON 87427 was N =9. The sample size for each of the individual site

analyses of MON 87427 was N = 3. The sample size was N = 8 for the combined site analyses of the control, because one of the three replicates from the IARL site may have been contaminated with other events and was excluded from the analysis.

Table VI-1. Summary of Differences (α=0.05) for the Comparison of Maize Component Levels for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	Test[2] Mean[3] (S.E.)	Control[4] Mean (S.E.)	Mean Difference (Test minus Control) Mean Difference (% of Control)	Mean Difference (Test minus Control) Significance (p-Value)	Test Range	Conventional Tolerance Interval[5]
Statistical Differences Observed in Combined-Site Analysis						
Grain Proximate (% dw)						
Total Fat	3.50 (0.13)	3.69 (0.13)	-5.09	0.036	3.13 - 3.83	2.12, 5.35
Grain Fatty Acid (% Total FA)						
16:0 Palmitic	10.91 (0.26)	10.54 (0.26)	3.52	<0.001	10.44 - 11.52	6.42, 15.23
18:0 Stearic	1.97 (0.091)	1.90 (0.091)	3.67	0.038	1.81 - 2.17	0.87, 2.88
18:1 Oleic	24.28 (0.92)	23.52 (0.92)	3.22	0.010	22.84 - 26.62	11.30, 43.27
18:2 Linoleic	60.84 (1.28)	62.06 (1.28)	-1.96	0.002	57.61 - 62.70	41.35, 74.78
20:0 Arachidic	0.42 (0.030)	0.41 (0.030)	4.00	0.005	0.37 - 0.48	0.15, 0.67
Grain Anti-nutrient (% dw)						
Phytic Acid	0.96 (0.031)	1.02 (0.031)	-5.92	0.008	0.87 - 1.04	0.73, 1.23

Table VI-1 (continued). Summary of Differences (α=0.05) for the Comparison of Maize Component Levels for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	Test[2] Mean[3] (S.E.)	Control[4] Mean (S.E.)	Mean Difference (Test minus Control) Mean Difference (% of Control)	Mean Difference (Test minus Control) Significance (p-Value)	Test Range	Conventional Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Grain Fatty Acid (% Total FA)						
16:0 Palmitic Site ARNE	11.49 (0.056)	10.99 (0.056)	4.53	<0.001	11.47 - 11.52	6.42, 15.23
16:0 Palmitic Site IARL	10.72 (0.053)	10.44 (0.063)	2.66	0.007	10.58 - 10.85	6.42, 15.23
16:0 Palmitic Site ILWY	10.54 (0.054)	10.21 (0.054)	3.25	<0.001	10.44 - 10.65	6.42, 15.23
18:1 Oleic Site ARNE	26.34 (0.14)	25.35 (0.14)	3.93	<0.001	26.16 - 26.62	11.30, 43.27
18:1 Oleic Site IARL	22.91 (0.13)	21.95 (0.16)	4.41	0.002	22.84 - 22.98	11.30, 43.27
18:1 Oleic Site ILWY	23.58 (0.12)	23.24 (0.12)	1.44	0.043	23.29 - 23.78	11.30, 43.27
18:2 Linoleic Site ARNE	57.94 (0.16)	59.56 (0.16)	-2.72	<0.001	57.61 - 58.13	41.35, 74.78
18:2 Linoleic Site IARL	62.57 (0.14)	63.90 (0.17)	-2.09	<0.001	62.49 - 62.70	41.35, 74.78

Table VI-1 (continued). Summary of Differences (α=0.05) for the Comparison of Maize Component Levels for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	Test[2] Mean[3] (S.E.)	Control[4] Mean (S.E.)	Mean Difference (Test minus Control) Mean Difference (% of Control)	Mean Difference (Test minus Control) Significance (p-Value)	Test Range	Conventional Tolerance Interval[5]
Statistical Differences Observed in More than One Individual Site						
Grain Fatty Acid (% Total FA)						
18:2 Linoleic Site ILWY	62.01 (0.18)	62.72 (0.18)	-1.13	0.005	61.68 - 62.32	41.35, 74.78
Grain Amino Acid (% dw)						
Methionine Site ARNE	0.29 (0.0052)	0.27 (0.0052)	6.48	0.043	0.28 - 0.29	0.11, 0.29
Methionine Site IARL	0.23 (0.0038)	0.25 (0.0046)	-7.29	0.018	0.22 - 0.23	0.11, 0.29
Grain Fatty Acid (% Total FA)						
18:3 Linolenic Site ARNE	1.15 (0.014)	1.19 (0.014)	-3.92	0.033	1.13 - 1.17	0.78, 1.52
18:3 Linolenic Site IARL	1.24 (0.0078)	1.20 (0.0096)	3.35	0.014	1.22 - 1.26	0.78, 1.52
Grain Vitamin (mg/kg dw)						
Vitamin B2 Site ARNE	3.27 (0.17)	2.36 (0.17)	38.30	0.004	3.05 - 3.56	0, 4.47
Vitamin B2 Site IARL	1.41 (0.13)	1.93 (0.16)	-26.71	0.042	1.17 - 1.60	0, 4.47

Table VI-1 (continued). Summary of Differences (α=0.05) for the Comparison of Maize Component Levels for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	Test[2] Mean[3](S.E.)	Control[4] Mean (S.E.)	Mean Difference (Test minus Control) Mean Difference (% of Control)	Mean Difference (Test minus Control) Significance (p-Value)	Test Range	Conventional Tolerance Interval[5]
Statistical Differences Observed in One Individual Site						
Grain Proximate (% dw)						
Carbohydrates Site IARL	84.24 (0.32)	83.11 (0.39)	1.36	0.047	83.60 - 84.96	80.77, 89.46
Moisture (% fw) Site IARL	10.93 (0.14)	10.40 (0.17)	5.13	0.043	10.90 - 11.00	7.56, 14.80
Protein Site IARL	10.60 (0.30)	11.73 (0.35)	-9.64	0.019	9.91 - 11.35	5.79, 13.43
Grain Fiber (% dw)						
Acid Detergent Fiber Site ILWY	3.78 (0.18)	3.05 (0.18)	23.75	0.020	3.33 - 4.27	1.84, 4.39
Grain Amino Acid (% dw)						
Arginine Site IARL	0.48 (0.015)	0.53 (0.017)	-9.19	0.033	0.45 - 0.49	0.24, 0.68
Cystine Site IARL	0.24 (0.0036)	0.26 (0.0042)	-5.95	0.012	0.24 - 0.25	0.14, 0.30
Serine Site IARL	0.49 (0.017)	0.56 (0.021)	-11.21	0.037	0.46 - 0.51	0.24, 0.66

Table VI-1 (continued). Summary of Differences (α=0.05) for the Comparison of Maize Component Levels for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	Test[2] Mean[3] (S.E.)	Control[4] Mean (S.E.)	Mean Difference (Test minus Control) Mean Difference (% of Control)	Mean Difference (Test minus Control) Significance (p-Value)	Test Range	Conventional Tolerance Interval[5]
Statistical Differences Observed in One Individual Site						
Grain Amino Acid (% dw)						
Tryptophan Site ARNE	0.062 (0.0019)	0.052 (0.0019)	19.32	0.006	0.059 - 0.064	0.032, 0.069
Grain Fatty Acid (% Total FA)						
18:0 Stearic Site ARNE	2.17 (0.021)	2.04 (0.021)	6.43	0.002	2.16 - 2.17	0.87, 2.88
20:0 Arachidic Site ARNE	0.48 (0.0035)	0.46 (0.0035)	4.63	0.002	0.47 - 0.48	0.15, 0.67
22:0 Behenic Site ARNE	0.21 (0.0042)	0.19 (0.0042)	11.00	0.007	0.21 - 0.23	0, 0.32
Grain Mineral						
Calcium (% dw) Site ARNE	0.0077 (0.00024)	0.0067 (0.00024)	14.03	0.024	0.0075 - 0.0079	0.0019, 0.0076
Zinc (mg/kg dw) Site IARL	23.54 (0.55)	26.51 (0.67)	-11.20	0.010	22.45 - 24.61	11.46, 30.37
Grain Vitamin (mg/kg dw)						
Folic Acid Site IARL	0.36 (0.024)	0.45 (0.028)	-19.59	0.020	0.31 - 0.40	0.11, 0.61

Table VI-1 (continued). Summary of Differences (α=0.05) for the Comparison of Maize Component Levels for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	Test[2] Mean[3] (S.E.)	Control[4] Mean (S.E.)	Mean Difference (Test minus Control) Mean Difference (% of Control)	Mean Difference (Test minus Control) Significance (p-Value)	Test Range	Conventional Tolerance Interval[5]
Statistical Differences Observed in One Individual Site						
Grain Anti-nutrient (% dw)						
Raffinose Site ARNE	0.11 (0.0066)	0.13 (0.0066)	-18.51	0.031	0.11 - 0.11	0.024, 0.29
Forage Proximate (% dw)						
Carbohydrates Site IARL	86.46 (0.60)	84.12 (0.72)	2.78	0.029	86.21 - 86.75	80.13, 94.05
Moisture (% fw) Site IARL	69.90 (1.03)	74.71 (1.21)	-6.44	0.008	67.70 - 71.20	51.70, 86.22
Protein Site IARL	7.03 (0.40)	8.63 (0.49)	-18.59	0.037	6.75 - 7.40	1.34, 11.57

[1]dw = dry weight; fw = fresh weight; FA = fatty acid, S.E. = Standard Error

[2] MON 87427 treated with glyphosate, Combined site N= 9, Individual site N=3.

[3]Mean = least-square mean.

[4]Control refers to the near isogenic, conventional control, Combined-site N =8, ARNE and ILWY sites N=3, IARL site N=2.

[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial references. Negative limits were set to zero.

6. p110 and other parts of the petition. Your analyses and evaluations were based on comparing MON 87427 with untransformed corn hybrids. Why was the obvious treatment "glyphosate-treated MON 87427" not used as comparator: Please explain.

The purpose of the safety and risk assessment data presented in Section VII of the USDA petition is to determine whether the introduction of MON 87427 into the environment would have adverse effects on human and animal health and the environment. For comparative plant assessments used for risk assessment decisions, the test and control substances should be grown under similar conditions with minimal environmental and/or treatment differences between them. The preferred approach to achieve this objective is to compare the effect of the inserted DNA in MON 87427 corn with a conventional control in a similar genetic background. A strategy of comparing unsprayed MON 87427 with its conventional parental hybrid provides the most accurate assessment of the effect of the inserted DNA on the plant itself; both test and control are untreated, and any differences found can be attributed to the trait alone with no confounding interactions. Although other comparisons might be used to address questions that are not of primary interest in the risk assessment, this strategy is aligned with published guidance documents for environmental risk assessments (EFSA, 2010).

REFERECNES:

EFSA. 2010. Guidance on the environmental risk assessment of genetically modified plants. European Food Safety Authority, Parma, Italy.

7. p149. Last paragraph, last sentence. Pollen viability differences between MON 87427 and control are perceived as "non-biologically meaningful." Please clarify why this is so and provide relevant references, if available.

In Section IX.B.3.3 where the evaluation for pollen morphology and viability are reported, no statistically significant difference was detected for pollen diameter. However, MON 87427 had a statistically significant higher percent pollen viability than the conventional control (99.7 vs. 98.9%, respectively), and this value was slightly outside for the reference range (99.2-99.6%, see Table VII-7, page 118). Both MON 87427 and the control demonstrated a high level of pollen viability, and the difference was small in magnitude (0.8%). A difference of this magnitude can be interpreted as being not biologically meaningful because, under normal agronomic production, pollen is rarely if ever limiting. A corn plant can produce approximately 2 million to 5 million pollen grains when actively flowering. Furthermore, the number of pollen grains produced far exceeds the number of receptive silks, which can range from approximately 700-1000 (Hoeft, et al., 2000). Thus, a difference of 0.8% in pollen viability from a base of 2 million to 5 million pollen grains would have minimal to no impact on the number successful pollinations under field conditions. Therefore, the difference of 0.8% in pollen viability between MON 87427 and the control is interpreted as being not biologically meaningful.

REFERECNES:

Hoeft, R.G., E.D. Nafziger, R.R. Johnson and S.R. Aldrich. 2000. Corn as a crop. Pages 1-29 in Modern Corn and Soybean Production. MCSP Publications, Champaign, Illinois.

8. p150. The second paragraph states that CP4 EPSPS has been extensively assessed. However, you provide only one reference that is also 15 years old. Please provide current literature if available. Also, note that Hammond et al. 1996 in the same issue of the reference provided, is a more complete study than the one you provided.

The studies described by Harrison et al. (1996) included the *in vitro* digestive stability and acute oral toxicity of purified CP4 EPSPS protein produced in *E. coli*. The data in this paper also demonstrated the *E. coli* produced CP4 EPSPS protein is equivalent to the CP4 EPSPS protein produced in GM crops. The results of studies on animals fed glyphosate-tolerant soybeans are described by Hammond et al. (1996).

More recent studies on the safety of the CP4 EPSPS protein have also been published. The results and conclusions of the *in vitro* digestive stability of purified CP4 EPSPS protein from *E. coli* corroborate the results and conclusions of the Harrison paper (Shim, et al., 2010). There have also been reports on 13-week safety assurance studies with rats fed grain from corn containing CP4 EPSPS protein (Hammond, et al., 2004; Healy, et al., 2008). The results of these studies complement extensive agronomic, compositional, and farm animal feeding studies, and confirm that the grain from this GM corn is as safe and nutritious as the grain from existing commercial corn hybrids.

The safety of CP4 EPSPS proteins present in biotechnology-derived crops has also been demonstrated through a history of safe use. Several Roundup Ready crops that produce the CP4 EPSPS protein have been reviewed by USDA. The CP4 EPSPS protein expressed in MON 87427 is identical to the CP4 EPSPS proteins in other Roundup Ready crops including Roundup Ready soybeans, Roundup Ready 2 Yield soybeans, Roundup Ready corn 2, Roundup Ready canola, Roundup Ready sugar beet, Roundup Ready cotton, Roundup Ready Flex cotton and Roundup Ready alfalfa. The Environmental Protection Agency (EPA) also reviewed the safety of the CP4 EPSPS protein and has established a tolerance exemption for the protein and the genetic material necessary for its production either in or on all raw agricultural commodities (U.S. EPA, 1996). A history of safe use is supported by the lack of any documented reports of adverse effects since the introduction of Roundup Ready crops in 1996.

REFERENCES:

Hammond, B.G., J.L. Vicini, G.F. Hartnell, M.W. Naylor, C.D. Knight, E.H. Robinson, R.L. Fuchs and S.R. Padgette. 1996. The Feeding Value of Soybeans Fed to Rats, Chickens, Catfish

and Dairy Cattle Is not Altered by Genetic Incorporation of Glyphosate Tolerance. The Journal of Nutrition 126: 717-727.

Hammond, B., R. Dudek, J. Lemen and M. Nemeth. 2004. Results of a 13 week safety assurance study with rats fed grain from glyphosate tolerant corn. Food and Chemical Toxicology 42: 1003-1014.

Harrison, L.A., M.R. Bailey, M.W. Naylor, J.E. Ream, B.G. Hammond, D.L. Nida, B.L. Burnette, T.E. Nickson, T.A. Mitsky, M.L. Taylor, R.L. Fuchs and S.R. Padgette. 1996. The expressed protein in glyphosate-tolerant soybean, 5-enolpyruvylshikimate-3-phosphate synthase from Agrobacterium sp. strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. Journal of Nutrition 126: 728-740.

Healy, C., B. Hammond and J. Kirkpatrick. 2008. Results of a 13-week safety assurance study with rats fed grain from corn rootworm-protected, glyphosate-tolerant MON 88017 corn. Food and Chemical Toxicology 46: 2517-2524.

Shim, S.-M., M.-H. Choi, S.-H. Park, Y.-U. Gu, J.-M. Oh, S. Kim, H.-Y. Kim, G.-H. Kim and Y. Lee. 2010. Assessing the digestibility of genetically modified soybean: Physiologically based in vitro digestion and fermentation model. Food Research International 43: 40-45.

U.S. EPA. 1996. Plant pesticide inert ingredient CP4 enolpyruvylshikimate-3-D and the genetic material necessary for its production in all plants. Federal Register 61: 40338-40340.

9. p196. The last sentence states that the data are "within the previously set acceptance criterion [of ± 35%]." Please provide a reference for this statement.

The assay acceptance criteria used to assess equivalence of the *E. coli* and MON 87427-produced proteins are based on the following factors: 1) extensive experience with the analytical procedures used to assess protein equivalence and, 2) when available, public literature addressing method variability. For immunoequivalence, literature references for acceptance criteria as they relate to the characterization of agricultural biotechnology products are lacking. The acceptance criterion for immunoequivalence of ±35% reflects the historical level of variability found for this analysis as part of the equivalence assessments for proteins expressed in agricultural biotechnology products.

10. p208. Section 10.2., states that the data are "within the previously set acceptance criteria." Please provide a reference for this statement.

The assay acceptance criteria used to assess equivalence of the *E. coli* and MON 87427-produced proteins are based on the following factors: 1) extensive experience with the analytical

procedures used to assess protein equivalence and, 2) when available, public literature addressing method variability. For functional activity assessment, literature references for acceptance criteria as they relate to the characterization of agricultural biotechnology products are lacking. The acceptance criterion for functional activity equivalence (2-fold) was based on our extensive functional activity data set for CP4 EPSPS, generated through the characterization (and on-going re-certifications) of the *E. coli*-produced protein (Orion lot 10000739, historical APS lot 20-100015).

11. p211. Section D.3., describes that "the experimental arrangement had no impact on analyses." Provide a reference and/or the methods used for determining this.

The analyses consist of a calculation of summary statistics, i.e., mean, standard deviation, and range. The experimental design or experimental arrangement in the field has no impact on simple summary statistics because the formulas which are used to calculate simple summary statistics are independent of the experimental design.

12. p224. Why were "extreme" values not removed prior to control analysis: Please clarify.

Extreme data points that are outside of the ± 6 studentized PRESS residual range are determined to be statistical outliers. Statistical outliers should never be automatically excluded since they can be reasonable or real values relative to what can be expected from the analytical method. Instead each statistical outlier should be examined for either inclusion or exclusion. Six fatty acids from two conventional references at the ILWY site had PRESS residual values outside of ± 6 range. In the analysis of the combined-site datasets for these 6 fatty acids, the data points from the ILWY site were not the extreme highest or lowest values within the dataset. As a result, they were not considered analytical outliers and were not removed from the statistical analysis.

13. p225, Table E-3. Please complete table with data of the amino acids left out (e.g. Methionine to Tryptophan).

This portion of Table E-3 was mistakenly omitted during the preparation of the MON 87427 dossier, and is included below as page 227a.

Table E-3 (continued). Statistical Summary of Site ARNE Grain Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Amino Acid (% dw)						
Methionine	0.29 (0.0052) (0.28 - 0.29)	0.27 (0.0052) (0.26 - 0.27)	0.017 (0.0073) (0.011 - 0.024)	0.00064, 0.034	0.043	0.11, 0.29 (0.17 - 0.25)
Phenylalanine	0.55 (0.020) (0.53 - 0.57)	0.51 (0.020) (0.49 - 0.53)	0.038 (0.029) (0.026 - 0.049)	-0.028, 0.10	0.220	0.23, 0.75 (0.39 - 0.66)
Proline	0.96 (0.033) (0.91 - 0.98)	0.91 (0.033) (0.86 - 0.95)	0.050 (0.047) (0.029 - 0.073)	-0.059, 0.16	0.318	0.40, 1.24 (0.66 - 1.07)
Serine	0.51 (0.018) (0.49 - 0.52)	0.48 (0.018) (0.46 - 0.50)	0.029 (0.025) (0.015 - 0.052)	-0.029, 0.088	0.280	0.24, 0.66 (0.38 - 0.59)
Threonine	0.38 (0.010) (0.38 - 0.39)	0.36 (0.010) (0.35 - 0.37)	0.026 (0.014) (0.015 - 0.033)	-0.0072, 0.059	0.108	0.20, 0.46 (0.28 - 0.41)
Tryptophan	0.062 (0.0019) (0.059 - 0.064)	0.052 (0.0019) (0.051 - 0.053)	0.010 (0.0027) (0.0061 - 0.013)	0.0037, 0.016	0.006	0.032, 0.069 (0.039 - 0.063)

14. p262, Table E-14, title. Is the site you refer to (ILWY) really IARL? Please clarify.

The titles of Tables E-11, E-12, E-13, and E-14 should all refer to the ILWY site. The corrected pages 259-261 are included below.

Table E-12. Statistical Summary of Site ILWY Grain Anti-nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Anti-nutrient (% dw)						
Phytic Acid	1.02 (0.024) (1.00 - 1.04)	1.09 (0.024) (1.03 - 1.12)	-0.071 (0.034) (-0.11 - -0.032)	-0.15, 0.0081	0.072	0.73, 1.23 (0.82 - 1.07)
Raffinose	0.20 (0.0076) (0.19 - 0.21)	0.20 (0.0076) (0.18 - 0.21)	0.0046 (0.011) (-0.017 - 0.025)	-0.020, 0.029	0.671	0.024, 0.29 (0.092 - 0.21)

[1]dw = dry weight.

[2] MON 87427 treated with glyphosate.

[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.

[4]Control refers to the near isogenic, conventional control.

[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial references. Negative limits were set to zero.

Table E-13. Statistical Summary of Site ILWY Grain Secondary Metabolite Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Secondary Metabolite (µg/g dw)						
Ferulic Acid	2317.21 (50.54) (2243.74 - 2354.95)	2368.37 (50.54) (2236.10 - 2500.00)	-51.16 (41.17) (-145.05 - 7.64)	-146.09, 43.77	0.249	1070.41, 2955.86 (1588.35 - 2630.98)
p-Coumaric Acid	193.24 (4.64) (184.51 - 198.39)	191.67 (4.64) (183.88 - 203.20)	1.57 (4.42) (-6.38 - 10.46)	-8.63, 11.76	0.731	58.74, 313.97 (124.16 - 250.30)

[1]dw = dry weight.

[2] MON 87427 treated with glyphosate.

[3]Mean (S.E.) = least-square mean (standard error); CI = confidence interval.

[4]Control refers to the near isogenic, conventional control.

[5]With 95% confidence, interval contains 99% of the values expressed in the population of commercial conventional references. Negative limits were set to zero.

Table E-14 Statistical Summary of Site ILWY Forage Nutrient Content for MON 87427 vs. the Conventional Control

Analytical Component (Units)[1]	MON 87427[2] Mean (S.E.)[3] (Range)	Control[4] Mean (S.E.) (Range)	Difference (Test minus Control) Mean (S.E.) (Range)	95% CI Lower, Upper	Significance (p-Value)	Commercial Tolerance Interval[5] (Range)
Proximate (% dw)						
Ash	4.79 (0.16) (4.53 - 5.13)	4.58 (0.16) (4.40 - 4.80)	0.21 (0.22) (-0.0096 - 0.33)	-0.31, 0.72	0.378	2.66, 6.48 (3.70 - 5.95)
Carbohydrates	88.18 (0.46) (87.27 - 89.23)	88.56 (0.46) (87.89 - 88.92)	-0.38 (0.47) (-1.59 - 0.31)	-1.45, 0.70	0.442	80.13, 94.05 (83.23 - 90.37)
Moisture (% fw)	65.50 (1.37) (62.70 - 67.90)	66.00 (1.37) (64.10 - 67.30)	-0.50 (1.54) (-1.40 - 1.30)	-4.04, 3.04	0.753	51.70, 86.22 (61.00 - 76.00)
Protein	5.46 (0.40) (4.48 - 6.17)	5.55 (0.40) (5.17 - 5.96)	-0.082 (0.50) (-1.48 - 0.66)	-1.23, 1.07	0.873	1.34, 11.57 (4.37 - 9.31)
Total Fat	1.57 (0.27) (1.09 - 1.85)	1.32 (0.27) (0.58 - 2.20)	0.25 (0.39) (-1.11 - 1.18)	-0.64, 1.14	0.535	0.44, 3.33 (0.78 - 3.16)
Fiber (% dw)						
Acid Detergent Fiber	27.86 (1.16) (26.42 - 29.00)	26.59 (1.16) (24.57 - 27.71)	1.27 (1.61) (-1.28 - 3.58)	-2.45, 4.99	0.452	14.84, 38.51 (21.33 - 35.92)

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March 29, 2012

RECEIVED
By APHIS BRS Document Control Officer at 10:06 am, Mar 30, 2012

Dr. John Turner
United States Department of Agriculture
Biotechnology Regulatory Services, APHIS
4700 River Road, Unit 147
Riverdale, MD 20737-1236

RE: Waiver of Confidential Business Information (CBI) Claim for Petition Number 10-281-01p (Determination of Nonregulated Status for MON 87427 Maize with Tissue-Selective Glyphosate Tolerance Facilitating the Production of Hybrid Maize Seed)

Dear Dr. Turner:

Monsanto has developed glyphosate tolerant maize MON 87427 that utilizes a specific promoter and intron combination (*e35S-hsp70*) to drive the tissue-selective expression of the same 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) protein that is present in commercial Roundup Ready® crop products. In MON 87427, this specific promoter and intron combination results in limited or no production of CP4 EPSPS protein in two key male reproductive tissues, which is useful for facilitating the production of hybrid maize seed. Monsanto has requested a determination from APHIS that MON 87427 be granted nonregulated status under 7 CFR Part 340. In support of this request, Monsanto submitted petition number 10-281-01p on October 7th, 2010, which is currently under review by APHIS.

Monsanto does not object to APHIS publishing for public comment, the un-redacted version of Monsanto's petition for the determination of nonregulated status for MON 87427 that Monsanto submitted to APHIS on October 7th, 2010, including the letter supplied on July 13th, 2011, that APHIS deemed complete on August 9th, 2011. As we explained in our letter and supporting analysis provided to APHIS on February 22nd, 2012, Monsanto's Confidential Business Information (CBI) claim for certain information in our draft petition extends until such time as: 1) APHIS determines the petition to be "complete"; and 2) APHIS makes the final petition available for public comment. Therefore, we hereby waive all prior CBI claims related to this completed petition upon APHIS' publication of the same for public comment.

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Should you have any questions concerning this letter, Petition #10-281-01p, or if you wish to set up a meeting for further discussion, please contact Daniel Jenkins, U.S. Agency Regulatory Affairs Lead, Washington D.C., at 202-383-2851, or myself, at 314-694-8921 or bradley.a.comstock@monsanto.com.

Sincerely,

Bradley A. Comstock
Regulatory Affairs Manager

cc: Daniel Jenkins/Monsanto
Regulatory files/10-CR-214U