USDA/APHIS Environmental Assessment

In response to Monsanto Petition 04-229-01P Seeking a Determination of Nonregulated Status for Lysine Maize line LY038

OECD Unique Identifier REN-ØØØ38-3

U.S. Department of Agriculture
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TABLE OF CONTENTS

I. SUMMARY 3

II. INTRODUCTION 3

A. DEVELOPMENT OF LYSINE CORN LY038 3
B. APHIS REGULATORY AUTHORITY 4
C. FOOD AND DRUG ADMINISTRATION (FDA) REGULATORY AUTHORITY 5

III. PURPOSE AND NEED 5

IV. ALTERNATIVES 5

A. NO ACTION: CONTINUATION AS A REGULATED ARTICLE 5
B. DETERMINATION THAT LY038 CORN PLANTS ARE NO LONGER REGULATED ARTICLES, IN WHOLE 5
C. DETERMINATION THAT LY038 PLANTS ARE NO LONGER REGULATED ARTICLES, IN PART 6

V. POTENTIAL ENVIRONMENTAL IMPACTS 6

A. ALTERNATIVE A: NO ACTION 6
B. ALTERNATIVE B: APPROVAL OF THE PETITION IN WHOLE 7
1. Plant pathogenic properties 7
2. Potential impacts based on the relative weediness of LY038 corn 11
3. Potential impacts from gene introgression from LY038 corn into its sexually - compatible relatives 12
4. Potential impacts on threatened or endangered species or non-target organisms including beneficial organisms 14
5. Potential impacts on biodiversity 15
6. Potential impacts on agricultural and cultivation practices 15
7. Potential impacts on organic farming 15
8. Potential impacts on raw or processed agricultural commodities. 16
C. ALTERNATIVE C. APPROVAL OF THE PETITION IN PART 16

VI. CONSIDERATION OF EXECUTIVE ORDERS, STANDARDS AND TREATIES RELATING TO ENVIRONMENTAL IMPACTS 17

VII. LITERATURE CITED 19

VIII. AGENCY CONTACTS 21

APPENDIX A: APHIS AUTHORIZATIONS FOR FIELD TESTS OF MONSANTO LY038 CORN 22

APPENDIX B: SUMMARY TABLE OF DATA SUBMITTED WITH PETITION 04-229-01P FOR LY038 CORN 23

I. Summary

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) in response to a petition (APHIS Number 04-229-01p) from Monsanto Company on behalf of Renessen LLC regarding the regulatory status of genetically engineered (transformed) lysine maize derived from their transformation event LY038. This maize (hereinafter referred to as corn) is currently a regulated article under USDA regulations at 7 CFR Part 340, and as such, interstate movements, importations, and field tests of LY038 corn have been conducted under permits issued or notifications acknowledged by APHIS. Monsanto Company petitioned APHIS requesting a determination that LY038 corn does not present a plant pest risk, and therefore LY038 corn and its progeny derived from crosses with other nonregulated corn should no longer be regulated articles under these APHIS regulations.

The LY038 corn has been genetically modified to express the *cordapA* gene from *Cornybacterium glutamicum*. This gene encodes for lysine-insensitive dihydrodipicolinate synthase (cDHDPS) enzyme. The expression of *cordapA* is under the control of the maize Glb1 promoter, which directs cDHDPS expression predominately in the germ of the seed, resulting in accumulation of lysine in the grain. Corn-soybean meal based diets formulated for poultry and swine are characteristically deficient in lysine and require the addition of supplemental lysine for optimal animal growth and production. Development of LY038 corn provides an alternative to direct addition of supplemental lysine to poultry and swine diets by increasing the amount of lysine in the corn component of feed.

Field trials with LY038 corn have been conducted under the APHIS notification procedure (7 CFR Part 340.3). Performance standards for such field trials require that the regulated article and its offspring must not persist in the environment after completion of the test. In accordance with APHIS procedures for implementing the National Environmental Policy Act (NEPA) (7 CFR Part 372), this EA has been prepared prior to issuing a determination of nonregulated status for LY038 corn in order to specifically address the potential for impact to the human environment through the unconfined cultivation and use in agriculture of the regulated article.

II. Introduction

A. Development of Lysine Corn LY038

Monsanto Company has submitted a "Petition for Determination of Non-regulated Status" to the USDA, requesting a determination from APHIS that corn subline LY038 and any progeny derived from crosses between this line and other nonregulated corn varieties, no longer be considered regulated articles under 7 CFR Part 340.

Human food and animal feed are derived from many different grains. These grains are often deficient in some of the ten essential amino acids which are required in an animal

diet. Corn is a preferred animal feed because it is a low cost energy source, but it is relatively poor in amino acid content; particularly lysine which is a dietary requirement for many animals. Due to the low lysine content of corn, it is necessary to supplement corn-based feed with synthetic lysine produced via fermentation (Leuchtenberger, 1996; Kircher and Pfefferle, 2001). Currently over 100,000 metric tons per year of synthetic lysine is added to feed as a nutritional supplement in the United States.

Monsanto produced line LY038 with high lysine content and higher nutritional value for use as a feed ingredient for animals; primarily poultry and swine. These corn plants were genetically engineered to produce high levels of lysine in the germ of the seed by inserting a gene from *Corynebacterium glutamicum* that codes for the enzyme dihydrodipicolinate synthase into the corn genome. This gene, along with its regulatory sequences, was introduced into these corn plants via a biolistic transformation protocol. This is a well-characterized procedure, which has been widely used for over a decade for introducing various genes of interest directly into the plant genome.

APHIS authorized the first field testing of these corn plants in 2000 and they have been field tested in the United States under APHIS authorization (listed in Appendix A) in subsequent years. LY038 corn and its progeny have been evaluated extensively to confirm that they exhibit the desired agronomic characteristics and do not present a plant pest risk. The field tests have been conducted in agricultural settings under physical and reproductive confinement conditions.

B. APHIS Regulatory Authority

APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority granted by the Plant Protection Act (7 U.S.C. 7701-7772), regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. An organism is no longer subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation and is also a plant pest, or if there is reason to believe that it is a plant pest. These corn plants have been considered regulated articles because they were originally engineered with regulatory sequences derived from plant pathogens.

Section 340.6 of the regulations, entitled "Petition for Determination of Nonregulated Status", provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk, and therefore should no longer be regulated. If APHIS determines that the regulated article is unlikely to present a greater plant pest risk than the unmodified organism, the Agency can grant the petition in whole or in part. In such a case, APHIS authorizations (i.e., permits or notifications) would no longer be required for field testing, importation, or interstate movement of the non-regulated article or its progeny.

C. Food and Drug Administration (FDA) Regulatory Authority

The FDA policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the Federal Register on May 29, 1992, and appears at 57 FR 22984-23005. Under this policy, FDA uses what is termed a consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g., labeling) are resolved prior to commercial distribution of bioengineered food. Monsanto submitted a food and feed safety and nutritional assessment summary for LY038 corn in August 2004. A final FDA decision is pending.

III. PURPOSE and NEED

APHIS has prepared this EA before making a determination on the status of LY038 corn as regulated articles under APHIS regulations. The developer of these corn plants, Monsanto and Renessen, submitted a petition to USDA-APHIS requesting that APHIS make a determination that these corn plants shall no longer be considered regulated articles under 7 CFR Part 340.

This EA was prepared in compliance with the National Environmental Policy Act (NEPA) of 1969 as amended, (42 USC 4321 *et seq.*) and the pursuant implementing regulations (40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 372).

IV. ALTERNATIVES

A. No Action: Continuation as a Regulated Article

Under the Federal "no action" alternative, APHIS would not come to a determination that these corn plants are not regulated articles under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would still be required for introductions of corn LY038 lines. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from the unconfined cultivation of corn engineered to produce a high level of lysine in the seed.

B. Determination That LY038 Corn Plants Are No Longer Regulated Articles, In Whole

Under this alternative, plants of LY038 corn would no longer be regulated articles under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would no longer be required for introductions of high lysine corn derived from these events. A basis for this determination would include a "Finding of No Significant Impact" under the National Environmental Policy Act of 1969, as amended (42 USC 4321 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 342).

C. Determination That LY038 Plants Are No Longer Regulated Articles, In Part

The regulations at 7 CFR Part 340.6 (d) (3) (I) state that APHIS may "approve the petition in whole or in part." There are two ways in which a petition might be approved in part:

1. Approval of some but not all lines requested in the petition. In some petitions, applicants request deregulation of lines derived from more than one independent transformation event. In these cases, supporting data must be supplied for each line. APHIS could approve certain lines requested in the petition, but not others. This request is for the one event LY038 and its progeny.

2. Approval of the petition with geographic restrictions. APHIS could determine that the regulated article poses no significant risk in certain geographic areas, but may pose a significant risk in others. In such a case, APHIS might choose to approve the petition with a geographic limitation stipulating that the approved line could only be grown without APHIS authorization in certain geographic areas.

V. POTENTIAL ENVIRONMENTAL IMPACTS

Potential impacts to be addressed in this EA are those that pertain to the use of LY038 corn and its progeny in the absence of confinement.

A. Alternative A: No Action

If APHIS takes no action, commercial scale production of LY038 corn and its progeny is effectively precluded. These plants could still be grown in field trials for variety development as they have been for the past several years under APHIS authorizations (notifications). APHIS has evaluated field trial data reports submitted on this event and progeny, and has noted no significant adverse effects on non-target organisms, no increase in fitness or weediness characteristics, and no effect on the health of other plants. The Agency expects that future field tests would perform similarly.

From a commercial perspective, if APHIS were to take no action, and growers do not have improved varieties of corn seed derived from corn line LY038, they may choose to plant another cultivar with similar properties as an alternative. For example, cultivars developed through conventional breeding derived from the recessive gene *opaque-2* also have high levels of lysine in the seed and are commercially available. However, in these cultivars, the lysine levels are higher in the endosperm, and not in the germ, as is the case in LY038 corn. The endosperm of *opaque-2* is softer than conventional dent corn making it more susceptible to damage during harvesting and cracking during drying. The soft, chalky endosperm can also result in greater susceptibility to ear and kernel rots in certain genetic backgrounds (Thomison, accessed 2005). The yields of *opaque-2* varieties have generally been lower than those of most popular conventional dent hybrids (Wright, 1987).

B. Alternative B: Approval of the Petition in Whole

If APHIS were to grant the petition for non-regulated status in whole, LY038 event and its progeny would no longer be considered regulated articles. The unrestricted cultivation and distribution of LY038 corn is compared to that for other corn not subject to regulation by APHIS under 7 CFR Part 340 in the following sections.

1. Plant pathogenic properties

APHIS considered the potential for the transformation process, the introduced DNA sequences or their expression products to cause or aggravate disease symptoms in LY038 corn and its progeny or in other plants. APHIS also considered whether the data indicated that unanticipated unintended effects would arise from engineering of these plants. APHIS considered information from the scientific literature as well as data provided by the developer when conducting their field trials.

a. Recipient organism

The plant material used for development of LY038 corn was a publicly available inbred line of corn, H99. The line was released in 1974 by the Indiana Agricultural Experiment Station at Purdue University. The initial transformant, selected from the transformation process, was designated LY038, and various breeding lines were developed from this event to provide the data presented in the petition. The breeding history and progeny resulting from the initial event LY038 can be found in Figure V-16, p. 63 of the petition and an updated version of Figure V-19 in the addendum on page 9. Corn is not listed as a Federal noxious weed or on other weed lists such as:
Federal Noxious Weed List (http://www.aphis.usda.gov/ppq/weeds/noxwdsa.html),
Washington State Weed Lists (http://www.nwcb.wa.gov/weed_list/weed_listhome.html),
California Weed Species Lists (http://www.extendinc.com/weedfreefeed/list-b.htm),
Montana County Noxious Weed List (http://www.weedawareness.org/weed%20list.html),
North Dakota Noxious Weeds (http://www.ext.nodak.edu/extpubs/plantsci/weeds/w1103w.htm).

b. Transformation system

LY038 corn was developed using microprojectile bombardment, also known as the biolistics transformation method. This is a well characterized transformation system which integrates the donor genes into the chromosome of the recipient plant cell (Batty and Evans, 1992). The system does not require the use of the plant pathogen, *Agrobacterium tumefaciens*, or other transformation vectors. The donor DNA sequences are stably and irreversibly integrated into the plant's chromosomal or organellar DNA, where they are maintained and inherited as any other genes of the plant cell.

c. DNA sequences inserted into LY038

LY038 corn was produced by integrating the *cordapA* coding sequence from *Corynebacterium glutamicum* into the corn genome using the biolistic transformation

system. Corn callus was transformed and transformed plants subsequently regenerated from the callus. The plasmid used for the initial biolistic transformation contained the *cordapA* cassette as well as an *nptII* cassette encoding resistance to the antibiotic paromomycin to facilitate selection of the transgenic plants containing both the *cordapA* and *nptII* coding sequences. The *nptII* gene was eliminated from subsequent progeny using the Cre-*lox* recombination system for marker removal (Hare and Chua, 2002; Zhang et al., 2003). The *cordapA* cassette consisted of a *Zea mays globulin 1* (Glb1) promoter, a rice actin (rAct1) intron, a *cordapA* coding sequence with maize DHDPS chloroplast transit peptide (DTP), and a *globulin 1* 3' untranslated region (Glb1 3" UTR). The second cassette consisted of the *nptII* coding region regulated by the CaMV 35S promoter and the nopaline synthase 3' (NOS 3') transcription termination sequence. The *nptII* cassette was flanked by *lox*P sites that allowed the cassette to be excised by Cre recombinase when plants regenerated were crossed with corn plants expressing the *cre* gene. The *cre* gene was subsequently segregated out by conventional breeding to produce the LY038 product from which the *nptII* was eliminated. The absence of both the nptII gene and cre gene were confirmed in subsequent generations using Southern blot analyses (pages 43 and 44 and Figures III-1b and V-11b in the petition). The *loxP* sequence that remains in the plants is DNA that is not expressed and therefore produces no corresponding RNA or protein. The *lox* gene is present in natural bacterial populations, including the flora of the gut, and is a part of normal animal and human exposures. Since the cre and nptII genes were eliminated through conventional breeding, the resulting progeny only contain the gene of interest (*cordapA*) and not the gene used for selection.

The *cordapA* gene from *Corynebacterium glutamicum* was inserted at a single site in the corn genome. Molecular characterization indicated that LY038 corn contains one intact copy of the *cordapA* gene cassette. Molecular analysis also confirmed that LY038 corn does not contain either intact or partial DNA fragments of the *nptII* cassette or the *cre* cassette. The absence of both the *nptII* and *cre* genes was confirmed over multiple generations of breeding in various points of the LY038 corn breeding tree.

This *cordapA* gene is derived from *Corynebacterium glutamicum*, a common soil bacterium that is widespread in the environment. Animals and humans are regularly exposed to this bacterium and its components without adverse effects. The *cordapA* gene encodes for dihydrodipicolinate synthase (cDHDPS) enzyme. DHDPS catalyzes the first enzymatic step in the lysine biosynthetic pathway and is found in higher plants, animals and bacteria. The DHDPS enzymes from higher plants and sporulating bacteria are feedback inhibited by L-lysine, *i.e.* L-lysine diminishes the activity of the enzyme which controls its synthesis. Plant enzymes are usually more sensitive to L-lysine levels than those from bacteria (Karsten, 1997). The cDHDPS enzyme from *C. glutamicum* is particularly less sensitive to lysine feedback inhibition. Therefore, by inserting the *cordapA* gene (which produces cDHDPS) into the genome of corn, and controlling the expression with a promoter that is specific to seed development (the *globulin 1* promoter), more lysine accumulates in the developing seed.

d. Evaluation of intended effects

As expected, introduction of the *cordapA* gene into the corn genome resulted in plants containing increased levels of lysine in the developing seed.

Analysis of inheritance: Data provided and reviewed by APHIS demonstrate stable integration and inheritance of the *cordapA* gene and its associated regulatory sequences over several breeding cycles. Analyses of inheritance showed the expected segregation and stability of the trait through subsequent generations in the breeding program (petition Section V, B page 61, Figure V-17, and updated Figure V-17, pp 8 and 9 of the addendum).

Analysis of gene expression: The *cordapA* gene in LY038 corn is under the control of the maize Glb1 promoter, which directs cDHDPS expression predominately in the germ of the seed. Data on cDHDPS (dihydrodipicolinate synthase) protein concentrations were collected from field trials conducted at multiple locations. Using standard laboratory ELISA techniques, protein concentrations in various corn tissues were determined (petition Table V-2, p. 68) on both a fresh weight and dry weight basis. cDHDPS protein concentrations on a dry weight basis for grain, forage, whole plant, forage root, root and pollen tissues averaged 26, 0.94, 0.081, 0.069, 1.5 and 0.78 µg/g respectively. The results confirmed that expression of cDHDPS is predominantly in the grain tissue, as expected.

cDHDPS enzymes are ubiquitous in plants, animals and microorganisms and have not been associated with hazards from consumption or to the environment. The cDHDPS protein belongs to a family of related DapA (DHDPS) proteins. These proteins have been isolated from a number of species. APHIS has reviewed information related to the exposure and protein characteristics of cDHDPS (petition section VI) and concludes exposure to the plants containing cDHDPS would have no harmful environmental effects. LY038 corn is also undergoing review by the FDA for use in food and feed (http://www.cfsan.fda.gov).

Analysis of possible unintended effects: Expression of cDHDPS is not expected to cause plant disease or influence susceptibility in LY038 corn or its progeny to diseases or other pests. The gene encodes protein activity already present in plants and results in a modest elevation of naturally-occurring amino acids. Numerous field trials were conducted (Appendix A of this EA and Appendix 7 of the petition) to evaluate LY038 corn. Standard field trials included an evaluation of dormancy and germination, ecological evaluations (plant interactions with insect pests, disease, and abiotic stresses), phenotypic evaluations, and compositional changes. Data addressing the above categories were collected in order to assess possible effects from introduction of the *cordapA* gene and its associated regulatory sequences. The petitioner has described these trials, conducted in 2002 and 2003 in a variety of locations, and presented these data in Section VII of the petition (starting on p. 73).

For dormancy and germination testing there were no differences found between LY038 corn and the reference hybrids (Section VII, 1 and Table VII -3; pp 77-78).

Data presented for ecological evaluations (plant interactions with insect pests, disease, and abiotic stresses) showed that there were slight qualitative differences found between LY038 corn and the control hybrids (petition Table VII-6, p 85), but the incidence of each pest or stressor was within the range of incidence observed for the reference hybrids. The data presented by the petitioner therefore support the conclusion that the ecological interactions with insect pests, diseases and abiotic stressors for LY038 corn were not unintentionally altered compared to the control.

For the phenotypic evaluations, there were 14 phenotypic characteristics evaluated during field testing (outlined on page 80 of the petition). While there were some small qualitative statistical differences found, such as decreased seedling vigor and a small increase in plant height; these varied between years and were all within the ranges observed for the reference corn hybrids (petition Tables VII-5 and VII-7, pp 84 and 88). Analysis of phenotypic characteristics data showed no significant biological differences between the reference corn control populations and LY038 corn, or differences outside the range of conventional corn norms. The only unusual observation was the appearance of a white-leaf phenotype in LY038 corn in some field test locations. The white-leaf phenotype occurs at germination and persists only to the V2 stage (when the second collar appears on the second leaf). A similar white-leaf or decreased chlorophyll content phenotype has been observed in other plant species that accumulate high concentrations of lysine (Coruzzi and Last, 2000). In some cases this trait is associated with a loss of apical dominance and other growth abnormalities. No such characteristics have been observed in the LY038 plants in these field tests. APHIS has reviewed the data in the petition related to this phenotype, and concurs with the petitioner that this phenotype would not contribute a negative impact on the environment, either thorough increased weediness or other effects on plant health; particularly since the trait is only transiently expressed from germination up to the second leaf stage, and only under certain planting conditions.

In addition to field studies on agronomic parameters, Monsanto/Renessen analyzed corn for compositional changes as part of their submission to FDA for the consultation process. While FDA uses these data as indicators of possible nutritional changes, APHIS views them as general indicators of possible unintended changes. Compositional analyses evaluating 85 different analytical components were assessed by Monsanto/Renessen (summarized on page 95 of the petition). Included in these analyses were the lysine metabolites cadaverine, α-aminoadipic acid, saccharopine, homoserine, L-pipecolic acid and 2,6-diaminopimelic acid. Eighteen analyates had more than 50% of the observations below the Limits of Quantitation and were excluded from statistical analysis. The values for the lysine catabolite, α-aminoadipic acid were summarized separately. Sixty six components were statistically assessed. A summary of the compositional components for which statistically significant differences were detected between LY038 corn and the reference hybrids is presented in Table VII-11 (pp 97-106) of the petition and the summary of grain composition is summarized in Appendix 6 of the petition. A summary of α-aminoadipic acid levels in grain is presented in the petition, Table VII-12 (p 107).

In forage samples, the data showed that the range of values for the components measured were within the range of the population of conventional reference varieties (99% tolerance interval)(page 96 of the petition). There was no difference found in lysine or its catabolites in forage tissue. This is expected since the gene directs expression primarily in the seed.

In the grain tissues, fourteen of the 22 statistically significant differences between LY038 corn and the control grain were outside of the range representing the population of commercial varieties and these were attributed to difference in grain lysine or free lysine and its catabolites. Of the remaining eight differences, only one, Total Dietary Fiber (TDF) fell outside the ranges reported in the literature. The difference in TDF was small (0.7% DW) and only one of 15 samples fell outside of the calculated range, indicating that this is not likely to be biologically significant.

The increase levels of lysine and the related increases in the two lysine catabolites, saccharopine and α-aminoadipic acid, in grain are expected since the gene codes for increased lysine in the seed. Lysine and its catabolites exist in many organisms that produce and metabolize lysine. Substantial levels of α-aminoadipic acid have been reported in lentils (790 mg/100 g FW), garden peas (310 mg/100 g FW) and lettuce (320 mg/100 g FW) (Nawaz and Sorensen, 1977; Rozan et al., 2001). Saccharopine is also found in asparagus (400 mg/100 g FW) and lettuce (400 mg/100 g FW) (Nawaz and Sorensen, 1977) and edible mushrooms (102 mg/gram) (Oka et al., 1981). Lysine content of common foods like meat and milk are substantially higher than the ~ 400 mg/100g total lysine in LY038 corn (for information see http://www.nal.usda.gov/fnic/foodcomp/search/index.html).

The germination, ecological, phenotypic and compositional data summarize above indicate that LY038 corn does not exhibit unexpected or unintended effects.

2. Potential impacts based on the relative weediness of LY038 corn

APHIS assessed whether LY038 corn is any more likely to become a weed than the nontransgenic recipient corn line, or other corn currently cultivated. The assessment encompasses a thorough consideration of the basic biology of corn and an evaluation of unique characteristics of LY038 corn.

Almost all definitions of weediness stress as core attributes the undesirable nature of weeds from the point of view of humans; from this core, individual definitions differ in approach and emphasis (Baker, 1965; de Wet and Harlan, 1975; Muenscher, 1980) (Booth et al., 2003). The parent plant in this petition, *Zea mays* L., is not listed as a serious weed in A *Geographical Atlas of World Weeds* (Holm et al., 1991) or as a weed in *World Weeds: Natural Histories and Distribution* (Holm et al., 1997), *Weeds of the North Central States* (http://www.ag.uiuc.edu/~vista/html_pubs/WEEDS/list.html), *Weeds of the Northeast* (Uva et al., 1997), or *Weeds of the West* (Whitson et al., 1992) nor is it listed as a noxious weed species by the U.S. Federal Government (7 CFR Part 360). Corn has been grown throughout the world without any report that it is a serious

weed. Cultivated corn is unlikely to become a weed. It does not persistent in undisturbed environments without human intervention. Although corn volunteers are not uncommon, they are easily controlled by herbicides or mechanical means and rarely reappear in a second season. Corn also possesses few of the characteristics of plants that are notably successful weeds (Baker, 1965; Keeler, 1989).

As part of a bilateral agreement between the United States and Canada, USDA/APHIS and the Canadian Food Inspection Agency (CFIA) have generated documents that outline basic data requirements for developers of genetically engineered plants (http://www.aphis.usda.gov/brs/international_coord.html). One of these documents, Appendix II, outlines the environmental characterization data requirements for unconfined releases. As a part of the entire package requesting a determination of non-regulated status, these data are designed to address characteristics that influence both reproductive biology and survival biology of the transgenic plant compared to its non-transgenic counterpart.

Monsanto/Renessen conducted dormancy and germination testing on LY038 corn and conducted agronomic field trials at a total of 17 unique locations in the U.S. Corn Belt during the 2002 and 2003 growing seasons. Dormancy and germination testing showed that there were no differences in percent germinated (categorized as percent normal germinated and percent abnormal geminated, percent viable hard (dormant), percent dead, and percent viable firm swollen seed (petition Table VII-3, p 79).

Field trial data (Tables VII-5 and VII-7, pp 84 and 88) indicated that LY038 corn does not exhibit characteristics that would cause it to be more weedy than the parental corn line. At the 17 locations, the range of values for agronomic parameters was within the range of values expected for traditional corn hybrids. In addition, data showed no significant biological differences between line LY038 corn and the non-transgenic counterparts for disease and pest susceptibility. Traits evaluated include: seedling vigor, early stand count, days to 50% pollen shed, days to 50% silking, stay green, ear height, plant height, dropped years, stalk lodged plants, root lodged plants, final stand count, grain moisture, test weight and yield.

The introduced trait, increased lysine accumulation in the grain, is not expected to cause LY038 corn to become a weed. None of the characteristics of weeds described by Baker involve increased levels of an amino acid in the seed, and there is no reason to expect that this trait would result in increased weediness. There were no effects on seed dormancy or germination or other plant fitness characteristics, and the susceptibility to insects, diseases and abiotic stressor remain unchanged.

3. Potential impacts from gene introgression from LY038 corn into its sexually-compatible relatives.

APHIS evaluated the potential for gene introgression to occur from LY038 corn to sexually compatible wild relatives and considered whether such introgression would result in increased weediness. Cultivated corn, or maize, *Zea mays* L. subsp. *mays*, is

sexually compatible with other members of the genus *Zea*, and to a much lesser degree with members of the genus *Tripsacum*.

Wild diploid and tetraploid members of *Zea* collectively referred to as teosinte are normally confined to the tropical and subtropical regions of Mexico, Guatemala, and Nicaragua. A few isolated populations of annual (*Zea mexicana*) and perennial (*Zea perennis*) teosinte have been reported to exist in the past in Texas, Florida, South Carolina, Georgia and Maryland (USDA, 2004); but are likely no longer in existence (EPA, 2000), or are small isolated occurrences. None of these teosinte species have been shown to be aggressive weeds in their native or introduced habitats. The Mexican and Central America teosinte populations primarily exist within and around cultivated corn fields; they are partially dependent on agricultural niches or open habitats, and in some cases are grazed upon or fed to cattle which distribute the seed. While some teosinte may be considered to be weeds in certain instances, they are also used by some farmers for breeding improved corn (Sánchez and Ruiz, 1997).

All teosinte members can be crossed with cultivated corn to produce fertile F_1 hybrids (Wilkes, 1967; Doebley, 1990a). In areas of Mexico and Guatemala where teosinte and corn coexist, they have been reported to produce hybrids. Of the annual teosintes, *Z. mays* subsp. *mexicana* forms frequent hybrids with maize. *Z. luxurians* hybridizes only rarely with maize, whereas populations of *Z. mays* subsp. *parviglumis* are variable in their ability to form hybrids (Wilkes, 1977; Doebley, 1990a). Research on sympatric populations of maize and teosinte suggests introgression has occurred in the past, in particular from maize to *Z. mays* subsp. *luxurians* and *Z. mays* subsp. *diploperennis* and from annual Mexican plateau teosinte (*Z. mays* subsp. *mexicana*) to maize (Kato, 1997) and references therein).

In the wild, introgressive hybridization from maize to teosinte is currently limited, in part, by several factors including distribution, differing degrees of genetic incompatibility, differences in flowering time in some cases, block inheritance, developmental morphology and timing of the reproductive structures, dissemination, and dormancy (Galinat, 1988; Doebley, 1990a, 1990b). First-generation hybrids are generally less fit for survival and dissemination in the wild, and show substantially reduced reproductive capacity which acts as a significant constraint on introgression.

Teosinte has coexisted and co-evolved in close proximity to corn in the Americas over thousands of years, but corn and teosinte maintain distinct genetic constitutions despite sporadic introgression (Doebley, 1990a). The potential for gene introgression from LY038 corn into teosinte would increase if varieties are developed, and approved for cultivation in locations where these teosintes are located. It has been noted that populations of teosinte have been in decline for several decades due to increased grazing and urbanization in Mexico (Wilkes, 1995). A limited potential can also occur through smuggling unapproved seeds or from imported grain for planting. Since LY038 corn does not exhibit characteristics that cause it to be any more weedy than other cultivated corn, its potential impact due to the limited potential for gene introgression into teosinte is not expected to be any different from that of other cultivated corn varieties.

The genus *Tripsacum* contains up to 16 recognized species, most of which are native to Mexico, Central and South America, but three exist or have existed as wild and/or cultivated species in the U.S. (Hitchcock, 1971). *Tripsacum* floridanum is native to the southern tip of Florida (USDA, 2004). Though many of these species occur where corn might be cultivated, gene introgression from LY038 corn under natural conditions is highly unlikely or impossible. Hybrids of *Tripsacum* species with *Zea* are difficult to obtain outside of a laboratory. Crosses between *Z. mays* and *Tripsacum* result in male sterile progeny and fertility can only be restored after several generations of backcrossing. None of the hybrids are able to withstand even the mildest winters (Beadle, 1980; Galinat, 1988). If the LY038 plants were to naturally outcross with *T. floridanum*, the F1 progeny would be male sterile, so the persistence of the trait in the environment would be unlikely (Dewald and Sims, 2003). None of the sexually compatible relatives of corn in the U.S. are considered to be weeds in the U.S. (Holm et al., 1991) (Holm et al., 1997), therefore, the unlikely acquisition of the *cordapA* gene would not be expected to transform them into weeds.

4. Potential impacts on threatened or endangered species or non-target organisms including beneficial organisms

APHIS evaluated the potential for deleterious effects or significant impacts on non-target organisms, including those on the Federal Threatened and Endangered Species (TES) list of the U.S. Fish and Wildlife Service (FWS) (http://endangered.fws.gov/wildlife.html#Species), from cultivation of LY038 corn and its progeny. The gene that codes for the enzyme cDHDPS which leads to an increase in lysine in the grain of the seed is from the bacterium *Corynebacterium glutamicum. Corynebacterium glutamicum* is a common soil bacterium that is widely distributed in the environment, and is not a human or animal pathogen. This is the same bacterium used in the fermentation process to produce commercial lysine sources to supplement the feed for poultry and swine diets. DHDPS is the first enzyme unique to lysine biosynthesis in bacteria and higher plants (Galili, 1995) and the DHDPS protein has been isolated from a number of species. The applicant's assessment of the potential impact of cDHDPS on animal and human health is based upon the extensive characterization of the cDHDPS protein and its functional homology to other DHDPS proteins commonly found in a wide variety of animal feed and human food sources. These feed and food sources have a history of safe consumption and exposure. The applicant has shown the similarity of cDHDPS to DHDPS from other organisms (petition Section VI, Table VI-I and Appendix 3), and has also shown that there was no detectable glycosylation of the plant-produced cDHDPS protein. A summary of cDHDPS feed and food safety assessment is presented in section VI-E which demonstrates that this protein is not known to have any toxic properties.

The higher levels of lysine and it catabolites, saccharopine and α-aminoadipic acid in the seed tissues are expected due to the function of the gene. Since expression of the *cordapA* gene is directed towards the seed, the levels of lysine in all other tissues is within the range found in conventional varieties. Lysine levels in the pollen in LY038 corn (addendum page 13) are within the range reported in the literature for pollen from

field corn (Lundgren and Wiedenmann, 2004) so insects feeding on the pollen would not be adversely affected. Field observations of LY038 corn and its progeny revealed no negative effects on non-target organisms. The lack of known toxicity for this enzyme, lysine, and its catabolites suggests no potential for deleterious effects on beneficial organisms such as bees and earthworms. The high specificity of the enzyme for its substrate makes it unlikely that the introduced enzyme would metabolize endogenous substrates to produce compounds toxic to beneficial organisms.

BRS has reviewed the data in accordance with a process mutually agreed upon with the U.S. Fish and Wildlife Service ("FWS") to determine when a consultation, as required under Section 7 of the Endangered Species Act, is needed. APHIS has reached a determination that the release following a determination of nonregulated status would have no effects on listed threatened and endangered species and consequently a written concurrence or formal consultation with FWS is not required for this EA.

5. Potential impacts on biodiversity

Our analysis concludes that line LY038 corn exhibits no traits that would cause increased weediness, that its cultivation should not lead to increased weediness of other cultivated corn or other sexually compatible relatives, and it is unlikely to harm non-target organisms common to the agricultural ecosystem or threatened or endangered species recognized by the U.S. Fish and Wildlife Service. Based on this analysis, APHIS concludes that there is no potential for significant impact to biodiversity from a determination of non-regulated status as requested in the petition.

6. Potential impacts on agricultural and cultivation practices

Our analysis of the biology of corn leads to the conclusion that the cultivation of LY038 corn and its progeny would have no impact on agricultural or cultivation practices. The engineered line shows no significant differences from its parental line, in all aspects investigated, except for its production of high levels of lysine. The varieties of corn that would be derived from this line will be grown and cultivated in the same way as any other variety of corn.

7. Potential impacts on organic farming

The National Organic Program (NOP) administered by USDA's Agricultural Marketing Service (AMS) requires organic production operations to have distinct, defined boundaries and buffer zones to prevent unintended contact with prohibited substances from adjoining land that is not under organic management. Organic production operations must also develop and maintain an organic production system plan approved by their accredited certifying agent. This plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition on the use of excluded methods. Excluded methods include a variety of methods used to genetically modify organisms or influence their growth and development by means that are not possible under natural conditions or processes.

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 their approved organic system plan. Organic certification of a production or handling operation is a process claim, not a product claim.

It is not likely that organic farmers, or other farmers who choose not to plant transgenic varieties or sell transgenic grain, will be significantly impacted by the expected commercial use of this product since: (a) nontransgenic corn will likely still be sold and will be readily available to those who wish to plant it; (b) farmers purchasing seed will know this product is transgenic because it will be marketed as high lysine corn. This particular product should not present new and different issues than those with respect to impacts on organic farmers. APHIS has considered that corn is open-pollinating and it is possible that the engineered genes could move via wind-blown pollen to an adjacent field. All corn, whether genetically engineered or not, can transmit pollen to nearby fields, and a very small influx of pollen originating from a given corn variety does not appreciably change the characteristics of corn in adjacent fields. The rate of cross-pollination from one field to another is expected to be quite low, even if flowering times coincide. Using proper isolation distances can reduce potential cross pollination (http://www.agry.purdue.edu/ext/corn/news/articles.00/GMO_Issues-000309.html). The frequency of cross pollination decreases with increasing distance from the pollen source such that it is sufficiently low at 660 feet away to be considered adequate for production of certified corn seeds. Methods are currently available to prevent or minimize and test for cross-contamination.

8. Potential impacts on raw or processed agricultural commodities.

APHIS' analysis of data on agronomic performance, disease and insect susceptibility, and compositional profiles of the kernels indicate no differences between LY038 corn and its non-transgenic hybrid counterparts that would be expected to cause either a direct or indirect plant pest effect on any raw or processed plant commodity from deregulation of line LY038.

C. Alternative C. Approval of the Petition in Part

Approval of some but not all of lines requested in the petition. The petition requested a determination of nonregulated status only for lines derived from the one transformation event, designated as LT038. Therefore, APHIS can consider only that one line for approval.

Approval of the petition with geographic restrictions. APHIS has not identified any potential effects from LY038 corn on non-target organisms, including threatened or endangered species, or any adverse impacts on related plant species or plant pest effects that would warrant placing geographic restriction on planting of LY038 corn.

VI. CONSIDERATION OF EXECUTIVE ORDERS, STANDARDS AND TREATIES RELATING TO ENVIRONMENTAL IMPACTS

Executive Order (EO) 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations," requires Federal agencies to conduct their programs, policies, and activities that substantially affect human health or the environment in a manner so as not to exclude persons and populations from participation in or benefiting from such programs. It also enforces existing statutes to prevent minority and low-income communities from being subjected to disproportionately high and adverse human health or environmental effects.
EO 13045, "Protection of Children from Environmental Health Risks and Safety Risks," acknowledges that children may suffer disproportionately from environmental health and safety risks because of their developmental stage, greater metabolic activity levels, and behavior patterns, as compared to adults. The EO (to the extent permitted by law and consistent with the agency's mission) requires each Federal agency to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children. Each alternative was analyzed with respect to EO 12898 and 13045. None of the alternatives are expected to have a disproportionate adverse effect on minorities, low-income populations, or children.

EO 13112, "Invasive Species", states that federal agencies take action to prevent the introduction of invasive species and provide for their control and to minimize the economic, ecological, and human health impacts that invasive species cause. Nonengineered corn as well as different varieties on engineered corn are widely grown in the United States. Based on historical experience with these varieties and the data submitted by the applicant and reviewed by APHIS, the engineered plant is sufficiently similar in fitness characteristics to other corn varieties currently grown, and it is not expected to have an increased invasive potential.

Executive Order 12114, "Environmental Effects Abroad of Major Federal Actions" requires Federal officials to take into consideration any potential environmental effects outside the U.S., its territories and possessions that result from actions being taken. APHIS has given this due consideration and does not expect a significant environmental impact outside the United States should nonregulated status be determined for corn line LY038 or if the other alternatives are chosen. It should be noted that all the considerable,

existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new corn cultivars internationally, apply equally to those covered by an APHIS determination of nonregulated status under 7 CFR Part 340. Any international traffic in LY038 corn subsequent to a determination of non-regulated status for line LY038 would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed under the International Plant Protection Convention (IPPC).

The purpose of the IPPC "is to secure a common and effective action to prevent the spread and introduction of pests of plants and plant products, and to promote appropriate measures for their control" (https://www.ippc.int/IPP/En/default.jsp). The protection it affords extends to natural flora and plant products and includes both direct and indirect damage by pests, including weeds. The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (137 countries as of April 2005). In April, 2004, a standard for pest risk analysis of living modified organisms (LMOs) was adopted at a meeting of the governing body of the IPPC as a supplement to an existing standard, International Standard for Phytosanitary Measure No. 11 (ISPM-11; Pest Risk Analysis for Quarantine Pests). The standard acknowledges that all LMOs will not present a pest risk, and that a determination needs to be made early in the PRA for importation as to whether the LMO poses a potential pest risk resulting from the genetic modification. APHIS pest risk assessment procedures for bioengineered organisms are consistent with the guidance developed under the IPPC. In addition, issues that may relate to commercialization and transboundary movement of particular agricultural commodities produced through biotechnology are being addressed in other international forums and through national regulations.

The Cartagena Protocol on Biosafety is a treaty under the United Nations Convention on Biological Diversity (CBD) that established a framework for the safe transboundary movement, with respect to the environment and biodiversity, of LMOs, which includes those modified through biotechnology. The Protocol came into force on September 11, 2003 and 119 countries are parties to it as of April 14, 2005 (see http://www.biodiv.org/biosafety/default.aspx). Although the United States is not a party to the CBD, and thus not a party to the Cartagena Protocol on Biosafety, US exporters will still need to comply with domestic regulations that importing countries that are parties to the Protocol have put in place to comply with their obligations. The first intentional transboundary movement of LMOs intended for environmental release (field trials or commercial planting) will require consent from the importing country under an advanced informed agreement (AIA) provision, which includes a requirement for a risk assessment consistent with Annex III of the Protocol, and the required documentation. LMOs imported for food, feed or processing (FFP) are exempt from the AIA procedure, and are covered under Article 11 and Annex II of the Protocol. Under Article 11 Parties must post decisions to the Biosafety Clearinghouse database on domestic use of LMOs for FFP that may be subject to transboundary movement. To facilitate compliance with obligations to this protocol, the US Government has developed a website that provides the status of all regulatory reviews completed for different uses of bioengineered products

(http://usbiotechreg.nbii.gov). These data will be available to the Biosafety Clearinghouse.
APHIS continues to work toward harmonization of biosafety and biotechnology consensus documents, guidelines and regulations, including within the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the United States and in the Organization for Economic Cooperation and Development. NAPPO has completed three modules of a standard for the *Importation and Release into the Environment of Transgenic Plants in NAPPO Member Countries* (see http://www.nappo.org/Standards/Std-e.html). APHIS also participates in the North American Biotechnology Initiative (NABI), a forum for information exchange and cooperation on agricultural biotechnology issues for the U.S., Mexico and Canada. In addition, bilateral discussions on biotechnology regulatory issues are held regularly with other countries including: Argentina, Brazil, Japan, China, and Korea. Many countries, e.g. Argentina, Australia, Canada, China, Japan, Korea, Philippines, South Africa, Switzerland, the United Kingdom, and the European Union have already approved genetically engineered corn varieties to be grown or imported for food or feed (http://www.agbios.com/dbase.php).

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Appendix A: APHIS authorizations for field tests of Monsanto LY038 corn

2000 Field Trials

00-098-02n
00-256-06n

2001 Field Trials

01-047-10n
01-088-04n
01-267-01n
01-267-03n
01-332-02n

2002 Field Trials

02-031-01n
02-037-05n
02-042-12n
02-046-32n
02-052-05n
02-058-05n
02-066-15n
02-087-08n
02-087-09n
02-212-07n
02-212-10n
02-220-09n
02-220-11n
02-220-12n
02-263-08n

2003 Field Trials

03-052-17n
03-052-31n
03-052-32n
03-052-33n
03-052-34n
03-052-35n
03-052-36n
03-052-37n
03-052-38n
03-052-39n
03-052-40n
03-052-41n
03-058-08n
03-133-06n
03-133-07n
03-258-15n
03-258-17n
03-338-04n

2004 Field Trials

04-006-01n
04-006-02n
04-014-04n
04-014-07n
04-022-08n
04-022-09n
04-022-10n
04-022-11n
04-022-12n
04-022-13n
04-023-12n
04-023-15n
04-023-16n
04-023-17n
04-028-08n
04-028-20n
04-030-01n
04-030-12n
04-070-09n
04-099-02n

Appendix B: Summary table of data submitted with petition 04-229-01p for LY038 Corn

Molecular genetic characterization data	Figure/ table number and page in petition
Plasmid map of PV-ZMPQ76	Fig. III-1a p. 30, Fig. III-1b p. 31
Summary of genetic elements in PV-ZMPQ76	Table IV-1, p. 34
Cre-loxP recombination system	Fig. IV-1, p. 35
DNA insert diagram with restriction sites and predicted fragment sizes	Fig. V-1 p. 40
Southern blot analyses of genetic elements in PV-ZMPQ76 verifying insert and copy number, intactness of insert, promoters, intron, coding region, UTR and loxP elements, absence of NOS 3' Polyadenylation sequence, absence of nptII cassette, and absence of PV-ZMPQ76 backbone	Fig. V-2, p 46, See also addendum p. 5 Fig. V-3, p. 47 Fig. V-4, p. 48 Fig. V-5, p 49 Fig. V-6, p. 50 Fig. V-7, p. 51 Fig V-8, p. 52 Fig V-9, p. 53 Fig V-10, p. 54
Plasmid map of cre plasmid PV-ZM003	Fig. V-11a, p. 55, Fig V-11b, p. 56
Southern blot analyses of genetic elements in cre plasmid PV-ZM003, verifying absence of T-DNA, absence of cre cassette, absence of nptII cassette, and absence of plasmid backbone	Fig V-8, p. 52 Fig V-9, p. 53 Fig V-12, p. 57 Fig. V-13, p. 58 Fig. V-14, p. 59, See also addendum p. 7
Confirmation of the organization of the insert by PCR analysis	Fig. V-15, p. 60
Statistical analysis of genetic segregation pattern of LY038	Table V-1. p. 62
Southern blots verifying stability of inheritance of the *cordapA* gene over multiple generations	Fig. V-17, p. 65 Fig. V-18, p. 66 See also addendum page 8
cDHDPS protein level expression in various tissue types	Table V-2, p. 68
Comparison of amino acid sequence of cDHDPS and representative DHDPS proteins	Table VI-1, p. 74
Field site planting information	Table VII-4, p. 82
Seed germination and dormancy	Table VII-3, p. 79
Phenotypic characterization data	Table VII-5, p. 84 Table VII-7, p. 88 Table VII-8, p. 89
Diseases, Insects, and Abiotic stresses	Table VII-6, p. 85 Table VII-9, p. 90
Pollen characterization	Table VII-10, p. 92
Compositional analyses	Table VII-11, p. 97-106 Table VII-12, p. 107