Pioneer Hi-Bred International, Inc.
Seed Production Technology (SPT)
Process DP-32138-1 Corn

OECD Unique Identifier:
DP-32138-1

Final Environmental Assessment

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Acronyms and Abbreviations

32138 SPT maintainer	Maize line containing the DP-32138-1 event
32138 SPT insertion	The DNA from plasmid PHP24597 that has been integrated into the genome of the 32138 SPT maintainer
AMS	USDA Agricultural Marketing Service
AOSCA	American Organization of Seed Certifying Agencies
APHIS	Animal and Plant Health Inspection Service
BRS	Biotechnology Regulatory Services (within USDA–APHIS)
Bt	*Bacillus thuringiensis* protein
CAA	Clean Air Act
CFR	Code of Federal Regulations (United States)
CH_4	methane
CO_2	carbon dioxide
CWA	Clean Water Act
DP-32138-1	Event DP-32138-1, also known as 32138 SPT maintainer
DsRed2(Alt1)	A specific fluorescent marker gene; a variant of Clontech's *DsRed* gene
DsRed2	A specific fluorescent protein expressed by *DsRed2(Alt1)* gene
EA	environmental assessment
EO	Executive Order
EPA	U.S. Environmental Protection Agency
FDA	U.S. Food and Drug Administration
FFDCA	Federal Food, Drug, and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FR	Federal Register
GE	genetically engineered
GHG	greenhouse gas
IP	identity preservation
IPCC	Intergovernmental Panel on Climate Change
ISPM	International Standard for Phytosanitary Measure
IPPC	International Plant Protection Convention
LMO	living modified organisms
Ms45	A specific (dominant) male-fertility gene in maize
MS45	A specific protein expressed by the *Ms45* gene in maize
ms45	A mutant allele (recessive) of the *Ms45* gene in maize

Acronyms and Abbreviations (continued)

NMFS	National Marine Fisheries Service
NO_2	nitrogen dioxide
N_2O	nitrous oxide
NEPA	National Environmental Policy Act of 1969 and subsequent amendments
NOP	National Organic Program
NPC	new protein consultation
OSTP	Office of Science and Technology Policy
PRA	pest risk analysis
R&D	research and development
SPT	Seed Production Technology
sq. ft.	square feet
TES	threatened and endangered species
TSCA	Toxic Substances Control Act
U.S.	United States
USDA	U.S. Department of Agriculture
USDA-ERS	U.S. Department of Agriculture-Economic Research Service
USDA-FAS	U.S. Department of Agriculture-Foreign Agricultural Service
USDA-NASS	U.S. Department of Agriculture-National Agricultural Statistics Service
USDA-NOP	U.S. Department of Agriculture-National Organic Program
U.S.C.	United States Code
USFWS	U.S. Fish and Wildlife Service
ZM-AA1	A specific α-amylase protein expressed in 32138 SPT maintainer and comprised of maize endogenous protein sequences from the Brittle-1 transit peptide and the ZM-AA1 protein.

I. Purpose and Need

Regulatory Authority

"Protecting American agriculture" is the basic charge of the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS). APHIS provides leadership in ensuring the health and care of plants and animals. The agency improves agricultural productivity and competitiveness, and contributes to the national economy and the public health. USDA asserts that all methods of agricultural production (conventional, organic, or the use of genetically engineered (GE) varieties) can provide benefits to the environment, consumers, and farm income.

Since 1986, the United States government has regulated genetically engineered (GE) organisms pursuant to a regulatory framework known as the Coordinated Framework for the Regulation of Biotechnology (Coordinated Framework) (51 FR 23302, 57 FR 22984). The Coordinated Framework, published by the Office of Science and Technology Policy, describes the comprehensive federal regulatory policy for ensuring the safety of biotechnology research and products and explains how federal agencies will use existing Federal statutes in a manner to ensure public health and environmental safety while maintaining regulatory flexibility to avoid impeding the growth of the biotechnology industry. The Coordinated Framework is based on several important guiding principles: (1) agencies should define those transgenic organisms subject to review to the extent permitted by their respective statutory authorities; (2) agencies are required to focus on the characteristics and risks of the biotechnology product, not the process by which it is created; (3) agencies are mandated to exercise oversight of GE organisms only when there is evidence of "unreasonable" risk.

The Coordinated Framework explains the regulatory roles and authorities for the three major agencies involved in regulating GE organisms: USDA's Animal and Plant Health Inspection Service (APHIS), the Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA).

APHIS is responsible for regulating GE organisms and plants under the plant pest authorities in the Plant Protection Act of 2000, as amended (7 USC § 7701 *et seq*.) to ensure that they do not pose a plant pest risk to the environment.

The FDA regulates GE organisms under the authority of the Federal Food, Drug, and Cosmetic Act. The FDA is responsible for ensuring the safety and proper labeling of all plant-derived foods and feeds, including those that are genetically engineered. To help developers of food and feed derived from GE crops comply with their obligations under Federal food safety laws, FDA encourages them to participate in a voluntary consultation process. All food and feed derived from GE crops currently on the market in the United States have successfully completed this consultation process. 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 (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.

The EPA regulates plant-incorporated protectants under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and certain biological control organisms under the Toxic Substances

Control Act (TSCA). The EPA is responsible for regulating the sale, distribution and use of pesticides, including pesticides that are produced by an organism through techniques of modern biotechnology.

Regulated Organisms

The APHIS Biotechnology Regulatory Service's (BRS) mission is to protect America's agriculture and environment using a dynamic and science-based regulatory framework that allows for the safe development and use of GE organisms. APHIS regulations at 7 Code of Federal Regulations (CFR) part 340, which were promulgated pursuant to authority granted by the Plant Protection Act, as amended (7 United States Code (U.S.C.) 7701–7772), regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. A GE organism is no longer subject to the plant pest provisions of the Plant Protection Act or to the regulatory requirements of 7 CFR part 340 when APHIS determines that it is unlikely to pose a plant pest risk. A GE 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 (7 CFR 340.2) and is also considered a plant pest. A GE organism is also regulated under Part 340 when APHIS has reason to believe that the GE organism may be a plant pest or APHIS does not have information to determine if the GE organism is unlikely to pose a plant pest risk.

A person may petition the agency that a particular regulated article is unlikely to pose a plant pest risk, and, therefore, is no longer regulated under the plant pest provisions of the Plant Protection Act or the regulations at 7 CFR 340. The petitioner is required to provide information under § 340.6(c)(4) related to plant pest risk that the agency may use to determine whether the regulated article is unlikely to present a greater plant pest risk than the unmodified organism. A GE organism is no longer subject to the regulatory requirements of 7 CFR part 340 or the plant pest provisions of the Plant Protection Act when APHIS determines that it is unlikely to pose a plant pest risk.

Petition for Determination of Nonregulated Status: Pioneer 32138-1 Seed Production Technology (SPT) Maintainer

Pioneer Hi-Bred International, Inc. (Pioneer) of Johnston, IA has submitted a petition to APHIS seeking a determination that their "Seed Production Technology" (SPT) corn maintainer event (hereafter referred to as DP-32138-1 is unlikely to pose a plant pest risk and, therefore, should no longer be a regulated article under regulations at 7 CFR Part 340.

According to Pioneer, DP-32138-1 is engineered to produce male sterile female inbred plants for the generation of hybrid corn seed that is non-transgenic (Pioneer 2009). As detailed in the petition, the carefully controlled expression of a seed color marker gene and pollen fertility and sterility genes allows for the generation of red transgenic seed for seed increase of male sterile female inbred lines. The multistep process yields a non-transgenic male-sterile female parent. This non-transgenic material can then be used for hybrid seed production (Pioneer 2009). DP-32138-1 is currently regulated under 7 CFR part 340. Interstate movements and field trials of DP-32138-1 have been conducted under permits issued or notifications acknowledged by APHIS.

DP-32138-1 corn is currently regulated under 7 CFR Part 340. Interstate movements, importations, and field testing of DP-32138-1 corn have been conducted under notifications acknowledged by APHIS.

DP-32138-1 has been field tested in the U.S. since 2005 as authorized by APHIS. Associated notifications acknowledged and permits issued by APHIS are listed in the petition (Pioneer 2009). The list compiles a total of 11 test sites in diverse regions of the U.S. including the major corn growing area of the Midwest and winter nurseries in Hawaii and Puerto Rico. Field tests conducted under APHIS oversight allow for evaluation in agricultural settings under confinement measures designed to minimize the likelihood of persistence in the environment after completion of the field trial. Under confined field trial conditions, data are gathered on multiple parameters and used by applicants to evaluate agronomic characteristics and product performance. These data are also valuable to APHIS for assessing the potential for a new variety to pose a plant pest risk. The evaluated data may be found in the APHIS Plant Pest Risk Assessment (USDA-APHIS 2010).

Purpose of Product

Pioneer seeks determination of nonregulated status of DP-32138-1 corn that was developed to facilitate a novel method of seed production to produce male sterile/female inbred plants for the generation of hybrid corn seed that is non-transgenic (Pioneer 2009). As detailed in the petition, the carefully controlled expression of a seed color marker gene and pollen fertility and sterility genes allows for the generation of red transgenic seed for seed increase of male sterile female inbred lines that are used for non-transgenic hybrid commercial seed production. Typically, detasseling is needed in corn seed production, and confers substantial expense, lower seed yield and lower genetic purity. Use of DP-32138-1 would eliminate detasseling and lead to increased seed yield and higher genetic purity during seed increase operations. As detailed in the petition, the process predictably and reliably results in a commercial product which does not contain the DP-32138-1 transgenes (Pioneer 2009).

Since the 1930's, corn productivity in the U.S. has been greatly enhanced by the use of hybrid corn seed (Sleper and Poehlman 2006). Corn hybrids are characterized by increased resistance to diseases and enhanced agronomic characteristics compared with the parental lines (Brewbaker 1964). The production of hybrid corn seed involves a cross between two inbred lines[1], where the pollen from the tassel/male parent is used to fertilize the ear/female parent. Because corn is mostly self pollinated (OECD 2003), hybrid corn seed is typically produced by the removal of male flowers (tassels) from the female parent plant either mechanically or by hand. These methods of detasseling reduce yields and are expensive. Mechanical detasseling may result in up to 40 % reduction in seed yield compared to hand detasseling treatments (Wych 1988). Additionally, female plants may escape detasseling or develop secondary tassels after manual detasseling resulting in female plants that are self-pollinated. Thus, the seed of female inbred plants is harvested along with the hybrid seed resulting in reduced genetic purity and lower seed yield for the final hybrid variety.

To overcome the expense, yield reduction and reduced genetic purity associated with detasseling, numerous genetic strategies have been attempted to achieve male sterility[2] as alternative

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[1] Inbred lines are populations of identical or nearly identical plants used as stocks for the creation of hybrid lines.

[2] Male sterility is the inability to produce functional pollen.

approaches to detasseling (Skibbe and Schnable 2005). Over 40 genetic elements are associated with male sterility; some of which are located on the nuclear chromosomes while others are located on the mitochondria chromosomes (Skibbe and Schnable 2005). A major drawback to male sterile genetic approaches is the difficulty in generating male sterile inbred lines, because no functional pollen is produced in a male sterile plant line.

APHIS' Response to Petition for Nonregulated Status

Under the authority of the plant pest provisions of the Plant Protection Act and 7 CFR Part 340, APHIS has issued regulations for the safe development and use of GE organisms. As required by 7 CFR 340.6, APHIS must respond to petitioners who request a determination of the regulated status of GE organisms, including GE plants such as DP-32138-1. When a petition for nonregulated status is submitted, APHIS must make a determination if the GE organism is unlikely to pose a plant pest risk. If APHIS determines based on its Plant Pest Risk Assessment (PPRA) that the genetically engineered organism is unlikely to pose a plant pest risk, the genetically engineered organism is no longer subject to the plant pest provisions of the Plant Protection Act and 7 CFR part 340.

APHIS has prepared this environmental assessment (EA) to consider the potential environmental effects of an agency determination of nonregulated status consistent with Council of Environmental Quality's (CEQ) regulations implementing NEPA (40 CFR Parts 1500-1508, 7 CFR 1b, and 7 CFR Part 372), and the USDA and APHIS NEPA implementing regulations and procedures. This EA has been prepared in order to specifically evaluate the effects on the quality of the human environment[3] that may result from the deregulation of DP-32138-1 corn.

Coordinated Framework Review

DP-32138-1 is not designed for human and animal consumption. Discarded parent seed, whether it is outdated or discarded from the color sorter, and seed byproducts, such as husks and cobs, will be disposed by placing in landfills, composting, or incineration (Pioneer 2009). According to Pioneer policy, seed producers will not feed discard corn to animals (Hubbard, 2011 - Public Comment on EA for DP-32138-1). However, animals may accidentally gain access to corn fields, to discarded corn seed or by-products and therefore may also be subject to regulation by FDA. A new protein consultation (NPC) for the DsRed2 protein color marker was submitted to FDA on October 11, 2006 with the follow up letter of January 29, 2010 received from FDA (Appendix A). The DsRed2 protein is the only non-corn protein in DP-32138-1. A NPC for the ZM-AA1 protein, normally found in germinating corn seeds, was submitted to FDA on June 18, 2009. The FDA considers Pioneer's consultation on ZM-AA1 alpha-amylase protein to be complete (Appendix B). Because DP-32138-1 does not contain any GE pesticides or the genetic machinery necessary to produce them, or tolerance to herbicides, EPA consultation is not required.

Public Involvement

APHIS-BRS routinely seeks public comment on draft environmental assessments prepared in response to petitions for nonregulated status of GE organisms. APHIS-BRS does this through a

[3] Under NEPA regulations, the "human environment" includes "the natural and physical environment and the relationship of people with that environment" (40 CFR §508.14).

notice published in the Federal Register. The issues discussed in this EA were developed by considering public concerns as well as issues raised in public comments submitted for other environmental assessments of genetically engineered organisms, concerns raised in lawsuits, as well as those issues that have been raised by various stakeholders. These issues, including those regarding the agricultural production of corn using various production methods, and the environmental and food/feed safety of genetically engineered plants were addressed to analyze the potential environmental impacts of DP-32138-1.

This EA, the petition submitted by Pioneer, and APHIS's Plant Pest Risk Assessment, will be available for public comment for a period of 60 days (7 CFR § 340.6(d)(2)). Comments received by the end of the 60-day period will be reviewed and used to inform APHIS's determination decision of the regulated status of DP-32138-1corn and to assist APHIS in determining whether an Environmental Impact Statement is required prior to the determination decision of the regulated status of this corn line.

Issues Considered

As stated above, the issues considered in this EA were developed based on APHIS' determination to deregulate certain genetically engineered organisms, and for this particular EA, the specific deregulation of DP-32138-1 for seed production.

Management Considerations:
- Acreage and Areas of Corn Production
- Cropping Practices
- Seed Production
- Organic Farming
- Specialty Corn Production

Environmental Considerations:
- Water Use
- Soil
- Air Quality
- Climate Change
- Animals
- Plants
- Biological Diversity
- Gene Movement

Public Health Considerations:
- Human Health
- Worker Safety

Socioeconomic Considerations:
- Domestic Economic Environment
- Trade Economic Environment
- Social Environment

II. Affected Environment

Agricultural Production of Corn

Acreage and Areas of Corn Production

Corn (*Zea mays* L.) is the world's most widely grown cereal, reflecting its ability to adapt to a wide range of production environments (Morris 1998). Corn is an annual plant typically grown in zones of abundant rainfall and fertile soils (Morris 1998). In the U.S., the moisture levels and number of frost-free days required to reach maturity are ideal for corn to be grown within temperate regions. Corn varieties having a relative maturity of 100 to 115 days are typically grown in the U.S. Corn Belt, which includes Iowa, Illinois, Nebraska, and Minnesota. Approximately 50% of all corn grown in the U.S. is from these four states. The Corn Belt also includes parts of Indiana, South Dakota, Kansas, Ohio, Wisconsin, and Missouri. Approximately 80% of all corn acres grown in the U.S. are within these top ten corn growing states; however, corn is grown in all states of the U.S. except Alaska (USDA-NASS 2010a).

Conventional farming, as defined in this document, includes any farming system where synthetic pesticides or fertilizers may be used. This definition of conventional farming includes the use of genetically engineered varieties that have been deregulated by APHIS. Conventional farming covers a broad scope of farming practices, ranging from farmers who only occasionally use synthetic pesticides and fertilizers to those farmers whose harvest depends on regular pesticide and fertilizer inputs.

U.S. corn production for 2009, including production of non-genetically engineered and genetically engineered corn varieties, was 13.2 billion bushels from 79.6 million harvested acres (USDA-NASS 2009a). In 2007 and 2008, growers harvested 13.0 billion bushels of grain from 86.56 million acres and 12.1 billion bushels from 78.6 million acres, respectively. Of the total corn acres planted in 2009, 85% were GE corn varieties (USDA-NASS 2009a) up from 80% in 2008 (USDA-NASS 2009a), 73% in 2007 (USDA-NASS 2009a), and 61% in 2006 (USDA-NASS 2009a).

Cropping Practices

Today, growers can choose from hundreds of corn hybrids marketed by companies that produce seed including GE varieties (NCGA 2010). Pioneer, the petition applicant, itself offers over 250 varieties (Pioneer 2010), which in turn implies the total number of corn hybrids available industry wide to be approximately 800-1000 since Pioneer has approximately a 30% market share (Pioneer 2008). Another estimate of total number of hybrids available for the industry is 4000 (Monsanto 2010).

Hybrids differ generally in agronomic characteristics, including disease and pest resistance and length of growing period. The optimum planting date for corn, usually between April and May, is influenced by factors such as the locality, environmental conditions, seed growing period, and seed variety. Harvesting generally occurs from mid-to-late September through November. The use of a combine (mechanical harvesting) is the standard practice for grain production (Olson and Sander 1988).

Crop rotations (successive planting of different crops on the same land) are used to optimize soil nutrition and fertility, and reduce pathogen loads (Hoeft, Nafziger et al. 2000). Crops used in rotation with corn vary regionally, but there has been an increase in the number of fields that have a corn-to-corn rotation, as opposed to rotation to another crop (Erickson and Lowenberg-DeBoer 2005). In some areas, the corn-to-corn rotation requires increased levels of fertilizer inputs (Sawyer 2007). Insect pests may also increase in corn-to-corn rotations as this system may provide a continual host environment for some insects and diseases. However, in a corn-soybean rotation, continuously growing corn for multiple growing seasons can decrease populations of soybean pests, such as soybean cyst nematode (Hoeft, Nafziger et al. 2000).

Corn production typically involves the extensive use of agronomic inputs and technology (Rooney and Serna-Saldivar 1987; Shaw 1988; Pollak and White 1995; White and Pollak 1995), and the main emphasis is placed on obtaining the best yield (Thomas 2007). Depending on the region and practices used, corn production includes inputs such as fertilizer (e.g., synthetic fertilizers, manure, and compost containing nitrogen and phosphorus) and pesticides (synthetic or NOP-approved insecticides, herbicides and fungicides), as well as irrigation. Each of these inputs can affect segments of the environment including, but not limited to, waterways by increases in nutrient pollution, biodiversity because pesticide inputs cause species changes, the water table because of excessive irrigation practices or productive fields because irrigation increases salinity (Hoeft, Nafziger et al. 2000).

Weed control methods differ depending on a number of factors including locality, grower resources, and crop trait; the techniques may be direct (e.g. mechanical[4] and chemical[5]) or indirect (e.g. cultural[6]) (Olson and Sander 1988). Pest control (weeds and insects) in corn production is essential in order to obtain good crop yield (Olson and Sander 1988; Hoeft, Nafziger et al. 2000). Generally, growers will manage a range of pests simultaneously. Therefore, growers will likely chose from a number of techniques to effectively and efficiently manage pests in their fields. In 2005, the most prevalent pest management practice was pesticide use (USDA-ERS 2005). Ultimately, the management practices utilized by a grower will depend on the types of pests in their field, level of infestation, cropping system, type of soil, cost, weather, time, and labor. Practices to cope with pests, nutrient needs, and moisture and temperature requirements vary regionally. All agricultural production systems, including corn production, may affect the surrounding environment.

Seed Production

Once a new variety has been developed by the plant breeder, seed providers need to increase seed so that commercial fields can be planted by farmers that wish to take advantage of new traits. This increase is necessary for all varieties of any plant species, including corn. The increase starts with a single seed, a single plant or a handful of seed. In the case of corn, the seed of all varieties combined must be able to plant over 90 million acres (USDA-NASS 2009a) each year. Since seed companies typically overproduce by 30-40% to assure enough seed for the

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[4] Mechanical techniques include tillage and mowing.

[5] Chemical techniques include herbicide application.

[6] Cultural techniques include crop rotation/spot spraying of herbicide/hand removal of weeds.

coming year, about 50-60 million bushels of seed are needed to meet the projected seed needs of the next year (Wych 1988). The precise number of bushels of seed needed for a given year is not known until all fields have been planted. A number of factors may influence the number of final planted acres including: weather, anticipated future commodity prices of corn and competing crops, changing goals for the amount of ethanol to be needed in future years, final crop yields in the U.S. and other countries that are harvested in the year the seed is produced, changing goals of government programs such as the Conservation Reserve Program, etc. (Farnham 2001; Nielsen and Thomison 2002; Brock 2007; Schnitkey 2008; Casselman 2010; Ortiz 2010; UK 2010).

Corn fields in the U.S. are generally planted with hybrid seed because hybrid vigor allows for maximizing grain yield. Beginning in the 1920's, when the first corn hybrids were introduced commercially, and continuing for 40-60 years, hybrids were generally double crosses (Wych 1988). If seed of double cross hybrids were used to plant 90 million acres, the 50-60 million bushels of seed could be harvested from about 500,000 acres since the seed would be harvested from female lines that are single cross hybrids. These 500,000 acres would have to be detasseled or some other method to control fertile pollen production on these female plants (Wych1988).

Starting in the late 1950's, the hybrids transitioned from double crosses to single crosses. Currently, single crosses are used almost exclusively (Wych 1988). This transition to single crosses occurred because the single crosses out-yielded double crosses and the farmers demanded the higher yielding single crosses (Wych1988). To produce 50-60 million bushels of single crosses, the seed is harvested from female parents that are inbred lines. Since inbred lines produce only about 40-60% of the single cross hybrid, then about one million acres would be needed to produce the seed for the 90 million acres of corn grain production. These one million acres of seed production (Pioneer 2009) would have to be detasseled or some other method used to control fertile pollen production on these female plants. To plant these one million acres, about 400,000-500,000 bushels of inbred seed would be needed and would be produced on about 10,000-20,000 acres (Pioneer 2009).

A new hybrid variety could potentially be released in about 2-4 years after the inbred lines have been developed. Since inbred lines are maintained and reproduced using self pollination, these 10,000-20,000 acres do not have to be detasseled. These 10,000-20,000 acres of seed increase of inbred lines are subdivided by the estimated 800-4,000 conventional corn hybrids with about 5-15 acres per variety. To plant these 5-15 acre fields requires only 2-5 bushels of seed, which only requires less than 0.1 acres (Pioneer 2009) to produce this seed. To produce enough seed to plant these 0.1 acres requires only 2-4 ears from 2-4 plants of the promising inbred line. With good planning and use of favorable growing locations, such as winter nurseries in Hawaii and Puerto Rico, 2-3 generations of corn can be grown in one calendar year.

The production of hybrid seed requires stringent control over the pollen sources. The utilization of hybrid vigor was practical in corn since the male portion (tassel) of the corn plant was completely separate at the top of the plant from the female portion (ear) in the central region of the plant. In order to assure that no self pollination occurs and obtain hybrid seed in corn, the tassel must be removed (detasseled) from the seed producing plant (female) while allowing nearby "male" plants with tassels to pollinate the female plants. Tassels are removed by hand or by machine, but both methods are expensive and result in major plant injury with accompanying lower seed yields. In 1988, detasseling costs ranged from \$100 to \$130 per acre (Wych 1988). Much of the cost of detasseling is personnel costs. Assuming that detasselers earn approximately minimum wages and based on minimum hourly wage rates of \$3.35 in 1988 and

$7.25 in 2009 (US-DOL 2010), it can be estimated that 2009 detasseling costs range from $216 to $281 per acre. Yield reduction due to detasseling has been estimated to range from 1.5% to 13.5% for hand detasseling and 2%-45% for mechanical detasseling (Wych 1988). To estimate cost of yield losses from detasseling, several assumptions can be made. For the production of any seed that requires detasseling, contracts are signed with farmers willing to grow the low yielding inbred plants along with the challenges of growing corn to exact planting, growing and harvesting standards and schedules. In return for these apparent disadvantages of growing seed corn, paying premiums and minimums are expected and standard parts of the contract. The minimum standards are likely to be equivalent to the returns that would be expected from growing conventional corn, which would be based on the average yields in the area and the commodity corn price (Wych 1988). For example, the average yield might be 170 bushels with a commodity price of $4.00 per acre [yields and prices based on approximate national averages for 2009 (USDA-NASS 2009a)] making the minimum standard $680 per acre. A 10% yield reduction due to detasseling is approximately $68 per acre. From the calculations here, total costs of detasseling (cost of detasseling plus yield reduction) may be in the range of $280 to $350 per acre.

To minimize or to overcome impacts and high costs of conventional detasseling, various means of inducing male sterility have been explored including cytoplasmic male sterility, genic male sterility, and chemically induced male sterility. The cytoplasmic male sterility system, which is the only male sterility system that is widely used throughout the industry, tends to have problems with reduced restoration to male fertility in the commercial field (Wych 1988). The genic male sterility system is complicated, time consuming and expensive (Wych 1988). The chemically induced male sterility systems generally had problems with insufficient male sterility or with intolerable levels of female sterility (Wych 1988). Research has been conducted over the last 20 years to develop useful male sterility systems (Albertsen, Fox et al. 1993; Greenland, Bell et al. 1997; Unger, Betz et al. 2001; Unger, Cigan et al. 2002; Havey 2004). Six transgenic male sterility systems – Petition Nos. 95-228-01p, 97-148-01p, 97-342-01p, 98-278-01p, 98-349-01p and 01-206-01p – have been deregulated by APHIS (APHIS-BRS 2010) and none of these systems, other than the cytoplasmic male sterility system, have been widely adopted in commercial hybrid corn seed production (Pioneer 2009).

Organic Farming

Organic farming, as defined in this document, includes any production system that falls under the USDA National Organic Program (NOP) definition of organic farming and is a certified organic production system. The National Organic Program is administered by USDA's Agricultural Marketing Service (AMS). Organic farming operations, as described by the National Organic Program, require organic production operations to have distinct, defined boundaries and buffer zones to prevent unintended contact with excluded methods 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. In organic systems, the use of synthetic pesticides, fertilizers, and genetically engineered crops, such as DP-32138-1 corn, is strictly limited.

An accredited organic certifying agent conducts an annual review of the certified operation's organic system plan and makes on-site inspections of the certified operation and its records. Organic growers must maintain records to show that production and handling procedures comply with USDA organic standards. Practices growers may use to exclude genetically engineered products include planting only organic seed, planting earlier or later than neighboring farmers who may be using GE crops so that the crops will flower at different times, and employing adequate isolation distances between the organic fields and the fields of neighbors to minimize the chance that pollen will be carried between the fields. 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 (USDA-AMS 2010). 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.

Certified organic corn acreage is a small percentage of overall corn production. The most recently available data show 143,000 acres of organic corn production in 2007. This is 0.17% of the total 86.2 million acres of corn planted in 2007 (USDA-ERS 2008). In 2005, 131,000 acres of organic corn were planted resulting in a 9% increase in organic corn acreage over the 2 year period from 2005 to 2007 (USDA-ERS 2010a).

Specialty Corn Systems

The vast majority of corn grown in the U.S. is grown as grain for animal feed, ethanol, and industrial uses (USGC 2008). However, approximately 8% of corn grown is specialty corn produced for a specific market or use (USGC 2006). Examples of specialty corn include white corn, waxy corn, hard endosperm, high oil, non-GE, and organic corn (USGC 2006). The uses of these corn varieties include human consumption, food processing (waxy corn) or specialty product (white corn, blue corn). These corn varieties are specified by buyers and end-users of corn for production, and premiums are paid for delivering a product that meets purity and quality standards for the corn variety. Product differentiation and market segmentation in the specialty corn industry includes mechanisms to keep track of the grain (traceability) for identity preservation (IP) and quality assurance processes (e.g., ISO9001-2000 certification), as well as contracts between growers and buyers that specify delivery agreements (Sundstrom, Williams et al. 2002). Systems used by specialty corn growers and end-users to maintain identity of the production include:

- Contracts – written agreements detailing responsibilities and duties of both parties, including premiums for reaching goals and penalties for failing to attain specifications.

- Tracking and Traceability Systems – correct labeling of all products (planting seeds and harvested material) and testing procedures for identifying and detecting acceptability of materials.

- Quality Assurance Processes – oversight on handling procedures, testing of planting seeds, and testing of harvested materials to determine acceptability of use and product requirements, and assuring testing procedures are appropriate.

- Closed-Loop Systems – end-user supplies the planting seeds and guarantees to purchase final products. May also include that the end-user conduct intermediate procedures such as planting, provide oversight during the growing season, harvesting and transportation to processing plant.

- Identity Preservation Systems – using systems of identity preservation that have been shown to be successful in the past, such as the seed certification systems conducted by members of the Association of Official Seed Certifying Agencies (AOSCA). To maintain the purity of the corn product, this production system is based on controlling, tracking and documenting each step from seed production to end use (processing plants).

Physical Environment

Water Resources

Corn plants use a substantial amount of water during growth. About 4,000 gallons of water are needed to produce 1 bushel of corn (NCGA 2007), and in 2008, 12.1 billion bushels of corn were harvested in the U.S (USDA-NASS 2010a). In 2008, about 12.0 million corn acres were irrigated, which is approximately 15% of all corn acres planted for grain and the rest of corn acreage only receive the ambient rainfall in a given area (USDA-NASS 2008b). Since inbred lines are not as vigorous as the hybrid corn plants and the seed of the inbreds is more valuable that commercial corn grain, irrigation is used more frequently to lower the risk of drought induced seed reduction for growing inbred plants compared to the nearby commercial grain fields (Pioneer 2009).

Soil

Disturbance and exposure of the top soil surface layer by certain agronomic practices causes impacts and may leave crop soils prone to degradation (Hoeft, Nafziger et al. 2000). Two environmental impacts of soil degradation are the decline in water quality and the contribution to the greenhouse effect (Lal and Bruce 1999). A decline in soil quality and soil resilience[7] enhances the greenhouse effect through emissions of radiatively-active gases[8] (carbon dioxide (CO_2), nitrous oxide (N_2O)) and depletion of the soil carbon pool (Lal 2003; US-EPA 2010). In turn, a decrease in carbon aggregation and sequestration in the soil leads to increased runoff and soil erosion.

Because inbreds are less vigorous than hybrids, greater care is taken in preparing the seed bed to provide optimum germinating conditions for the inbred seed. This usually means that the soil is tilled more frequently, possibly resulting in more carbon loss and exposing the soil to the possibility of increased erosion. In the production of seed corn, when detasseling is required, severe damage to the soil structure can occur from the use of detasseler carriers and mechanical detasselers under wet soil conditions, resulting in poor water drainage, increased root damage and ineffective nutrient uptake resulting in lower seed yields (Kok et al.1996; Daum 1996)

7 Soil resilience is the ability of a soil to restore itself.

[8] Radiatively-active gases are gases that absorb incoming solar radiation or outgoing infrared in turn, affecting the temperature of the atmosphere.

Indirect effects of detasseling, such as soil compaction, could also impact the subsurface soil environment. The corn root system is closely associated with several microbial groups such as bacteria, fungi, protozoa, and mites (Bais, Weir et al. 2006) and probably modifies the ecology because of these associations. Bacteria typically represent the most abundant microbes in the soil followed by fungi (Hoeft, Nafziger et al. 2000). These microbial groups play an important and particular role in the ecology of the soil, including nutrient cycling and the availability of these nutrients for plant growth. In addition, certain microbial organisms may contribute to the protection of the root system against soil pathogens (OECD 2003).

Air Quality

Many agricultural activities affect air quality including smoke from agricultural burning, tillage, traffic and harvest emissions, pesticide drift from spraying, and nitrous oxide emissions from the use of nitrogen fertilizer (Hoeft, Nafziger et al. 2000; Aneja, Schlesinger et al. 2009). These agricultural activities individually have potentially adverse environmental impacts on air quality. Tillage contributes to the release of greenhouse gases (GHGs) because of the loss of CO_2 to the atmosphere, and the exposure and oxidation of soil organic matter (Baker, Southard et al. 2005). Emissions released from agricultural equipment (e.g., irrigation pumps and tractors) include carbon monoxide, nitrogen oxides, reactive organic gases, particulate matter, and sulfur oxides (US-EPA 2010). Nitrous oxide may also be released following the use of nitrogen fertilizer (US-EPA 2010). Aerial application of pesticides may cause impacts from drift and diffusion. Pesticides may volatilize after application to soil or plant surfaces and move following wind erosion (Vogel, Majewski et al. 2008). Agriculture, including land-use changes for farming, is responsible for an estimated 6 % of all human-induced GHG emissions in the U.S., and N_2O emissions from agricultural soil management are a large part of this, 68 % of all U.S. N_2O emissions (US-EPA 2010).

Climate Change

Climate change is possibly interrelated with agriculture in several relevant ways. Production of agricultural commodities is one of the many human activities that could contribute greenhouse gases to the air (Iserman 1993; Hoeft, Nafziger et al. 2000; Aneja, Schlesinger et al. 2009). First, this may occur through the combustion of fossil fuels to run farm equipment, the use of fertilizers, or the decomposition of agricultural waste products including crop residues and animal wastes. Second, the classes of crops planted are relevant to climate change, whether trees, grasses or field crops (Cole, Duxbury et al. 1997; Freibauer, Rounsevell et al. 2004). The location and the soil types in which they are planted also affect production of greenhouse gases (Flessa, Wild et al. 1998; Kamp, Steindl et al. 2001). Third, climate change itself may force changes to agricultural practices by extending the ranges of weeds and pests of agriculture (IPCC 2007). The influences that GE agricultural organisms may have on global climate change are unclear. Many of the indirect effects of these organisms will be determined by the traits engineered into organisms and the management strategies used in the production of these organisms. APHIS will continue to monitor developments that may lead to possible changes in the conventional production system likely to result from GE products brought to APHIS for approval. Some of the crops submitted by developers may clearly promote changes that may have impacts on greenhouse gases or the climate.

Climate changing greenhouse gas production will not be significant unless large amounts of crop plantings produce changes in measureable concentrations. The contribution of agriculture to climate change is largely dependent on the production practices employed to grow various commodities, the region in which the commodities are grown, and the individual choices made by growers. A recent Intergovernmental Panel on Climate Change (IPCC) forecast (IPCC 2007) for aggregate North American impacts on agriculture from climate change actually projects yield increases of 5-20% for this century. The IPCC report notes, however, that certain regions of the U.S. will be more heavily impacted because water resources may be substantially reduced. While agricultural impacts on existing crops may be significant, North American production is expected to adapt with improved cultivars and responsive farm management (IPCC 2007).

Animal and Plant Communities

Animals

Corn fields have been known to be visited by birds, deer and small mammals (e.g. deer mice), and other types of wildlife species. Although many birds visit row-crop fields such as corn, numbers are low and few nest there (Patterson and Best 1996). The red-winged blackbird (*Agelaius phoeniceus*) is the most abundant bird in North America; they are often initially attracted to corn fields to feed on insect pests but then feed on the corn. Annually, this bird destroys over 360,000 tons of field corn and substantial amounts of sweet corn (Dolbeer 1990); other abundant species of birds that forage and/or nest on and around corn include the horned lark (*Eremophila alpestris*), the brown-headed cowbird (*Molothrus ater*), and the vesper sparrow (*Pooecetes gramineus*) (Patterson and Best 1996).

Deer, such as the white-tailed (*Odocoileus virginianus*), find field corn attractive because it functions both as food and cover throughout the latter half of the growing season (Vercauteren and Hygnstrom 1993). Deer can significantly damage or completely destroy small corn fields that are surrounded by woody or brushy areas; however, deer damage to large corn fields is often limited to a few rows closest to the wooded areas (Neilsen 2005). The deer mouse (*Peromyscus maniculatus*) is the most common small mammal in almost any agricultural field (Stallman and Best 1996; Sterner, Petersen et al. 2003). The deer mouse feeds on a wide variety of plant and animal matter depending on availability, but primarily feeds on seeds and insects. The deer mouse has been considered beneficial in agroecosystems because it consumes both weed and pest insect species. The meadow vole (*Microtus pennsylvanicus*) feeds primarily on fresh grass, sedges, and herbs, but also on seeds and grains (Sterner, Petersen et al. 2003). The meadow vole may also be considered beneficial for its role in the consumption of weeds, but can be a significant agricultural pest where abundant as they rely on cover absent from tilled agriculture. The lined ground squirrel (*Spermophilus tridecemlineatus*) feeds primarily on seeds of weeds and available crops, such as corn and wheat (Sterner, Petersen et al. 2003). This species has the potential to damage agricultural crops, although this ground squirrel can also be considered beneficial when eating pest insects, such as grasshoppers and cutworms.

Although many of the invertebrate organisms found in corn-producing areas are considered pests, such as the European corn borer (*Ostrinia nubilalis*) and the corn rootworm (*Diabrotica* spp.), many others are considered beneficial (Hoeft, Nafziger et al. 2000). Numerous insects and related arthropods perform valuable functions: they pollinate plants, contribute to the decay of organic matter, cycle soil nutrients, and attack other insects and mites that are considered to be pests.

Plants

The landscape surrounding a corn field varies depending on the region. In certain areas, corn fields may be bordered by other corn (or any other crop); fields may also be surrounded by woodland and/or pasture/grassland areas. Therefore, the types of weeds in and around a corn field depend on the immediate area in which the corn is planted. Those weed species present will also vary depending on the geographic region where the corn is planted. Weeds compete with crops for water, nutrients, light, and other growth factors. Each year in the U.S., corn yields are threatened by more than 200 weed species (Heap 2008). Weed species such as giant foxtail and barnyardgrass have been shown to reduce corn yields by up to 13 and 35%, respectively (Bosnic and Swanson 1997; Fausay, Kells et al. 1997). Common weeds that cause problems in corn fields include velvetleaf, common cocklebur, common lambsquarters (annuals) and quackgrass and Johnsongrass (perennials) (Hoeft, Nafziger et al. 2000).

Biological Diversity

Species diversity and abundance in corn agro-ecosystems may differ between the three corn production methods: conventional with GE varieties, conventional with non-GE varieties, and organic. Many studies over the last 10 years have investigated the differences in biological diversity and abundance between GE and non-GE fields, particularly those GE crops that are resistant to insects (e.g., Bt crops) or herbicides (e.g., glyphosate-tolerant or glufosinate-tolerant crops.) Different studies have indicated potential decreases in biological diversity or abundance due to GE crops, or the presence of a pesticidal protein in some GE crops (Bt) (e.g., (Hansen Jesse and Obrycki 2000; Ponsard, Gutierrez et al. 2002; Pilcher, Rice et al. 2005). Some studies investigating decreases in weed populations following the use of herbicides and herbicide-tolerant crops have observed decreases in animal populations using the weeds as a food or refuge source. Herbicide inputs in some cases may reduce overall biological diversity in farmed fields (Marshall, Brown et al. 2003). Other studies of GE crops, such as Bt corn, when compared to non-GE crops sprayed with insecticides demonstrate that GE crops do not cause any changes in arthropod abundance or diversity (e.g., (Bitzer, Rice et al. 2005; Torres and Ruberson 2005; Romeis, Meissle et al. 2006; Marvier, McCreedy et al. 2007; Chen, Zhao et al. 2008; Wolfenbarger, Naranjo et al. 2008)). Some reports show that GE crops may even increase biological diversity (e.g., (Romeis, Meissle et al. 2006; Marvier, McCreedy et al. 2007)) in agro-ecosystems. Herbicide-tolerant corn, when compared to non-GE corn production, may not result in changes in arthropod abundance and may even increase species diversity during different times of the year (e.g., (Brooks, Bohan et al. 2003; Haughton, Champion et al. 2003; Hawes, Haughton et al. 2003; Roy, Bohan et al. 2003; Wolfenbarger, Naranjo et al. 2008)). Since biological diversity can be defined and measured in many ways, APHIS considers determining the level of biological diversity in any crop to be complex and hard to achieve concurrence. Another difficulty with biodiversity studies is separating expected impacts from indirect impacts. For example, reductions of biological control organisms are seen in some Bt-expressing GE crops, but are caused by reduction of the pest host population following transgenic pesticide expression in the transformed crop plant.

Gene Movement

Corn plants are pollinated through wind movement of pollen to other receptive corn plants. In the U.S., there are no other species that can be pollinated by corn pollen without human intervention (e.g., manually forcing reproduction in the laboratory) (USDA-APHIS 2010).

Thus, the public concern surrounding gene movement for GE corn is between GE and non-GE corn plants. Of the total corn acres planted in 2009, 85% were GE corn varieties (USDA-NASS 2009a). Specialty corn, those with traits of particular interest to various markets such as blue corn, waxy corn or organic corn, are typically grown with various management practices that intend to limit corn pollen from reaching the specialty corn crop during the period of time that the specialty corn crop is receptive to pollen. For example, the NOP has requirements for organic plans to address pollen flow from GE crops (Kuepper 2002; Krueger 2007; Kuepper, Born et al. 2007). The Association of Official Seed Certifying Agencies (AOSCA) also has information for specialty corn crops, and a protocol for growing non-GE corn (AOSCA 2008). There is a price premium associated with growing these types of specialty crops in conjunction with the extra regimens in place to maximize the purity of these specialty crops. For example, in 2007, conventional corn averaged $4.19/bushel (USDA-NASS 2010b), whereas organic corn averaged $7.08/bushel (USDA-NASS 2010c).

A recent paper reviewed studies investigating gene flow and cross-fertilization studies in corn grain production fields, and using the data from these studies recommended 50m (approx. 164ft) as the distance needed to isolate GE corn from non-GE corn (Sanvido, Widmer et al. 2008). The authors limited their analysis to studies that confirmed fertilization in the non-GE corn plants, and excluded studies on pollen dispersal (e.g., (Raynor, Ogden et al. 1972; Di Giovanni, Kevan et al. 1995; Aylor, Schultes et al. 2003)) that only measured pollen flow, because pollen flow does not necessarily result in fertilization. Successful cross-fertilization requires many different biological and physical factors, such as synchrony of flowering between corn fields, viability of pollen, and presence of physical barriers, and thus pollen dispersal is not equivalent to cross fertilization. Sandivo et al. (2008) analyzed existing studies and found that the cross-fertilization rate in non-GE corn typically remained below 0.5% at this distance. This result was validated when large scale studies were analyzed for cross-fertilization events (e.g. (Henry, Morgan et al. 2003; Weber, Bringezu et al. 2007).

However, there are studies that refute the success of distance as an isolation strategy. Jones and Brooks (1950) found cross-fertilization to be as high as 2.5 % at 660ft, which is the isolation distance used by AOSCA to isolate corn fields for seed production (AOSCA 2004). One potential reason for the discrepancy between this study and many other gene flow studies in corn may be due to the type of corn used in the Jones and Brooks study. Jones and Brooks (1950) investigated the appropriate isolation distance for seed production in open-pollinated varieties, and not in hybrid varieties. Due the biology of open-pollinated varieties, these types of plants may be more receptive to pollen over a longer period of time than hybrid corn plants (Sanvido, Widmer et al. 2008), allowing for a greater chance of pollination events. Thus the results from Jones and Brooks (1950) may be an overestimation of cross-fertilization potential for hybrid corn plants.

Public Health

Public health concerns surrounding GE field corn, like DP-32138-1 corn, focus primarily on human and animal consumption. Non-GE corn varieties, both those developed for conventional use and for use in organic production systems, do not require routine evaluation by any regulatory agency in the U.S. for food or feed safety prior to release in the market. Under the Federal Food, Drug, and Cosmetic Act (FFDCA), it is the responsibility of food and feed manufacturers to ensure that the products they market are safe and properly labeled. Food and feed derived from DP-32138-1 must be in compliance with all applicable legal and regulatory requirements**.** GE organisms for food and feed may undergo a voluntary consultation process with the FDA prior to release onto the market. Although a voluntary process, applicants who wish to commercialize a GE variety that will be included in the food supply invariably complete a consultation with the FDA. In a consultation, a developer who intends to commercialize a bioengineered food meets with the agency to identify and discuss relevant safety, nutritional, or other regulatory issues regarding the bioengineered food and then submits to FDA a summary of its scientific and regulatory assessment of the food; FDA evaluates the submission and responds to the developer by letter (US-FDA 2010).

Socioeconomic

The last three decades have been marked by significant transition in the seed industry (Fernandez-Cornejo 2004). Before the 1970's, seed development occurred in the public domain. Most private seed companies focused on cleaning, handling, storing packaging, and selling seed (Fernandez-Cornejo 2004). However, following the passage of the 1970 Plant Variety Protection Act, more than 50 seed companies were acquired by pharmaceutical, petrochemical, and food firms (Fernandez-Cornejo 2004). Mergers and acquisitions created a new seed industry structure and, by the early 1980s, several international firms were among the top seed sellers worldwide (Fernandez-Cornejo 2004). The seed company can be viewed in terms of different functions including plant breeding research and development (R&D), seed production, seed conditioning, and seed marketing and distribution. The high costs associated with large-scale R&D limit this activity to Federal Government agencies, land-grant colleges and universities, and a small number of large companies. Given the size of their R&D investments, these plant breeders play a central role in managing the entire production, distribution, and marketing processes in the seed industry (Fernandez-Cornejo 2004). In 1999-2002, approximately 300 firms were involved with seed corn production with the top five companies having 64% market share, the next 10 companies having an additional 16% market share, and the rest of the companies having the remaining 20% market share (Kalaitzandonakes and Magnier 2004).

The top five companies involved in seed production are national or multinational companies with their own R&D departments developing their own genetics and their own production, processing and distribution facilities. The next 10 largest companies are regional with a dependence on foundation seed firms and university developed lines in addition to their own proprietary genetics along with their own production and distribution facilities. The remaining smaller firms depend almost solely on purchased genetics and others for production and concentrate on distribution (Kalaitzandonakes and Magnier 2004). These smaller seed companies with a marketable seed product typically contract out the production and multiplication processes to farmers, farmers' associations, and private firms such as Illinois Crop Improvement Association (www.ilcrop.com), Indiana Crop Improvement Association (http://www.indianacrop.org/), and Iowa Crop Improvement Association (http://www.agron.iastate.edu/ICIA).

In all cases, seed companies closely manage the production of seed by contract growers. Seed companies want to ensure that the desirable plant characteristics are carried through to subsequent generations and to prevent problems that could affect the quality of the product (Fernandez-Cornejo 2004). After harvesting certified seed, the seed is conditioned, which includes the drying, cleaning, and sorting of seed. Seeds are also treated with insecticides and fungicides and packaged for distribution and sale to farmers (Fernandez-Cornejo 2004). Large seed companies play a more direct role in assuring their seed meets at least the minimum standards for certified seed as well as in marketing and distributing their end product to regional, national, and international markets. Many companies also license or outsource marketing and distribution to private firms and individuals to improve access to local markets (Fernandez-Cornejo 2004).

Technical developments in the genetic engineering of plants has been one of the prime motivations for the consolidation of the seed corn companies (Kalaitzandonakes and Hayenga 1999; Fernandez-Cornejo 2004; Kalaitzandonakes and Magnier 2004) with high biotechnology regulatory/compliance costs being the major reason for limiting the development and marketing of new biotech corn varieties to the large national/multinational companies (Fulton and Giannakas 2001; Kalaitzandonakes and Magnier 2004). The greater the regulatory requirements in an industry, the more concentrated the industry is expected to be (Fulton and Giannakas 2001) with accumulating evidence suggesting that biotechnology compliance costs have increased over time (Kalaitzandonakes, Alston et al. 2007).

In the production of hybrid seed corn, the task that is one of the most difficult but essential to the overall success is the detasseling of the corn. Manual detasseling of corn is a physically demanding job consisting of pulling tassels out of the top of thousands of tall corn plants by each detasseler each day. Detasseling operations are conducted under challenging conditions, such as those environmental conditions that persist in July in the U.S. Midwest. Detasseling is a temporary job lasting only one to four weeks in mid-summer. The job is time sensitive because tassels need to be pulled within a day of emergence before any pollen is released; a high level of flexibility for the task is required because the emergence of tassels depends on the weather. Hot humid weather speeds up the emergence of tassels by one to more weeks and cold wet weather slows down the emergence of tassels by one or more weeks. Thousands of people are needed to complete the operation in timely basis. Because of the short duration and physical demands of the job, the detasseling operation mainly attracts high school students during the summer break. Thousands of high school students are hired for detasseling each year (Byron 2002; Pioneer 2007; Gustafson 2008; Holloway 2010; Schernikau 2010).

There are many challenges for the farmers whose land is contracted to grow the low yielding inbred plants including growing corn to meet exacting planting, growing and harvesting standards and schedules. Additionally, pay premiums and minimums are standard parts of the contract. The minimum standards are likely to be equivalent to the returns that would be expected from growing conventional corn, and based on the average yields in the area and the commodity corn price. For example, the average yield might be 170 bushels with a commodity price of $4.00 per acre making the minimum standard $680 per acre. These contract conditions also require that the farmers need to accept the large ruts that are left by detasseling machines used in muddy conditions. These ruts destroy soil structure, increase tillage requirements, increase erosion, result in major yield reduction in the corn crop and possibly in the crop the following year as well, and result in damage to harvesting and tillage machinery (Daum 1996; Kok, Taylor et al. 1996; Gelderman 2009; Tilghman 2009; Roegge 2010).

For the company that wants to produce and sell the hybrid seed corn at a profit, detasseling is an expensive operation whether it is manual or mechanical. Mechanical detasselers reduce the need for manual (human) detasselers, but they tend to reduce yields even more than manual detasseling operations and manual detasseling is still required to completely finish the job (Wych 1988).

The United States is the largest seed market worldwide, followed by China and Japan (Fernandez-Cornejo 2004). Seed expenditures by U.S. farmers rose from about $500 million per year in 1960 to nearly $7 billion in 1997 (Fernandez-Cornejo 2004). The United States is a net exporter of seed. In 1996, the U.S. seed trade surplus was $384 million: $698 million in seed exports were mainly sent to Mexico, Canada, Italy, Japan, and Argentina. About $314 million in seed imports, were received mainly from Canada, Chile, the Netherlands, and China (Fernandez-Cornejo 2004).

III. Alternatives

This document analyzes the potential environmental consequences of a determination of nonregulated status of DP-32138-1 corn. To respond favorably to a petition for nonregulated status, APHIS must determine that DP-32138-1 corn is unlikely to pose a plant pest risk. Based on its risk assessment APHIS has concluded that DP-32138-1 corn is unlikely to pose a plant pest risk (USDA-APHIS 2010). Therefore APHIS must determine that DP-32138-1 corn is no longer subject to 7 CFR part 340 or the plant pest provisions of the Plant Protection Act.
Two alternatives will be evaluated in this EA: (1) no action and (2) determination of nonregulated status of DP-32138-1 corn. APHIS has assessed the potential for environmental impacts for each alternative in the "Environmental Consequences" section

No Action: Continuation as a Regulated Article

Under the No Action Alternative, APHIS would deny the petition. DP-32138-1 corn and progeny derived from DP-32138-1 corn would continue to be regulated articles under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would still be required for introductions of DP-32138-1 corn and measures to ensure physical and reproductive confinement would continue to be implemented. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from the unconfined cultivation of DP-32138-1 corn.

This alternative is not the Preferred Alternative because APHIS has concluded through a Plant Pest Risk Assessment that DP-32138-1 corn is unlikely to pose a plant pest risk (USDA-APHIS 2010). Choosing this alternative would not satisfy the purpose and need of making a determination of plant pest risk status and responding to the petition for nonregulated status.

Preferred Alternative: Determination that DP-32138-1 is No Longer a Regulated Article

Under this alternative, DP-32138-1 corn and progeny derived from them would no longer be regulated articles under the regulations at 7 CFR Part 340. DP-32138-1 corn is unlikely to pose a plant pest risk (USDA-APHIS 2010). Permits issued or notifications acknowledged by APHIS would no longer be required for introductions of DP-32138-1 corn and progeny derived from this event. This alternative best meets the purpose and need to respond appropriately to a petition for nonregulated status based on the requirements in 7 CFR part 340 and the agency's authority under the plant pest provisions of the Plant Protection Act. Because the agency has concluded that DP-32138-1 corn are unlikely to pose a plant pest risk, a determination of nonregulated status of DP-32138-1 corn is a response that is consistent with the plant pest provisions of the PPA, the regulations codified in 7 CFR part 340, and the biotechnology regulatory policies in the Coordinated Framework. Under this alternative, growers may have future access to DP-32138-1 corn and progeny derived from this event if the developer decides to commercialize DP-32138-1 corn.

Alternatives Considered but Rejected from Further Consideration

APHIS assembled a list of alternatives that might be considered for DP-32138-1 corn. The agency evaluated these alternatives in light of the agency's authority under the plant pest provisions of the Plant Protection Act, and the regulations at 7 CFR part 340, with respect to environmental safety, efficacy, and practicality to identify which alternatives would be further considered for DP-32138-1 corn. Based on this evaluation, APHIS rejected several alternatives. These alternatives are discussed briefly below along with the specific reasons for rejecting each.

Prohibit any DP-32138-1 from Being Released

In response to public comments that stated a preference that no GE organisms enter the marketplace, APHIS considered prohibiting the release of DP-32138-1, including denying any permits associated with the field testing. APHIS determined that this alternative is not appropriate given that APHIS has concluded that DP-32138-1 is unlikely to pose a plant pest risk(USDA-APHIS 2010).

In enacting the Plant Protection Act, Congress found that

> [D]ecisions affecting imports, exports, and interstate movement of products regulated under (the Plant Protection Act) shall be based on sound science… §402(4) (see 7 U.S. C. §7701(4)).

On March 11, 2011, in a Memorandum for the Heads of Executive Departments and Agencies, the White House Emerging Technologies Interagency Policy Coordination Committee developed broad principles, consistent with Executive Order 13563, to guide the development and implementation of policies for oversight of emerging technologies (such as genetic engineering) at the agency level. In accordance with this memorandum, agencies should adhere to Executive Order 13563 and, consistent with that Executive Order, the following principle, among others, to the extent permitted by law, when regulating emerging technologies:

> "[D]ecisions should be based on the best reasonably obtainable scientific, technical, economic, and other information, within the boundaries of the authorities and mandates of each agency"

Based on our Plant Pest Risk Assessment (USDA-APHIS 2010) and the scientific data evaluated therein, APHIS concluded that DP-32138-1 are unlikely to pose a plant pest risk. Accordingly, there is no basis in science for prohibiting the release of DP-32138-1.

Approve the petition in part

The regulations at 7 CFR 340.6(d)(3)(i) state that APHIS may "approve the petition in whole or in part." For example, a determination of nonregulated status in part may be appropriate if there is a plant pest risk associated with some, but not all lines described in a petition. Because APHIS has concluded that DP-32138-1 corn is unlikely to pose a plant pest risk, there is no regulatory basis under the plant pest provisions of the Plant Protection Act for considering approval of the petition only in part.

Isolation Distance between DP-32138-1 and Non-GE Corn Production

In response to public concerns of gene movement between GE and non-GE plants, APHIS considered requiring an isolation distance of DP-32138-1 corn and non-GE corn production. However, because APHIS has concluded that DP-32138-1 corn is unlikely to pose a plant pest risk (USDA-APHIS 2010), an alternative based on requiring isolation distances would be inconsistent the statutory authority under the plant pest provisions of the Plant Protection Act and regulations in 7 CFR part 340. APHIS also considered geographically restricting the production of DP-32138-1 corn based on the location of production of non-GE corn in organic production systems or production systems for GE-sensitive markets in response to public concerns regarding possible gene movement between GE and non-GE plants. However, as presented in APHIS' plant pest risk assessment for DP-32138-1 corn, there are no geographic differences associated with any identifiable plant pest risks for DP-32138-1 corn (USDA-APHIS 2010). This alternative was rejected and not analyzed in detail because APHIS has concluded that DP-32138-1 corn does not pose a plant pest risk, and will not exhibit a greater plant pest risk in any geographically restricted area. Therefore, such an alternative would not be consistent with APHIS' statutory authority under the plant pest provisions of the Plant Protection Act and regulations in Part 340 and the biotechnology regulatory policies embodied in the Coordinated Framework.

Based on the foregoing, the imposition of isolation distances or geographic restrictions would not meet APHIS' purpose and need to respond appropriately to a petition for nonregulated status based on the requirements in 7 CFR part 340 and the agency's authority under the plant pest provisions of the Plant Protection Act. Nevertheless, APHIS is not expecting significant effects. However, individuals might choose on their own to geographically isolate their non-GE corn production systems from DP-32138-1 corn or to use isolation distances and other management practices to minimize gene movement between corn fields. Information to assist growers in making informed management decisions for DP-32138-1 corn is available from Association of Official Seed Certifying Agencies (AOSCA 2010).

Requirement of Testing For Event 32138 Corn

During the comment periods for other petitions for nonregulated status, some commenters requested USDA to require and provide testing for GE products in non-GE production systems. APHIS notes there are no nationally-established regulations involving testing, criteria, or limits of GE material in non-GE systems. Such a requirement would be extremely difficult to implement and maintain. Additionally, because DP-32138-1 corn does not pose a plant pest risk (USDA-APHIS 2010), the imposition of any type of testing requirements is inconsistent with the plant pest provisions of the Plant Protection Act, the regulations at 7 CFR part 340 and the biotechnology regulatory policies embodied in the Coordinated Framework. Therefore, imposing such a requirement for DP-32138-1 corn would not meet APHIS' purpose and need to respond appropriately to the petition in accordance with its regulatory authorities.

Comparison of Alternatives

Table 1, below, briefly summarizes the results for each of the issues raised in the Environmental

Consequences (Section IV) by each of the alternatives described in the Alternatives section (Section III).

Table 1. Issues of Potential Impacts and Consequences of Alternatives.

Attribute/Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
Meets Purpose and Need and	**No**	**Yes**
Unlikely to Pose a Plant Pest Risk	Satisfied through use of regulated field trials	Satisfied—plant pest risk assessment (USDA-APHIS 2010)
Management Practices		
Acreage and Areas of Corn Production	Unchanged	Unchanged
Cropping Practices	Unchanged	Unchanged
Pesticide Use	Unchanged	Unchanged
Seed Corn Production	Unchanged	Minimal - may decrease need for seed corn acreage due to increased yields efficiency
Organic Farming	Unchanged	Unchanged
Impact to Specialty Corn	Unchanged	Unchanged
Environment		
Water use	Unchanged	Unchanged
Soil	Unchanged	Unchanged
Air Quality	Unchanged	Unchanged
Climate Change	Unchanged	Unchanged
Animals	Unchanged	Unchanged
Plants	Unchanged	Unchanged
Biological Diversity	Minimal	Minimal
Gene Movement	Minimal	Minimal
Human and Animal Health		
Public Health: Risk to Human Health	Unchanged	Unchanged
Public Health: Risk to Worker Safety	Minimal – detasseling carries some risk to workers	Minimal – fewer safety risks with less detasseling
Public Health: Risk to Animal Feed	Unchanged	Unchanged
Socioeconomic		
Domestic Economic Environment	Unchanged	Minimal – seed producers save 4% of retail sales price; growers – possible small seed cost decrease Unchanged – immeasurably small impact on cost of commodity corn
Trade Economic Environment	Unchanged	Unchanged

Attribute/Measure	Alternative A: No Action	Alternative B: Determination of Nonregulated Status
Social Environment	Unchanged	Minimal – fewer part time detasselers hired for summer work
Other Regulatory Approvals		
U. S.	Completed new protein consultations with FDA	Completed new protein consultations with FDA
Compliance with Other Laws		
CWA, CAA. EOs	Fully compliant	Fully compliant

IV. Environmental Consequences

This analysis of potential environmental consequences addresses the potential impact to the human environment from the alternatives analyzed in this EA, namely taking no action and a determination by the Agency that DP-32138-1 corn does not pose a plant pest risk.

Potential environmental impacts from the No Action Alternative and the Preferred Alternative for DP-32138-1 are described in detail throughout this section. A cumulative effects analysis is also included for each environmental issue. Certain aspects of this product and its cultivation would be no different between the alternatives: those are described below.

Scope of the Environmental Analysis

Although the preferred alternative would allow for new plantings of DP-32138-1 to occur anywhere in the U.S., APHIS will limit the environmental analysis to those areas that currently support corn production. To determine areas of corn production, APHIS used data from the National Agricultural Statistics Service (NASS) 2007 Census of Agriculture to determine where corn is produced in the United States (USDA-NASS 2010d). Corn grain was produced in all states except for Alaska.

The scope of possible impacts is limited in some ways by the relatively small area of potential use. DP-32138-1 lines containing transgenes only have utility in seed production and are not intended to be a commercial product to be used to plant conventional corn acres (Pioneer 2009). The total acreage of DP-32138-1 planted in the U.S. each year under close supervision is expected to be less than 5,000 acres (Pioneer 2009). If the DP-32138-1 is licensed to third parties and adopted across the entire U.S. seed industry, the total acreage is not expected to exceed 20,000 acres each year (Pioneer 2009).

Other Assumptions

The environmental consequences of the different alternatives described above will be analyzed under the assumption that seed producers who produce corn seed using DP-32138-1 corn and farmers who produce conventional corn or corn using organic methods are using reasonable, commonly accepted best management practices for their chosen system and varieties during agricultural corn production. However, APHIS recognizes that not all farmers follow these best management practices for corn. Thus, the analyses of the environmental effects will also include the assumption that some farmers do not follow these best management practices.

Agricultural Production of Corn

One of APHIS' missions is to improve American agricultural productivity. Best management practices, such as planting dates, seeding rates, and harvest times are commonly accepted, practical ways to grow corn, regardless of whether the corn farmer is using conventional practices with non-GE or GE varieties, or organic practices. These well-established, widely-practiced means to produce corn can be obtained through local Cooperative Extension Service offices and their respective websites (NSFC-IPM 2010).

GE and non-GE corn varieties are continually under development. From 2004 to 2009, the corn acreage ranged from a low of 78.3 million acres planted in 2006 to a high of 93.5 million acres planted in 2007. Most of the corn acreage in the U.S. is planted to GE varieties. Of the 86.3 million acres of corn planted in the U.S. in 2009, 85% of the acreage was planted with GE varieties (USDA-NASS 2009a). Based upon these trends, conventional production practices that use GE varieties will likely continue to dominate in terms of acreage, or perhaps increase in acreage, with or without a determination of nonregulated status of DP-32138-1.

Organic Production of Corn

APHIS recognizes that producers of non-GE corn, particularly producers who sell their products to markets sensitive to genetically engineered traits (e.g. organic or some export markets) can be reasonably assumed to be using practices on their farm to protect their crop from unwanted substances and maintain their price premium. For example, the National Organic Program (NOP) has recognized the feasibility of protecting organically-produced crops, and the investment farmers put into their production practices, by requiring that organic production plans include practical methods to protect organically-produced crops.

"Organic crops must be protected from contamination by prohibited substances used on adjoining lands (for example, drifting pesticides, fertilizer-laden runoff water, and pollen drift from genetically engineered…)" (NCAT 2003).

Typically, growers use more than one method under organic practices to prevent unwanted material from entering their fields including: isolation of the farm, physical barriers or buffer zones between organic production and non-organic production, as well as formal communications between neighboring farms (NCAT 2003). The organic plan used as the basis for organic certification should include a description of practices used to prevent or reduce the likelihood of unwanted substances, like GE pollen or seed, at each step in the farming operation, such as planting, harvesting, storing and transporting the crop (Riddle 2004; Krueger 2007; Kuepper, Born et al. 2007). Organic plans should also include how the risk of GE pollen or co-mingling of seed will be monitored (Kuepper, Born et al. 2007). Farmers using organic methods are requested to let neighboring farmers know that they are using organic production practices and request that the neighbors also help the organic farmer reduce contamination events (NCAT 2003; Krueger 2007). Recommended organic production practices for field corn are also readily available (Kuepper 2002). Thus, commonly used production practices for corn, and the practical methods typically used by organic corn farmers to protect their crop and maximize their profits and price premiums from corn under organic production, currently provide many measures that greatly reduce the likelihood of accidental gene flow between DP-32138-1 corn and non-GE corn fields. APHIS will use the assumption that farmers are already using, or have the ability to use, these common, reasonable practices as its baseline for the analyses of the following alternatives below.

Acreage and Areas of Corn Production

No Action: Acreage and Areas of Corn Production

The amount of GE corn planted in conventional systems in the U.S. is increasing. Since 2000 when 25% of the total corn acreage was devoted to GE varieties, the share of total corn acres devoted to GE corn varieties has risen each year to the present 85% level in 2009 (USDA-NASS 2009a).

Conventional corn production with GE varieties will likely continue to increase without a determination of nonregulated status of DP-32138-1 under the No Action Alternative, based on current acreage trends. Seed for currently available conventional and GE varieties will remain the same under the No Action Alternative, except progeny of DP-32138-1 will be unavailable. Corn is currently produced commercially in 49 states, excluding only Alaska according to the 2007 Census of Agriculture (USDA-NASS 2009b) and under the No Action Alternative, this range of production will be unchanged.

Preferred Alternative: Acreage and Areas of Corn Production

In 2009, GE corn was planted on 85% of all corn acres currently in production in the U.S., and the use of GE corn has been steadily increasing over the last 10 years (USDA-NASS 2009a). Most corn is planted in fields that have been in crop production for many years. A determination of nonregulated status of DP-32138-1under the Preferred Alternative is not expected to alter the range of corn cultivation as the new GE trait (DP-32138-1) does not change the growth habits compared to conventional varieties (USDA-APHIS 2010). Additionally, because DP-32138-1 is a technique for more efficiently producing seed that will be used to plant conventional corn production acres without introducing new transgenes to these production acres, even widespread use of DP-32138-1 will have no significant effect on increasing or decreasing the use of GE corn or on total corn production acreage.

Thus, under the preferred alternative, a determination of nonregulated status of DP-32138-1 would not increase corn production, either by its availability alone or accompanied by other factors, or cause an increase in overall GE corn acreage. Impacts would be similar to the No Action Alternative.

Cumulative Effects: Acreage and Areas of Corn Production

Cumulative effects of a determination of nonregulated status of DP-32138-1 are unlikely. Neither the No Action Alternative nor a determination of nonregulated status of DP-32138-1 will directly cause an increase in agricultural acreage devoted to corn production, or those corn acres devoted to GE corn cultivation. DP-32138-1 will also not change cultivation areas for corn production in the U.S. There are no anticipated changes to the availability of GE and non-GE corn varieties on the market under either alternative.

Cropping Practices: Crop Rotation, Tillage, and Pesticide Use

The current economics of corn production are driving the change or perceived change in crop rotation practices (such as corn-to-corn cropping, Erickson and Lowenberg-DeBoer, 2005). Growers make choices to plant certain corn varieties and use certain crop rotation practices based on factors such as yield, weed and disease pressures, cost of seed and other inputs, technology fees, human safety, potential for crop injury, and ease and flexibility of the production system (Olson and Sander 1988; Giannessi 2005). Therefore, when taking into account these factors, growers will ultimately base their choice of inputs and agronomic practices on individual wants and needs. There may be more tillage and use of pesticides involved with the production of seed corn than with commercial corn grain. The low vigor of seed and plants of inbred lines compared to hybrid corn generally means a better seed bed is needed (generally requiring more tillage) along with the protection from diseases and insects provided by pesticide used on the seed and the plants (Pioneer 2009).

No Action: Cropping Practices: Crop Rotation, Tillage, Production, and Pesticide Use

Producing seed corn requires taking into account the crop rotation on the farm on which the seed production is taking place as well as the crop rotations on surrounding farms. Adequate isolation must be assured to maintain high genetic purity. Corn after corn rotation is discouraged to help avoid the possibility of lowering genetic purity from volunteer corn plants arising from a previous corn crop (Wych 1988).

Conventional tillage and the removal of plant residue from soil are considered agriculture practices that accentuate loss of soil organic carbon (Lal and Bruce 1999). This loss has negative impacts on the atmosphere and increases soil erosion, among other effects. Because of numerous benefits to growers and the agricultural environment (Fawcett and Caruana 2001), it is not likely that DP-32138-1 will require a change from the increasingly prevalent practice of low-till production of corn. Under the No Action Alternative, the use of tillage methods in U.S. agricultural production of seed corn and commercial corn will remain unchanged.

Under the No Action Alternative, corn production and pesticide use will remain as it is practiced today by the farming community. Growers make choices to use certain pesticides based on weed, insect and disease pressures, cost of seed and other inputs, technology fees, human safety, potential for crop injury, and ease and flexibility of the system (Olson and Sander 1988; Giannessi 2005). Growers will ultimately base their choice of these inputs on individual wants and needs. As an example of the pesticides used during the production of field corn, the Pesticide Action Network has an online database, including a detailed description of all the pesticides used in corn agriculture in California (Kegley, Hill et al. 2010). It lists the top 50 pesticides (e.g., herbicides, insecticides, fungicides) used in California corn production. Any effects due to crop rotation, tillage and pesticide use in the agricultural production of seed corn and commercial corn will remain the same under the No Action Alternative.

Preferred Alternative: Crop Rotation, Tillage, Production, and Pesticide Use

As stated above, the current economics of corn production are driving the change or perceived change in crop rotation practices (such as corn-to-corn cropping, Erickson and Lowenberg-DeBoer, 2005). A determination of nonregulated status of DP-32138-1 is unlikely to change the entire pricing scheme of corn commodities in the U.S. Prices will continue to be set by market demand, without regard to the number or type of corn varieties available on the market, nor to decreased costs of seed production. Based on information provided by the applicant, DP-32138-1 lines containing transgenes are expected to reach an industry wide maximum of only 20,000 acres in seed corn production, which is only 0.02% of the approximately 90 million acres planted to corn each year. DP-32138-1 is not likely to affect a farmer’s decision to either stop using a corn-to-corn rotation or other type of rotation, or to increase the overall use of corn-to-corn rotation or other type of rotation as a cropping strategy with the U.S. farming community.

Adoption of DP-32138-1 will not change current production or cultivation practices. Crop rotation schemes that assure adequate isolation to maintain high genetic purity and tillage for seed corn production will remain the same. In those cases where seed producers employ conventional tillage for the inbred crops used in seed production, planting of DP-32138-1 will not change those practices, either. A determination of nonregulated status of DP-32138-1 will not change the loss of soil organic carbon due to tillage in seed corn production systems or in commercial production of corn in which DP-32138-1 was used in the production of hybrid seed.

DP-32138-1 seed corn production uses the same agricultural inputs (e.g., pesticides, fertilizers) as seed corn currently grown. DP-32138-1 does not require different types of pesticides than are currently used in seed corn production. A determination of nonregulated status of DP-32138-1 will not have any effect on the pesticides used in the production of seed corn or commercial corn production in the U.S., compared to the No Action Alternative.

<u>Cumulative Effects: Crop Rotation, Tillage, Production, and Pesticide Use</u>

A determination of nonregulated status of DP-32138-1 will not have any cumulative effect on the crop rotation, tillage and crop production practices or on the pesticides used in the production of seed corn or commercial corn production in the U.S., compared to the No Action Alternative. The limited number of seed corn production acres that may use DP-32138-1 lines containing transgenes is expected to have no cumulative effect on crop rotations, tillage and crop production practices, or pesticide use. The requirements for crop rotation, tillage and crop production practices, and pesticide use for the non transgenic hybrid progeny resulting from DP-32138-1 seed production that will be used to plant conventional corn fields, will be exactly the same as those used for conventionally produced hybrids.

Seed Production

Seed corn production differs from commercial grain production because seed companies impose strict requirements to maintain seed identity and high levels of genetic purity of the final product. This purity is accomplished using contracts, tracking and traceability systems, quality assurance processes, record maintenance, auditing, proper labeling, appropriate sampling and testing and identity preservation systems (Sundstrom, Williams et al. 2002). An example of a successful identity preservation system are the Seed Certifications programs as used by the AOSCA which have maintained seed purity standards for national and international trade since the 1920s (Sundstrom, Williams et al. 2002).

Seed increase for a new corn variety encompasses larger and larger acreage for each successive generation. The initial generation of the new inbred line consists of one or only a few plants. These plants will produce a few ears. The seeds from these few ears are used to plant a small plot, approximately 0.1 acres (3000-6000 sq. ft.). Early generation plants are self pollinated and pollen control is generally achieved by using bags over the tassels and ears. Using this system of pollen control allows many lines to be grown in a small area with an extremely low possibility of cross pollination with other lines grown in the immediate area. The 2-10 bushels of seed harvested are used to plant 2-20 acres for the next increase. Plants at this stage are also self pollinated to increase the seed; however, the area is likely too large for bagging as a means of pollen control. Pollen control is achieved by means of temporal or physical isolation from other lines. This increase produces 100 to 1000 bushels of inbred line seed.

In the next stage, which produces single cross hybrid seed to be used for planting commercial corn grain fields, two different inbred lines (both are from the previous stage increase) are planted in the same field. One inbred line is considered the male line providing the pollen and the second line is the female line, from which the hybrid seed will be harvested. Procedures must be taken to prevent these plants of the female line from producing viable fertile pollen. Presently, detasseling or cytoplasmic male sterility systems are used to prevent self pollination of this stage female plants.

The Pioneer Hi-Bred SPT process is designed to produce non-transgenic male-sterile female inbred parent plants for hybrid corn seed production without the need for tassel removal or bagging. As a process, SPT uses two types of inbred corn lines: 1) nontransgenic male-sterile inbred lines (referred to as *ms45*) that cannot produce pollen but can produce fertile egg cells, and 2) a transgenic male-fertile line containing a DP-32138-1 SPT maintainer cassette within the *ms45* genetic background (referred to as DP-32138-1 corn) that permits pollen development in which half the pollen is non-transgenic and viable and the other half is transgenic but not viable. The maintainer can also produce fertile eggs which allows for production of new transgenic seeds of the maintainer line (see below). The DP-32138-1 SPT maintainer cassette contains three linked genes that are differentially expressed over time, including Ms45 (expressed in the anther), α-amylase (expressed in the pollen grain), and dsRed (expressed in the kernel).

The Pioneer Hi-Bred SPT process consists of three major steps (Figure 1): 1) DP-32128-1 seed increase, 2) non-transgenic *ms45* male sterile corn increase, and 3) hybrid seed production using non-transgenic seed from use of the SPT process. The steps of this process are described in additional detail, below.

<u>Step 1: 32138 SPT Maintainer Seed Increase</u>. The first major step of the Pioneer Hi-Bred SPT process propagates DP-32138-1 corn (see Step 1, Figure 1, and Figure 2). Propagation involves both the self-pollination of DP-32138-1 corn in the field and mechanical sorting to select for the desired seed. In contrast to a male sterile *ms45* corn line, DP-32138-1 corn is able to produce pollen by means of the fertility trait MS45 which was engineered into the DP-32138 SPT maintainer cassette. Half of the pollen produced by DP-32138-1 corn contains the DP-32138-1 SPT maintainer cassette and is not viable (pollen expresses a starch-consuming enzyme, ZM-AA1); however, the remaining half of the pollen does not contain the DP-32138-1 SPT maintainer cassette and is fully viable.

Both transgenic (where the DP-32138-1 SPT maintainer cassette is present) and non-transgenic (SPT absent) kernels are produced in 1:1 ratios, following the self-fertilization of DP-32138-1 corn (Figure 2). Seed that is normal (yellow) in color is non-transgenic for SPT (does not contain the DP-32138-1 SPT insertion). Pinkish-red seed that fluoresces a bright red color under appropriate illumination is transgenic for SPT (contains the DP-32138-1 SPT insertion). In order to separate these two kernel types, mechanical color sorting is used to detect the the 32138 SPT maintainer cassette (containing the dsRed fluorescent protein) (see Figure 3). The remaining kernels, non-transgenic due to the absence of the 32138 SPT maintainer cassette, are discarded after mechanical sorting. Pure seed of DP-32138-1 SPT maintainer is then available for the propagation of non-transgenic male-sterile female inbred parent seed (Figure 1, Step II). Yellow, non-transgenic seed collected from the first color sort will be discarded.

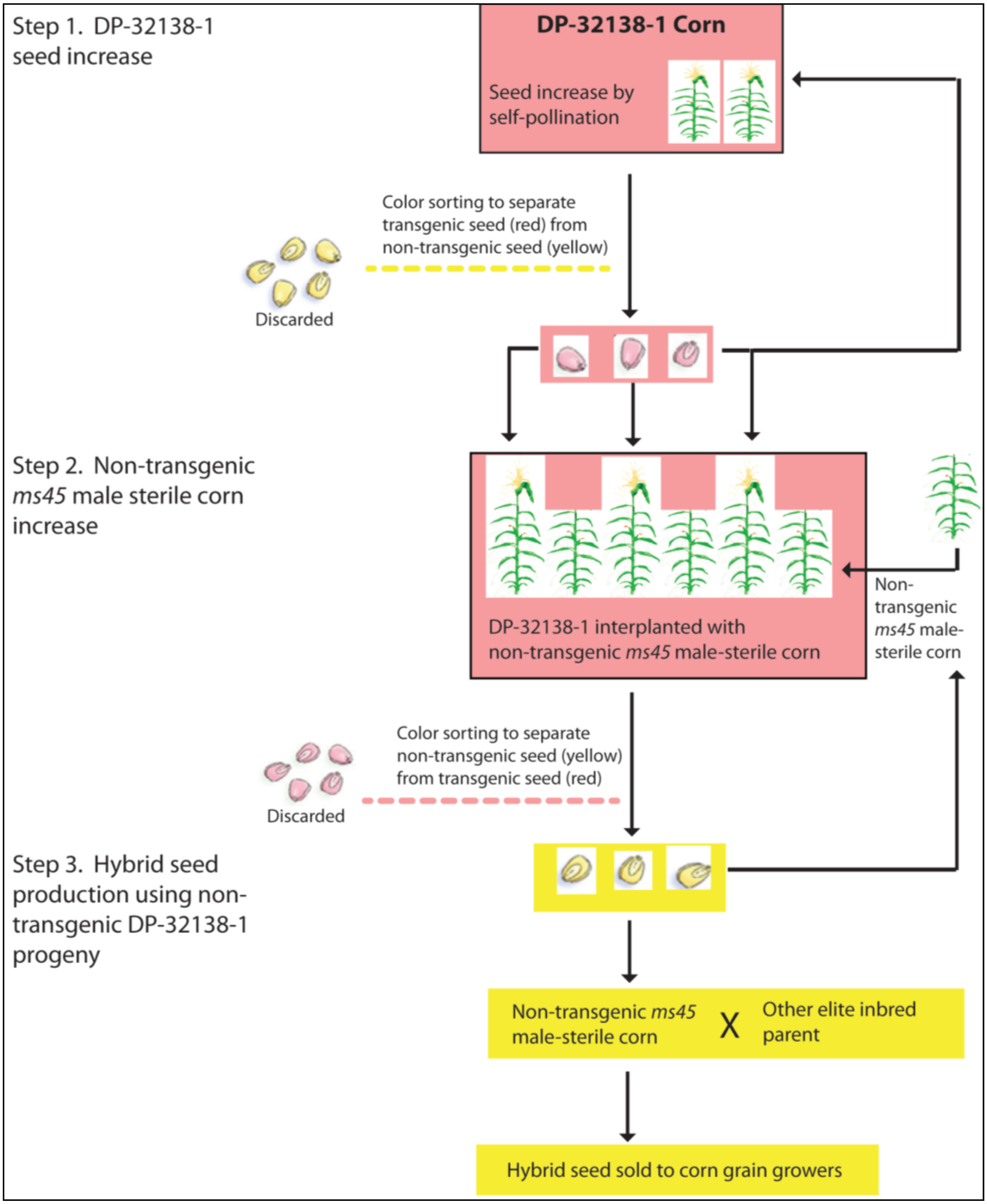
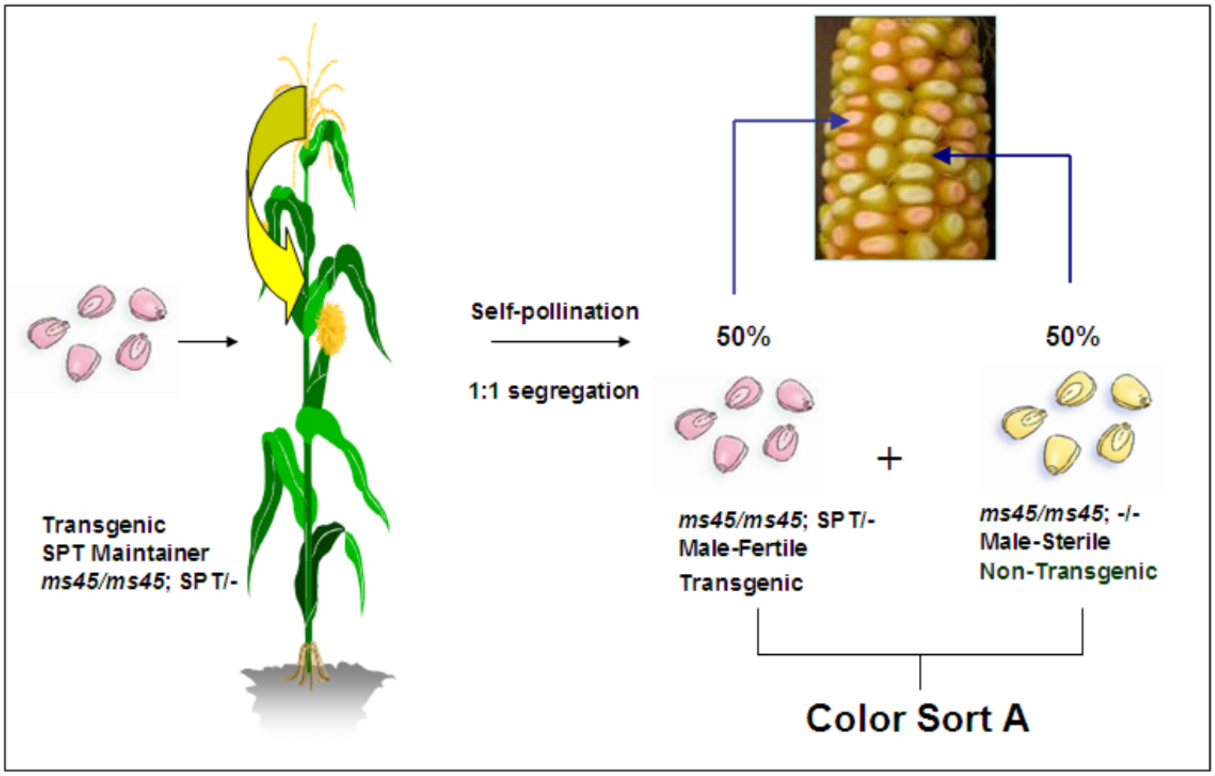


Figure 1. Outline of the SPT Process for Producing Hybrid Seed.



Source: (Pioneer 2009)

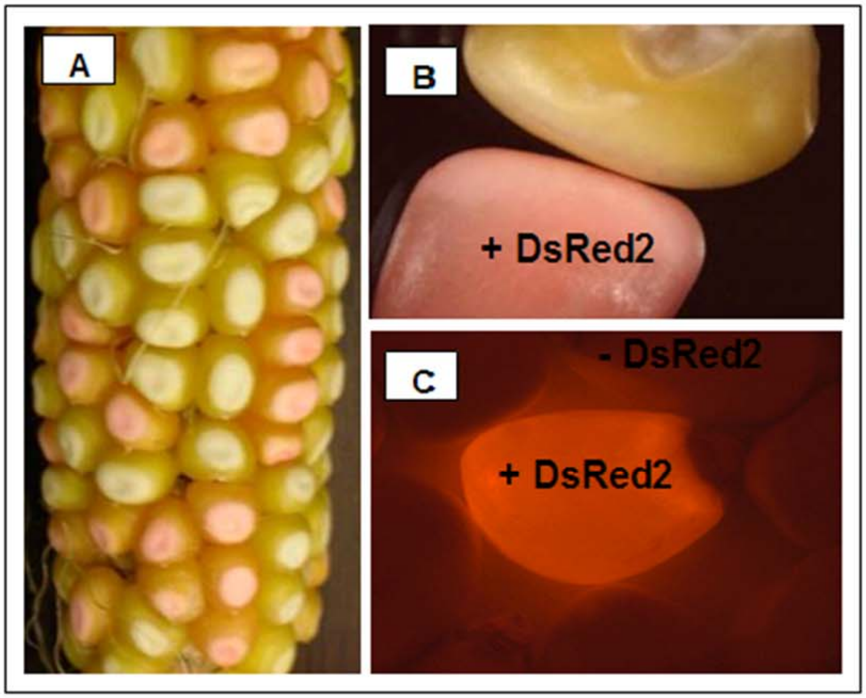
Figure 2. Production of the 32138 SPT Maintainer Line (Figure 1, Step I).

Step II: Male-Sterile Female Inbred Parent Seed Increase. The second major step of the Pioneer Hi-Bred SPT process produces male-sterile female inbred plants for use in hybrid seed production (Figure 1, Step II). This process involves the interplanting of both DP-32138-1 and *ms45* corn (non-transgenic). Within this cross, DP-32138-1 represents the only pollen donor. Consequently, DP-32138-1 corn may cross with itself or with *ms45* corn. Both transgenic (where the 32138 SPT maintainer cassette is present) and non-transgenic kernels (SPT cassette is absent) are produced following these crosses (Figure 4). Only the nontransgenic pollen is viable and can pollinate the male-sterile female inbred line.

Similar to the previous step of DP-32138-1 propagation, mechanical color sorting is used to distinguish transgenic kernels from non-transgenic kernels; in contrast, however, two rounds of mechanical color sorting are used to select for non-transgenic kernels (absence of the dsRed fluorsence protein, and thus, absence of the 32138 SPT maintainer cassette) for use in hybrid seed production (see Sort B, Figure 3). Kernels expressing DsRed, and thus containing the 32138 SPT maintainer cassette, are discarded after mechanical color sorting.

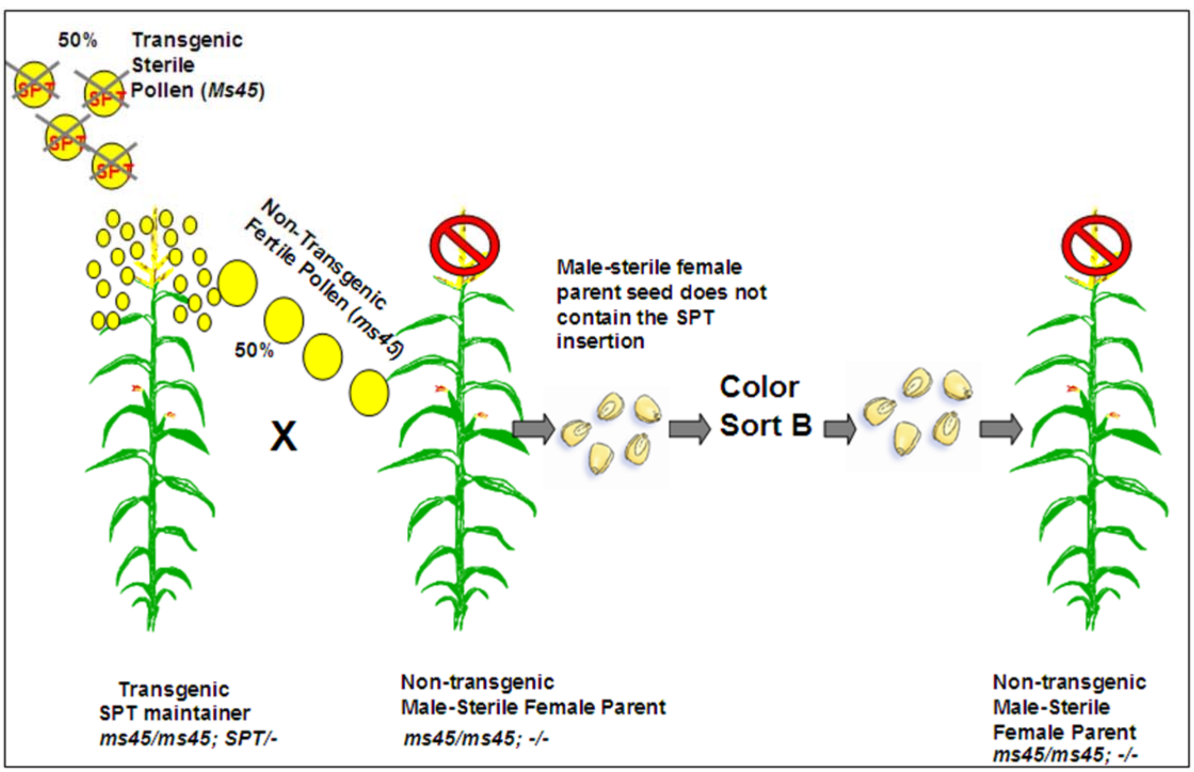
Kernels produced from the second major component of the Pioneer Hi-Bred SPT process are non-transgenic. Consequently, any hybrid corn lines produced from these kernels are also non-transgenic. Additionally, propagation of both the DP-32138-1 (Stage I) and *ms45* corn lines (Stage II) precedes commercial corn grain production by two generations. The final stage of of hybrid seed production is likely accomplished at a distant location relative to Stage I or II production, at seed production sites not directed by Pioneer (which handles the first stages of production). Consequently, because of the geographic separation of any seed that may contain

Figure 3. Visualization of the Red Marker, DsRed2 Expressed by the SPT Cassette.



Photograph A shows a corn ear with both the transgenic SPT cassette kernels/seeds (red coloring) and nontransgenic seeds (yellow coloring). Photograph B shows the SPT and the nontransgenic seed under standard illumination. Photograph C shows the SPT seed (+ DsRed2) and the non-transgenic seed (- DsRed2) under fluorescent illumination (Pioneer 2009).

Figure 4. Production of the Non-Transgenic Female Sterile Inbred (Step II).



Source: (Pioneer 2009)

the 32138 SPT maintainer cassette, admixture by mechanical means of commercial corn grain with DP-32138-1 corn is unlikely. This unlikely potential for admixture with DP-32138-1 corn is further reduced by the relatively small cultivation acreage that will use the SPT technology; fields containing DP-32138-1 are not expected to exceed 20,000 acres or approximately 0.023 percent of total U.S. corn grain producing acreage.

Step III: Commercial Corn Hybrid Seed Production. Commercial corn hybrid seed production (Figure 1, Step III) using the male-sterile seed (nontransgenic) prepared using SPT technology will be completed as it is for conventional production methods. The purity of the male-sterile female inbred plants produced from the SPT process is insured in Step II because of the built-in biological feature in which transgenic pollen is non-viable and because the seed was passed twice through mechanical color sorters (refer to Pioneer 2009, Appendix 3 for details on the color sorter). Therefore, the hybrid seed harvested and sold to growers for commercial grain production is non-transgenic for SPT and the resulting hybrid plants are fully fertile. The hybrid seed grown for grain is non-transgenic for SPT and therefore, the grain harvested is also non-transgenic for SPT.

The field sizes that will be used for any one corn variety at each step of increase can vary considerably depending on the present or expected market share of each variety, the level of testing to be conducted during the increase phase, and the expected yield of the inbred lines. In the case of corn, based on current production data, the amount of seed of all varieties combined must be sufficient to plant over 90 million acres each year (USDA-ERS 2010a). Since Pioneer, the applicant in this petition, has approximately a 30% market share and has approximately 250 different corn hybrids available, this implies the number of corn varieties available industry-wide is about 800-1000 (Pioneer 2008; Pioneer 2010) with other estimates of over 4000 hybrids available in the U.S. in 2009 (Monsanto 2010). The development of new inbreds along with the Steps I and II seed increases are the stages when the transgenic plants of DP-32138-1 are expected to be in the field. The 250 Pioneer varieties multiplied by the 2-20 acre Step II increase per variety is the "up to 5000 acres" maximum acreage of DP-32138-1 to be used by Pioneer (Pioneer 2009). The industry-wide assumed total of 1000 to 4000 varieties multiplied by the 2 to 20 acre Step II increase per variety equates to the "up to 20,000 acres" maximum acreage of DP-32138-1 to be utilized if adopted industry-wide (Pioneer 2009). In the Step III seed increase, DP-32138-1 confers its largest benefits through the planting of the non-transgenic, male sterile female inbred produced with DP-32138-1. Pioneer may avoid detasseling up to 300,000 acres, while other seed industry organizations may potentially have up to 1 million acres that would not require detasseling.

No Action: Seed Production

The availability of methods used to produce seed corn would be the same as currently used in seed corn production systems under the No Action Alternative.

Preferred Alternative: Seed Production

A determination of nonregulated status of DP-32138-1 corn under the Preferred Alternative would not change the use of presently available systems for seed corn production. DP-32138-1 will be produced in a manner similar to other seed corn inbreds and resulting hybrids. As discussed above, these inbreds and resulting hybrids are typically produced under identity preservation systems that include contracts with growers, traceability, product tracking, and process verification since Pioneer and other seed corn companies take precautions to insure that inbred parent lines are not misappropriated by third parties. These procedures greatly minimize any chances of commingling of the DP-32138-1 seed with other seed and, ultimately, commercial grain.

With the price premiums involved with production of any seed corn variety, including the DP-32138-1 corn, there is no benefit for modifying any of the well known procedures to maintain high genetic purity. Using DP-32138-1 in the increase of corn varieties could reduce the yield losses associated with detasseling and the consequence would be fewer acres of increase needed per variety. Additionally, there could be a decrease in the number of people required for detasseling resulting in reduced expenses and fewer safety hazards during the seed corn increase. The assumed maximum use of 20,000 acres industry wide for DP-32138-1 is only about 0.02% of the 80-90 million acres of annual corn production. The agronomic practices used for these seed production acres would be almost identical to the agronomic practices and locations used under the No Action alternative, so no overall effects are anticipated.

Discarded parent seed, whether it is outdated or discarded from the color sorter, and seed byproducts, such as husks and cobs, will be disposed by placing in landfills, composting, or incineration (Pioneer 2009). According to Pioneer policy, seed producers will not feed discard corn to animals (Public Comment on EA for DP-32138-1).

Since the DP-32138-1 insertion has three separate genes, each of which is required for the production system to work correctly, APHIS considered the effects if one or two of these three genes were lost through mutation or recombination, which was addressed and discussed in detail in the petition (Pioneer 2009). DP-32138-1 will maintain the propagation of the male sterile female inbred as well as eliminate detasseling. All of the plants in Steps I, II, and III are inspected for off-type characteristics and any off-types will be rogued out. Since Step I and II fields are very small, these fields can be expected to be inspected more heavily than the larger Step III fields. In conventional seed corn production, fields under Steps I, II, and III of seed production are similarly inspected for plants with off-type characteristics, so the inspection process is not an additional step required for DP-32138-1 corn. There is the possibility that the mutation or recombination could take place at a very low frequency during Step II allowing a very low frequency of female inbred plants that would also have male fertility in Step III. This frequency would be expected to be much lower than the incomplete detasseling that happens during conventional seed production. None of these plants would produce seeds with the DsRed2 color marker protein, the only non-native corn protein in DP-32138-1 corn. Please refer to the petition for the complete analysis (Pioneer 2009).

There is always the remote possibility for misdirection of DP-32138-1 in the handling, planting, harvesting, transportation or processing stream. Because DP-32138-1 has successfully completed the new protein consultation process for the DsRed2 color marker protein with FDA (Appendix A), there are no human or animal health concerns if DP-32138-1 enters the food or feed supply (see section on Public Health below for more discussion).

Cumulative Effects: Seed Production

Because the acreage, agronomic practices, and locations for seed production using DP-32138-1 are expected to be the same or similar to the No Action alternative, no cumulative effects have been identified for this issue.

Organic Farming

Certified organic corn acreage is a small percentage of overall corn production. The most recently available data show 143,000 acres of organic corn production in 2007. This is 0.17% of the total 86.2 million acres of corn planted in 2007 (USDA-ERS 2008). In 2005, 131,000 acres of organic corn were planted resulting in a 9% increase in organic corn acreage over the 2 year period from 2005 to 2007 (USDA-ERS 2010a). GE corn has been in production since the mid-1990's, and in 2009 accounted for 85% of the 86.7 million acres of corn planted (USDA-NASS 2009a). GE corn production and organic corn production in the U.S. are both increasing. Based upon trend data, it is anticipated that both production practices will likely continue to increase with or without a determination of nonregulated status of DP-32138-1. APHIS also recognizes that producers of non-GE corn, particularly producers who sell their products to markets sensitive to genetically engineered traits (e.g. organic or some export markets) can be reasonably assumed to be using practices on their farm to protect their crop from unwanted substances and maintain their price premium. For example, the NOP has recognized the practicality of protecting organically-produced crops, and the investment farmers put into their production practices, by requiring that organic production plans include methods to protect organically-produced crops. "Organic crops must be protected from contamination by prohibited substances used on adjoining lands (for example, drifting pesticides, fertilizer-laden runoff water, and pollen drift from genetically engineered...)" (NCAT 2003).

Typically, there is more than one method for farms under organic practices to prevent unwanted material from entering their fields including: isolation of the farm, physical barriers or buffer zones between organic production and non-organic production, as well as formal communications between neighboring farms (NCAT 2003). The organic plan used as the basis for organic certification should include a description of practices used to prevent or reduce the likelihood of unwanted substances, like GE pollen or seed, at each step in the farming operation, such as planting, harvesting, storing and transporting the crop (Riddle 2004; Krueger 2007; Kuepper, Born et al. 2007). Organic plans should also include mechanisms to monitor the risk of GE pollen or seed co-mingling with the organic crop (Kuepper, Born et al. 2007). Farmers using organic methods are requested to let neighboring farmers know that they are using organic production practices and request that the neighbors also help the organic farmer reduce contamination events (NCAT 2003; Krueger 2007). Thus, commonly used production practices for corn, and the practical methods typically used by corn farmers using organic methods currently provide many measures that greatly reduce the likelihood of accidental gene flow between DP-32138-1 and non-GE corn fields. These practices protect organic crops and thus maximize profits and price premiums accorded to corn under organic production. APHIS will assume that farmers are already using, or have the ability to use, these common practices as APHIS' baseline for the analyses of the following alternatives below. Recommended organic production practices for field corn are also readily available (Kuepper 2002). Without any requirements in place for GE corn varieties previously deregulated pursuant to Part 340 and the Plant Protection Act, the production of corn using organic methods continues to increase.

In agricultural systems, growers may choose to grow GE or non-GE corn, and obtain price premiums for growing varieties of corn for particular markets (e.g., using organic methods for corn production or producing a specialty corn variety for particular processing needs). For example, in 2007, conventional corn averaged $4.19/bushel (USDA-NASS 2010b), whereas organic corn averaged $7.08/bushel (USDA-NASS 2010c). USDA asserts that agricultural practices that use conventional means, organic production systems, or genetically engineered varieties can all provide benefits to the environment, consumers, and farm income.

No Action: Organic Farming

Current availability of seed for conventional (both GE and non-GE) corn varieties, and those corn varieties that are developed for organic production, are expected to remain the same under the No Action Alternative. Commercial production of conventional and organic corn will not change and will remain the same under the No Action Alternative. Planting and production of GE corn varieties and organic corn have both increased due to market demands over the last ten years, and these markets are likely to continue to increase under the No Action Alternative (USDA-NASS 2010a; USDA-NASS 2010c).

Preferred Alternative: Organic Farming

It is not likely that organic farmers who choose not to plant transgenic varieties or sell transgenic grain will be impacted from the use by the developer of DP-32138-1 corn or by other developers licensed to use the corn. Commonly used production practices for corn, and the practical methods typically used by corn farmers using organic methods to protect their crop under organic production (NCAT 2003) provide many measures that greatly reduce the likelihood of accidental gene flow between DP-32138-1 and non-GE corn fields. Pollen from DP-32138-1 corn containing SPT transgenes is not functional and as a result will not fertilize corn plants. Therefore, DP-32138-1 corn is not likely to affect neighboring farms. Similar to the No Action Alternative, the use of GE corn varieties and the use of organic corn production systems are likely to continue a trend toward increasing production under the Preferred Alternative.

According to the petition, no transgenes will be present in the seed for planting the Step III increase acres (Pioneer 2009). Likewise, after the seed harvest of Step III, the seed for planting Step IV will also contain no transgenes in either commercial corn grain fields or in the harvest from the grain fields (Pioneer 2009).

A determination of nonregulated status of DP-32138-1 will not impact the availability of seed corn for organic production systems. In accordance with NOP production standards (7 CFR 205.204a) "the producer must use organically grown seed, annual seedlings, and planting stock: except, that (1) Nonorganically produced, untreated seeds and planting stock may be used to produce an organic crop when an equivalent organically produced variety is not commercially available." Organic seed corn is commercially available and therefore in accordance with NOP organic seed corn must be used in certified organic corn production systems. The use of DP-32138-1 will have no impact on a grower's ability to obtain seed corn for organic production systems. The Organic Trade Association provides available resources to locate suppliers that sell organic seeds including corn (OTA 2010).

Cumulative Effects: Organic Farming

Under the No Action Alternative and under the Preferred Alternative, a determination of nonregulated status of DP-32138-1 will not change the market demands for GE corn or corn produced using organic methods. A determination of nonregulated status of DP-32138-1 will not add another GE corn variety to the conventional corn market, since the SPT genes are confined to the seed production process, and are not found in the commodity corn. The SPT technology will not change the amount of GE corn available for production, because it will only replace detasseling operations. Additionally, based upon recent trend information, the market changes of conventionally farmed corn varieties are not related to the ability of organic production systems to increase their market share. Between 2002 and 2008, acreage of conventionally farmed corn varieties increased by 12% (USDA-ERS 2010d) while the acreage associated with the organic production of corn rose 50% during this same time period (USDA-ERS 2008).

Specialty Corn Systems

Specialty corn, such as waxy corn, white corn, blue corn, and organic corn, comprises 8% of the U.S. market (USGC 2006). With 85% of conventional corn being GE varieties in 2009, a market exists for non-GE corn with low or no level of GE corn. The size of this non-GE corn market, generally for Japan and Europe, was 300,000 to 550,000 acres in 2005 with a flat projected growth (USGC 2006). These specialty corn products intensively use systems to maintain the purity of the corn product, based on the demands of the end-user (e.g., food processing plants).

<u>No Action: Specialty Corn Systems</u>

The availability of methods used to separate specialty corn products from corn used as grain would remain the same.

<u>Preferred Alternative: Specialty Corn Systems</u>

A determination of nonregulated status of DP-32138-1 under the Preferred Alternative would not change the availability of using contracts, tracking and traceability systems, quality assurance processes, closed-loop systems, and identity preservation systems to maintain product purity for specialty corn production. Impacts would be similar to the No Action Alternative.

<u>Cumulative Effects: Specialty Corn Systems</u>

The availability of methods used to separate specialty corn products from corn used as grain would be the same as currently used in corn production systems, and no changes are foreseeable. No cumulative effects have been identified for this issue.

Physical Environment

Water Resources

Since inbreds are much less vigorous than hybrids, irrigation can be preferred in the seed production of inbreds to help assure desired seed yields (Wych 1988), especially in those areas subject to frequent droughts.

<u>No Action: Water Use</u>

Under the No Action Alternative, DP-32138-1 interactions with water would be limited to the areas that were approved for regulated releases by APHIS. Irrigation practices associated with conventional seed corn production would not be affected. Under the No Action Alternative, there is no change in the conventional seed production procedures of irrigating corn inbred lines in drought prone areas and no change in irrigation practices of commercial corn production.

Preferred Alternative: Water Use

Impacts would be similar to the No Action Alternative. DP-32138-1 does not change cultivation practices for seed production of corn inbreds. A determination of nonregulated status of DP-32138-1 will not change the use of irrigation practices in seed corn production or commercial corn production. The very small number of acres involved is an additional reason that the use of DP-32138-1 in seed corn production is expected to have no effect on water use. Since hybrid corn will not contain the DP-32138-1 transgenes or non-native corn proteins at the commercial stage of planting and harvesting, the consequences of the Preferred Action Alternative on commercial corn production are the same as the No Action Alternative.

Cumulative Effect: Water Use

No cumulative effects have been identified for a determination of nonregulated status of DP-32138-1 corn. A determination of nonregulated status of DP-32138-1 will not change the current irrigation practices used in seed corn production or commercial corn production.

Soil

Certain agronomic practices such as cultivation cause disturbance and exposure of the top surface layer of soils and allow some soils to be prone to degradation. Two environmental impacts of soil degradation are the decline in water quality and the possible contribution of additional CO_2 towards climate change (Lal and Bruce 1999). One production step for corn seed that may have detrimental effects on soil are detasseling operations. Detasseling must take place, rain or shine, when the tassels start to appear on the plant. If the soil is wet, using heavy implements, such as the detasseler carriers and the mechanical detasselers, produce large ruts in the soil. These ruts result in damage to the corn roots and negatively impact soil structure which in turn change water movement and nutrient uptake by plants (Daum 1996; Kok, Taylor et al. 1996; Gelderman 2009; Tilghman 2009; Roegge 2010).

Detasseling operations resulting in soil compaction could also potentially affect subsurface soil and constituent organisms. The soil environment in and around corn fields is complex, and rich in microorganisms and arthropods. The corn root system modifies soil through its close association with several microbial groups such as bacteria, fungi, protozoa, and mites (Bais, Weir et al. 2006). Bacteria typically represent the most abundant microbes in the soil followed by fungi. These microbial groups play an important and particular role in the ecology of the soil, including nutrimental cycling and the availability of nutrients for plant growth (Hoeft, Nafziger et al. 2000; OECD 2003)

No Action: Soil

DP-32138-1 interactions with the soil would be limited to the areas that are approved by APHIS for regulated releases. Cultivation practices associated with conventional seed corn production would not be affected. The soil environment would be modified by corn roots and crop soils would still be affected by agronomic practices associated with conventional seed corn cultivation, including the negative impacts associated with detasseling when soils are wet. Cultivation practices associated with commercial production of corn would not be affected.

Preferred Alternative: Soil

In the event of a determination of nonregulated status of DP-32138-1 under the Preferred Alternative, soil interactions with DP-32138-1 would occur at a larger scale than under the regulated releases. DP-32138-1 seed corn production could have three possible effects on soils as compared to conventional seed corn production: no effects from detasseling, possible presence of additional ZM-AA1 alpha-amylase protein, and possible presence of DsRed2 color marker protein.

Cultivation practices associated with DP-32138-1 seed corn production would be the same as conventional seed corn production during seedbed preparation, planting and early plant development. The soil environment would be modified by corn roots and crop soils would still be affected by agronomic practices associated with conventional seed corn cultivation. However, for DP-32138-1 seed corn production, detasseling would no longer be required and the negative consequences of detasseling would be avoided, such as compaction and large ruts in the soil. Generally these seed corn production fields, including DP-32138-1 seed corn fields, are in rotation with other crops. These rotations with other non-corn crops generally result in averaging out any positive or negative impacts that may have resulted from the seed corn production. These positive or negative effects cannot be quantified because the DP-32138-1 seed corn fields are a very small proportion of commercial corn production. In comparison to commercial corn grain production in which no detasseling takes place, DP-32138-1 seed corn would be no different.

DP-32138-1 expresses small amounts of ZM-AA1 alpha-amylase protein during plant development and only at low levels in the seed at physiological maturity (Pioneer 2009). In both conventional and DP-32138-1 seed corn production, the rows of male plants are generally destroyed to aid in increasing seed yield of the female rows by decreasing plant competition and to decrease the chances of commingling of male and female seeds at harvest (Pioneer 2009). In DP-32138-1 corn, the plants that contain the transgenes (male fertile) would be destroyed following pollination, generally by chopping up the plant. These immature plants contain low levels of ZM-AA1 alpha-amylase protein throughout plant tissues. Consequently, there is a potential for ZM-AA1 alpha-amylase protein to be incorporated into agricultural soils. However, alpha-amylases are ubiquitous enzymes found in microorganisms, plants, and animals (Janeček, Lévêque et al. 1999).

If the DP-32138-1 plants are allowed to reach physiological maturity, these plants are generally harvested with most, if not all, of the seed removed from the field. For seed that may remain in the field after harvest, physiologically mature seed of DP-32138-1 will contain low levels of ZM-AA1 alpha-amylase protein. However, if soil moisture and temperatures are adequate to stimulate germination, seeds of both conventional and DP-32138-1 would produce similar amounts of ZM-AA1 alpha-amylase protein. Since the levels of ZM-AA1 alpha-amylase protein are essentially the same in conventional corn and DP-32138-1 corn, impacts of DP-32138-1 seed corn production on the soil would be similar to those in conventional corn seed production.

DP-32138-1 expresses small amounts of the DsRed2 color marker protein during plant development with the highest levels in the seed detected at physiological maturity (Pioneer 2009). During the production of non transgenic male sterile inbred for seed increase, plants that contain the transgenes may be chopped and left on the soil surface (Pioneer 2009). These immature plants express low levels of DsRed2 color marker protein throughout the plant. The DsRed2 color marker protein will be susceptible to degradation and adsorption to humus or minerals as are other proteins in agricultural soils (Bastida, Moreno et al. 2009). Soils contain numerous organisms and enzymatic activity from decomposed organisms that degrade a wide array of molecules including proteins (Bastida, Moreno et al. 2009). These processes would metabolize or sequester the DsRed2 color marker protein with no likely effect on the soil.

If the DP-32138-1 plants are allowed to reach physiological maturity, these plants are generally harvested with most, if not all, of the seed removed from the field. In the few seeds that may remain in the field after harvest, physiologically mature seed of DP-32138-1 will contain DsRed2 color marker protein. Similar to DsRed2 that originated in whole plants, normal soil degradation and adsorption would be expected to remove this protein.

For the preferred alternative of deregulating DP-32138-1 corn, the three possible effects of no detasseling, possible presence of additional ZM-AA1 alpha-amylase protein and possible presence of DsRed2 color marker protein combined with the very low presence in the environment (a maximum 20,000 acres), impacts on soil are expected to be similar to those from conventional corn seed production. Since hybrid corn will not contain the DP-32138-1 transgenes or non-native corn proteins at the commercial stage of planting and harvesting, the consequences of the Preferred Action Alternative on commercial corn production are the same as the No Action Alternative.

Cumulative Effects: Soil

APHIS has not identified any cumulative effects for this issue. The fields devoted to seed corn production, conventional as well as DP-32138-1 seed corn, are normally rotated with other crops with the maximum potential for 20,000 acres expected to be devoted to DP-32138-1 seed corn. DP-32138-1 seed corn acres would be only 0.02% of all corn production acres. APHIS concludes that adverse effects of the ZM-AA1 alpha-amylase protein or of the DsRed2 color marker protein are not likely, effects of avoiding detasseling are slightly positive, if any, and exposure of 0.02% of all annual corn production acres is small, and therefore with adverse impacts on soil unlikely, no cumulative impacts are likely. Since hybrid corn will not contain the DP-32138-1 transgenes or non-native corn proteins at the commercial stage of planting and harvesting, the effects of the the Preferred Action Alternative on commercial corn production are not significant and are therefore no cumulative effects are expected.

Air Quality

Tillage, traffic and harvest emissions, pesticide drift from spraying, and nitrous oxide emissions from the use of nitrogen fertilizer may affect air quality (Aneja, Schlesinger et al. 2009). Tillage contributes to the release of GHGs because of the loss of CO_2 to the atmosphere, and the exposure and oxidation of soil organic matter (Baker, Southard et al. 2005). Emissions released from agricultural equipment (e.g., irrigation pumps and tractors) include carbon monoxide, nitrogen oxides, reactive organic gases, particulate matter, and sulfur oxides (US-EPA 2010). Nitrous oxide may also be released following the use of nitrogen fertilizer (US-EPA 2010). Aerial application of pesticides may cause impacts from drift and diffusion. Pesticides may volatilize after application to soil or plant surfaces and move following wind erosion (Vogel, Majewski et al. 2008). Agriculture, including land-use changes for farming, is responsible for an estimated 6% of all human-induced GHG emissions in the U.S., and N_2O emissions from agricultural soil management are a large part of this, 68 % of all U.S. N_20 emissions (US-EPA 2010).

No Action: Air Quality

Under the No Action Alternative, DP-32138-1 interactions with the air would be limited to the areas that were approved for regulated releases by APHIS. Cultivation practices associated with conventional seed corn production or commercial corn production would not be affected. Air quality would still be affected by agronomic practices associated with conventional seed corn cultivation and commercial corn production such as tillage, pesticide application, and use of agricultural equipment.

Preferred Alternative: Air Quality

DP-32138-1 seed corn production does not change cultivation practices for seed production of corn inbreds. A determination of nonregulated status of DP-32138-1 will not change the use of tillage, use of agriculture equipment, irrigation, pesticide applications and fertilizer applications in seed production or commercial corn production. With DP-32138-1, no detasseling would be required, subsequently reducing the combustion gas emissions from detasseler carriers, mechanical detasselers and movement of detasselers between homes and seed fields resulting in a beneficial effect on air quality. Considering the very small number of acres involved, the use of DP-32138-1 in seed corn production is expected to have minimal effects on air quality.

Since hybrid corn will not contain the DP-32138-1 transgenes or non-native corn proteins at the commercial stage of planting and harvesting, the consequences of the Preferred Action Alternative on commercial corn production are the same as the No Action Alternative.

Cumulative Effects: Air Quality

APHIS has not identified any cumulative effects for this issue. The use of DP-32138-1 in seed corn production is expected to have no cumulative effect on air quality because of the very small number of acres that would be involved in DP-32138-1 seed corn production and there are no changes in cultivation practices. Since hybrid corn will not contain the DP-32138-1 transgenes or non-native corn proteins at the commercial stage of planting and harvesting, the effects of the Preferred Action Alternative on commercial corn production were not significant, and consequently there are unlikely to be any cumulative effects.

Climate Change

Production of agricultural commodities is one of the many human activities that could possibly GHGs that affect climate (see discussion in Affected Area, Physical Environment). CO_2, nitrogen dioxide (NO_2), and methane (CH_4) may be produced through the combustion of fossil fuels to run farm equipment, the use of fertilizers, or the decomposition of agricultural waste products including crop residues and animal wastes. Classes of crops planted are relevant to climate change, as are the locations and the soil types in which they are planted. Climate change itself may force changes to agricultural practices by extending the ranges of weeds and pests of agriculture (IPCC 2007). Indirect effects of new crops will be determined by the traits engineered into organisms and the management strategies used in the production of these organisms.

No Action: Climate Change

Under the No Action Alternative, environmental releases of DP-32138-1 would be under APHIS regulation. There would be no measurable effect from these confined environmental releases. Cultivation practices associated with conventional seed corn production or commercial corn production would not be affected. Agronomic practices associated with conventional seed corn cultivation or commercial corn production such as tillage, pesticide application, and use of agriculture equipment would remain the same.

Preferred Alternative: Climate Change

DP-32138-1 seed corn production does not change cultivation practices for seed production of corn inbreds. A determination of nonregulated status of DP-32138-1 will not change the use of tillage, use of agriculture equipment, irrigation, pesticide applications and fertilizer applications in seed production on commercial corn production. With DP-32138-1, no detasseling would be required, consequently reducing the energy requirements for detasseler carriers, mechanical detasselers and for movement of detasselers between homes and seed fields resulting in a reduced level of greenhouse gases. However, with the relatively small number of acres that would be involved with seed corn production using DP-32138-1 a slight decrease of GHG production is likely too small to calculate any meaningful impact for climate change. Since hybrid corn will not contain the DP-32138-1 transgenes or non-native corn proteins at the commercial stage of planting and harvesting, the consequences of the Preferred Action Alternative on commercial corn production are the same as the No Action Alternative.

Cumulative Effects: Climate Change

APHIS has not identified any cumulative effects for this issue. The use of DP-32138-1 in seed corn production is expected to have no cumulative effect on climate change because of the very small number of acres that would be involved in DP-32138-1 seed corn production and there are no changes in cultivation practices. Since hybrid corn will not contain the DP-32138-1 transgenes or non-native corn proteins at the commercial stage of planting and harvesting, the effects of the Preferred Action Alternative on commercial corn production were not significant, and consequently there are no cumulative effects expected.

Animal and Plant Communities

Animals

Corn production systems in agriculture are host to many animal species. Mammals and birds may seasonally use grain, and invertebrates can feed on the plant during the entire growing season. The cumulative effects analysis for this issue is found below at "Cumulative Effects: Plants, Animals, Biodiversity."

No Action: Animals

Under the No Action Alternative, environmental releases of DP-32138-1 would be under APHIS regulation. Animal incursions would be limited to regulated field trials. The new protein consultation with FDA was successfully completed for DsRed2 color marker protein, the only non native corn protein in DP-32138-1 (Appendix A), which addressed any concerns of composition. The consultation demonstrated a lack of toxicity and allergenicity of DP-32138-1 for human and animal consumption. Based upon the FDA's new protein consultation, APHIS supports Pioneer's conclusions that corn produced using Pioneer's SPT technology is considered safe for animal consumption.

Preferred Alternative: Animals

APHIS has reviewed and accepts the data submitted by the applicant, which are similar to the data submitted during the FDA new protein consultation process for DsRed2 color marker protein, the only non native corn protein in DP-32138-1 (Appendix A). Impacts would be similar to the No Action Alternative. The agronomic practices used to produce DP-32138-1 will be the same as those used to produce conventionally grown seed corn. Therefore, the discussion of effects on animals of DP-32138-1 will focus solely on the introduced proteins in DP-32138-1 corn, the ZM-AA1 alpha-amylase protein and the DsRed2 color marker protein.

Pioneer DP-32138-1 has not been genetically engineered to produce any pesticides. DP-32138-1 contains the ZM-AA1 protein in the developing pollen and in other parts of the plant at low levels except at physiological maturity when it has declined below the limits of quantitation (Pioneer 2009). The ZM-AA1 alpha-amylase protein is normally found predominantly in germinating kernels (sprouts) of conventional corn. Amylases may also be found in germinating pollen (Bhadula and Sawhney 1989; Castro and Clement 2007), including corn (Brewbaker 1971; Agarwala, Sharma et al. 1981; Wakhle, Phadke et al. 1983). In DP-32138-1, ZM-AA1 concentration is 400 times greater in pollen than in seed and 64 times greater than in R4 stage leaves. The alpha-amylases are ubiquitous enzymes present in many organisms including plants and insects (Campos, Xavier-Filho et al. 1989; Raimbaud, Buleon et al. 1989; Silva, Terra et al. 1999; Cristofoletti, Ribeiro et al. 2001). The alpha-amylases from multiple sources including plants, fungi and bacteria have a long history of safe consumption in foods (Pariza and Johnson 2001). Animals that feed primarily on corn kernels are seed-feeding insects and rodents found in agricultural fields. During field trials, the applicant found no differences in insect feeding damage (Pioneer 2009), indicating similar insect susceptibility of DP-32138-1 tissues and conventional corn.

DP-32138-1 contains the DsRed2 color marker protein in the seed (410 ng/mg tissue dry weight) but not in leaves or pollen of physiologically mature plants, and at lower levels in leaves (87 ng/mg during the vegetative growth stage and 160-210 ng/mg during the early reproductive stages of growth) of the growing plant (Pioneer 2009). Rodents, such as mice or squirrels, in some seasons may feed exclusively on corn kernels. Thus, these animals are most likely to have a diet containing large amounts of corn kernels. DsRed2 showed no signs of toxicity when used as a vital marker in mice (Figueiredo, Fernandes et al. 2008). In a feeding study of mice to determine acute toxicity level of the DsRed2 protein, a wide margin of safety was observed (Pioneer 2009). Other mammals, such as deer, would have even lower exposure to DsRed2 color marker protein and ZM-AA1 alpha-amylase protein because of feeding habits; for example, deer nibble on tips of corn ears as opposed to kernels (Steffey, Rice et al. 1999). Finally, transgenic plants stably expressing DsRed variants did not show any abnormalities in growth habits, development, fertility, germination or morphogenesis (Jach, Binot et al. 2001; Dietrich and Maiss 2002; Stuitje, Verbree et al. 2003; Wenck, Pugieux et al. 2003; Mirabella, Franken et al. 2004; Pioneer 2009). APHIS has reviewed this information and has determined that there would be no negative effects to animals that forage on DP-32138-1 corn (USDA-APHIS 2010).

Plants

Corn production acreage is host to many plant species as well. The landscape surrounding a corn field varies depending on the region. In certain areas, corn fields may be bordered by other corn (or any other crop); fields may also be surrounded by wooded or pasture/grassland areas. Therefore, the types of vegetation, including weeds, around a corn field depend on the area where the corn is planted. A variety of weeds dwell in and around corn fields; those species will also vary depending on the geographic region where the corn is planted. Corn itself is not sexually compatible with any other plant species found in the U.S. (USDA-APHIS 2010). The cumulative effects analysis for this issue is found below at "Cumulative Effects: Plants, Animals, Biodiversity."

No Action: Plants

Under the No Action Alternative, environmental releases of DP-32138-1 would be under APHIS regulation. Plant species that typically inhabit corn production systems will be managed as in conventional corn production, including the use of mechanical, cultural, and chemical control methods.

Preferred Alternative: Plants

In the event of a determination of nonregulated status of DP-32138-1, agricultural practices used for conventional corn would be used for plant management during the cultivation of DP-32138-1 corn. Impacts would be similar to the No Action Alternative. DP-32138-1 does not exhibit characteristics associated with weedy growth and will not compete with plants found outside of agricultural production (USDA-APHIS 2010). Weeds within fields of DP-32138-1 will be managed using mechanical, cultural, and chemical control, as weeds are now managed in conventional corn systems. As there are no toxic effects on animals (see Animals discussion above), there are no toxic effects on animals that could be pollinators of other plants in or around fields cultivated with DP-32138-1 corn. DP-32138-1 has not been genetically engineered to be tolerant to any herbicides.

Biological Diversity

Biological diversity, or the variation in species or life forms in an area, is highly managed in agricultural systems. Farmers typically plant crops that are genetically adapted to grow well in a specific area of cultivation and have been bred for a specific market. In the case of corn agriculture, varieties have been developed for food processing needs (e.g., waxy corn), consumer qualities (e.g., blue corn or white corn), or for use as a vegetable (e.g. sweet corn). In conventional agriculture, farmers want to encourage high yields from their corn crop, and will intensively manage the 'plant communities,' or weeds, found in corn crops through chemical, cultural or mechanical means. Animals, particularly insect and other pest species will also be managed through chemical and cultural controls to protect the crop from damage by certain animal pests. Therefore, the biological diversity in agricultural systems (the agro-ecosystem) is highly managed and may be lower than in the surrounding habitats. The cumulative effects analysis for this issue is found below at "Cumulative Effects: Plants, Animals, Biodiversity."

No Action: Biological Diversity

Under the No Action Alternative, environmental releases of DP-32138-1 would be under APHIS regulation. Animal and plant species that typically inhabit seed corn and commercial production systems will be continue to be affected by the management plan of typical conventional corn production, which includes the use of mechanical, cultural, and chemical control methods.

Preferred Alternative: Biological Diversity

The importance of corn as a food crop, and its dependence on human management, has produced a long history of effort to protect the integrity of the germplasm lines of corn. Decades prior to the introduction of transgenic corn products, the corn industry developed effective methods and means to maintain product segmentation and standards of genetic purity. Specialty corn crops, for example, were successfully isolated over long term cultivation and continue to be grown today, even with transgenic commodity corn widely adopted in the U.S. Moreover, with respect to both conventional and transgenic corn, the ability to protect and maintain the genetic purity of breeding lines is critical to seed companies and developers of new technologies such as DP-32138-1. Consequently, seed companies routinely apply standard breeding techniques, including physical and temporal isolation, which have proven effective at maintaining the genetic purity of breeding lines (Wych 1988).

Genetically engineered corn lines have been available on the market since 1994 and the preponderance of evidence in peer-reviewed literature does not establish any consistent adverse effect of its production on biodiversity (Bitzer, Rice et al. 2005; Torres and Ruberson 2005; Romeis, Meissle et al. 2006; Marvier, McCreedy et al. 2007; Chen, Zhao et al. 2008; Wolfenbarger, Naranjo et al. 2008). APHIS' review and analysis of Pioneer's data (USDA-APHIS 2010) indicate that the line DP-32138-1 exhibits no traits that would cause increased weediness, that its unconfined cultivation should not lead to increased weediness of other cultivated corn or other sexually compatible relatives, and that 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.

Cultivation of DP-32138-1 seed corn requires the same agronomic practices as conventional seed corn production. Animal and plant species that typically inhabit seed corn production systems will be managed as in conventional corn production, likely with the use of mechanical, cultural, and chemical control methods. Overall impacts would be similar to the No Action Alternative. Since hybrid corn will not contain the DP-32138-1 transgenes or non-native corn proteins at the commercial stage of planting and harvesting, the consequences of the Preferred Action Alternative on commercial corn production are the same as the No Action Alternative.

Cumulative Effects: Animals, Plants, Biodiversity

APHIS has determined that there are no impacts from past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action to create cumulative impacts or reduce the long-term productivity or sustainability of any of the resources associated with the ecosystem in which the DP-32138-1 is planted. DP-32138-1 has not been genetically engineered to produce a toxin or pesticide, and has not been genetically engineered to be tolerant to an herbicide. Although some studies have found both increases and decreases in animal and plant diversity and abundance in the agro-ecosystem due to the use of GE crops (Hansen Jesse and Obrycki 2000; Ponsard, Gutierrez et al. 2002; Brooks, Bohan et al. 2003; Haughton, Champion et al. 2003; Hawes, Haughton et al. 2003; Marshall, Brown et al. 2003; Roy, Bohan et al. 2003; Romeis, Dutton et al. 2004; Sisterson, Biggs et al. 2004; Bitzer, Rice et al. 2005; Pilcher, Rice et al. 2005; Torres and Ruberson 2005; Romeis, Meissle et al. 2006; Marvier, McCreedy et al. 2007; Chen, Zhao et al. 2008; Wolfenbarger, Naranjo et al. 2008), DP-32138-1 is unlikely to affect the animal or plant communities found in conventional corn production systems because of the lack of toxicity and allergenicity, and because there will be no change to agronomic practices caused by the cultivation of DP-32138-1corn.

Gene Movement

An environmental impact to consider as a result of planting this corn variety is the potential for gene flow (the transfer of genetic information between different individuals or populations). Pollen flow, or the movement of genes from one plant to another, occurs between plants that are sexually-compatible, or able to receive pollen at the appropriate time during the appropriate plant stage. Corn does not have sexually-compatible relatives found in 'natural' areas; in the U.S., corn is only able to reproduce with other cultivated corn plants (USDA-APHIS 2010).

Corn pollen moves by the wind (OECD 2003). Successful gene movement from one plant to another requires many different biological and physical factors, such as synchrony of flowering between corn fields, viability of pollen, and presence of physical barriers (OECD 2003); thus pollen movement is not equivalent to gene movement. A recent paper (Sanvido, Widmer et al. 2008) reviewed studies investigating gene flow and gene movement studies in corn grain production fields, and using the data found that the gene movement from GE corn to non-GE corn typically remained below 0.5% at 50m (approx. 164ft). In large scale studies, this same frequency of cross-fertilization events was similarly validated (Henry, Morgan et al. 2003; Weber, Bringezu et al. 2007).

One study found cross-fertilization rates higher at comparable distances than other studies (Jones and Brooks 1950). Jones and Brooks (1950) found successful gene movement to be as high as 2.5 % at 660ft. One potential reason for the discrepancy between this study and most other gene flow studies in corn may be a result of the type of corn used in this study. Jones and Brooks (1950) investigated the appropriate isolation distance for seed production in open-pollinated varieties, and not for hybrid varieties. Due the biology of open-pollinated varieties, these types of plants may be more receptive to pollen over a longer period of time than hybrid corn plants (Sanvido, Widmer et al. 2008), allowing for a greater chance of pollination events. Thus, the results from Jones and Brooks (1950) may overestimate the potential for cross-fertilization of hybrid corn plants.

<u>No Action: Gene Movement</u>

Under the No Action Alternative, DP-32138-1 would remain a regulated article and would require an APHIS permit or notification for release into the environment. Under regulated releases, regulated GE corn is typically separated from non-regulated corn by a distance of 660ft, based on distances set for seed production (AOSCA 2004), if distance is the only method used to prevent movement of pollen or genes. APHIS has concluded this separation distance is sufficient to substantially limit gene movement to other corn crops.

<u>Preferred Alternative: Gene Movement</u>

In 2009, GE corn was planted on 85% of all corn acres currently in production in the US, and has been steadily increasing (USDA-NASS 2009a). Concurrently, organic corn acreage has also increased by 9% over the 2 year period from 2005 to 2007 (USDA-ERS 2010a). Separation between the two types of corn varieties has been maintained by means of only recommended practices for organic growers and no mandated requirements for GE corn. Production of both organic and conventional corn will likely continue to increase with or without a determination of nonregulated status of DP-32138-1.

In agricultural systems, growers may choose to grow GE or non-GE corn, and obtain price premiums for growing these varieties of corn for particular markets (e.g., using organic methods for corn production or producing a specialty corn variety for particular processing needs). For example, in 2007, conventional corn averaged $4.19/bushel (USDA-NASS 2010b), whereas organic corn averaged $7.08/bushel (USDA-NASS 2010c). USDA asserts that agricultural practices that use conventional means, with GE or non-GE varieties, and organic production systems can all provide benefits to the environment, consumers, and farm income. Gene movement into and out of these specialized corn production systems have been managed using various types of buffer zones or isolation practices, such as differences in planting (which results in differences in flowering) or making sure fields are sufficiently distant from other compatible crops (such as using appropriate isolation distances).

In addition to the typical identity preservation and closed loop systems in place for specialty crops, those farmers using organic production also put into place measures to maintain purity of their crops. Typically, more than one method is used by farmers under organic production rules to prevent unwanted material from entering their fields including: isolation of the farm, physical barriers or buffer zones between organic production and non-organic production, and formal communications between neighboring farms (NCAT 2003). The plan used as the basis for organic certification should include a description of practices used to prevent or reduce the likelihood of unwanted substances, like GE pollen or seed, at each step in the farming operation, such as planting, harvesting, storing and transporting the crop (Riddle 2004; Krueger 2007; Kuepper, Born et al. 2007). Practical methods typically used by corn farmers will protect their crop and maximize their profits and premiums. The financial benefits accrue to organic corn produced using approved plans that are also successfully accomplished. For example, farmers using organic methods are requested to let neighboring farmers know that they are using organic production practices and request that the neighbors also help the farmer reduce contamination events (NCAT 2003; Krueger 2007). Thus, commonly used organic production practices for corn currently provide many measures that greatly reduce the likelihood of accidental gene flow. In addition, pollen containing the SPT transgene is not fertile, and gene flow between DP-32138-1 and non-GE corn fields is highly unlikely.

DP-32138-1 would be an additional GE corn variety that may be available to the seed production industry. The production area for DP-32138-1 seed is likely limited to less than 5000 acres if SPT is utilized only by Pioneer or no more than 20,000 acres if SPT is licensed to all seed corn producers in the U.S. Overall impacts of gene movement would be similar to the No Action Alternative.

Cumulative Effect: Gene Movement

DP-32138-1 would be an additional technology that may be available to the seed corn industry. The production area for DP-32138-1 seed is likely limited to less than 5000 acres if SPT is used only by Pioneer or no more than 20,000 acres if SPT is licensed to all seed corn producers in the U.S. The production of conventional, GE and organic corn is expected to continue. GE and organic acreage currently coexist, and there is no change in this coexistence with DP-32138-1 corn. The potential for movement of transgenes from GE crops into non GE varieties is not expected to change as a result of approving DP-32138-1.

Public Heath

Human Health

Under the FFDCA, it is the responsibility of food and feed manufacturers to ensure that the products they market are safe and properly labeled. Food and feed derived from DP-32138-1 must be in compliance with all applicable legal and regulatory requirements. GE organisms for food and feed may undergo a voluntary consultation process with the FDA prior to release onto the market.

No Action: Human Health

The FDA considers Pioneer's consultation on DsRed2 color marker protein, the only non-native corn protein in DP-32138-1 corn, and the consultation on the alpha-amylase ZM-AA1 to be complete (Appendix A). The status of the FDA consultation will not change under the No Action Alternative.

Preferred Alternative: Human Health

DP-32138-1 is genetically engineered to contain a sequence of DNA with three genes, *Ms45*, *zm-aa1*, and *DsRed2 (Alt1)*. *Ms45* is a corn gene that encodes the MS45 protein required for the production of fertile pollen and is controlled by a corn anther preferred promoter 5126. *zm-aa1* is a corn gene that encodes the ZM-AA1 alpha-amylase protein, which breaks down starch (Janeček 1994). *zm-aa1* is controlled by a corn polygalacturonase promoter (*Pg47*) that targets expression to pollen (Allen and Lonsdale 1993). When the ZM-AA1 protein is expressed in immature pollen, the depletion of starch reserves renders the pollen infertile. The *DsRed2* gene is from a marine coral-like anemone *Discosoma* sp. (Matz, Fradkov et al. 1999; Wasson-Blader 2001) that was modified by a single base pair substitution to produce *DsRed2(Alt1)*. The lipid transfer protein (*Ltp2*) promoter from barley targets expression to the aleurone layer of the seed (Kalla, Shimamoto et al. 1994). DP-32138-1 seeds expressing the DsRed2 color marker protein are colored pinkish-red, which will enable detection and sorting of transgenic red corn seeds from non-transgenic yellow seeds using a color sorting machine (Wasson-Blader 2001; Pioneer 2009).

The MS45 protein is a native corn fertility gene. The MS45 protein in DP-32138-1 was found only in the developing anther tissues during early pollen formation, the same location as conventional corn, and was not found in later stages of pollen formation or in other parts of the plant (Pioneer 2009). Pioneer also submitted to APHIS information on identity, function, characterization of genes, expression levels of gene products, as well as information on the potential allergenicity and toxicity of the expressed MS45 protein. APHIS' assessment of the safety of this product for humans and animals focuses on plant pest risk (USDA-APHIS 2010) and effects on wildlife and threatened and endangered species (section on Animals and Threatened and Endangered Species), and those analyses are based on the comparison of the GE-corn to its non-GE counterpart. No new issues appear to be associated with the *Ms45* transgene in DP-32138-1 corn.

In conventional corn, the alpha-amylase protein is expressed predominantly in the scutellum of germinating seed and minimal expression is observed in the developing endosperm. The endogenous corn alpha-amylase protein cannot be detected in non transformed corn pollen (Pioneer 2009). Alpha-amylases are ubiquitous in the environment, being naturally present in microorganisms, plants and animals (Janeček, Lévêque et al. 1999). Many types of commercial food processing, feed ingredient applications, and industrial applications also utilize alpha-amylase enzymes, including the production of fuel and potable alcohol (brewing, distillation processes), and corn syrups (Janeček, Lévêque et al. 1999; Lévêque, Janeček et al. 2000; Pariza and Johnson 2001; Olempska-Beer, Merker et al. 2006).

In DP-32138-1 corn, the ZM-AA1 protein is additionally found in the developing pollen and at low levels in other parts of the plant except at physiological maturity when it is below levels of quantitation (Pioneer 2009). Since the ZM-AA1 protein in DP-32138-1 is found in a new tissue location in corn, a new protein consultation was submitted to the FDA. Pioneer provided the FDA with information on identity, function, and characterization of the genes, as well the expression levels of the gene products. They also provided information on the potential allergenicity and toxicity of the expressed proteins. The FDA considers Pioneer's consultation on ZM-AA1 alpha-amylase protein to be complete (Appendix B). Pioneer also submitted information on identity, function, characterization of genes, expression levels of gene products, as well as information on the potential allergenicity and toxicity of the expressed proteins to APHIS. APHIS' assessment of the safety of this product for humans and animals focuses on plant pest risk (USDA-APHIS 2010) and effects on wildlife and threatened and endangered species (section on Animals and Threatened and Endangered Species). These analyses are based on the comparison of the GE-corn to its non-GE counterpart. No new issues appear to be associated with the *zm-aa1* transgene in DP-32138-1 corn.

The DsRed2 color marker protein is the only expressed transgenic protein in DP-32138-1 not derived from corn. In physiologically mature plants of DP-32138-1 corn, the DsRed2 protein is found in the seed, but not in leaves or pollen. In the growing plant, however, it is found in leaves (Pioneer 2009). Pioneer provided the FDA with information on identity, function, and characterization of the genes, as well the expression levels of the gene products. They also provided information on the potential allergenicity and toxicity of the expressed proteins. The FDA considers Pioneer's consultation on DsRed2 protein in DP-32138-1 to be complete (Appendix A). Pioneer also submitted information on identity, function, characterization of genes, expression levels of gene products, as well as information on the potential allergenicity and toxicity of the expressed proteins to APHIS. APHIS' assessment of the safety of this product for humans and animals focuses on plant pest risk (USDA-APHIS 2010) and effects on wildlife and threatened and endangered species (section on Animals and Threatened and Endangered Species), and those analyses are based on the comparison of the GE-corn to its non-GE counterpart. No new issues appear to be associated with the *DsRed2 (Alt1)* transgene in DP-32138-1 corn.

Based on the assessment of laboratory data provided by Pioneer in the submitted petition and an analysis of the scientific literature (USDA-APHIS 2010), along with the completion of the consultation process with FDA regarding DsRed2 color marker protein in DP-32138-1 (Appendix A), APHIS has concluded that under this alternative, the proposed action to approve nonregulated status for DP-32138-1 would have no significant impacts on human or animal health.

<u>Cumulative Effects: Human Health</u>

There are no significant impacts on human or animal health related to the No Action Alternative or a determination of nonregulated status of DP-32138-1 corn, and no cumulative effects have been identified.

Worker Safety

No Action: Worker Safety

During agricultural production of corn, farmers may be exposed to pesticides during application of these chemicals to crops. Under the No Action Alternative, exposure to these agricultural chemicals during corn production would remain the same. Under the No Action Alternative, workers continue to be exposed to safety hazards associated with farm equipment during detasseling operations.

Preferred Alternative: Worker Safety

Worker safety issues related to the use of pesticides during agricultural production of DP-32138-1 would remain the same as the No Action Alternative. As discussed under the issue of "Cropping Practices: Crop Rotation, Tillage, Production, and Pesticide Use", DP-32138-1 does not change the agronomic practices, or use of chemicals such as pesticides, associated with seed corn production. With DP-32138-1, detasseling would no longer be needed and therefore workers would no longer be exposed to safety hazards associated with farm equipment during detasseling operations.

Cumulative Effects: Worker Safety

Worker safety issues related to agronomic practices and the use of pesticides during agricultural production of DP-32138-1 would continue and remain the same under both alternatives. As discussed under the issue of "Pesticide Use", DP-32138-1 does not change the agronomic practices, or use of chemicals such as pesticides, associated with seed corn production. With DP-32138-1, detasseling would no longer be needed and therefore, workers would no longer be exposed to safety hazards associated with farm equipment during detasseling operations. There are no cumulative effects identified for this issue.

Animal Feed

More than 60 % of the corn produced in the US and much of the exported corn is used for animal feed (Hoeft, Nafziger et al. 2000). Under the FFDCA, it is the responsibility of feed manufacturers to ensure that the products they market are safe and properly labeled. Feed produced with DP-32138-1 technology must be in compliance with all applicable legal and regulatory requirements. GE organisms may undergo a voluntary consultation process with the FDA for feed safety prior to release onto the market.

No Action: Animal Feed

The FDA considers Pioneer's consultation on DsRed2 color marker protein, the only non-native corn protein in DP-32138-1 corn, to be complete (Appendix A). The FDA also considers Pioneer's consultation on ZM-AA1 alpha-amylase protein to be complete (Appendix B). This native corn protein is normally expressed in germinating seeds but in DP-32138-1 corn the modified amylase is expressed in the pollen. The status of the FDA consultations will not change under the No Action Alternative.

Preferred Alternative: Animal Feed

DP-32138-1 is genetically engineered to contain a sequence of DNA with three genes, *Ms45*, *zm-aa1*, and *DsRed2 (Alt1). Ms45* is a corn gene that encodes the MS45 protein required for the production of fertile pollen and is controlled by a corn anther preferred promoter 5126. *zm-aa1* is a corn gene that encodes the ZM-AA1 alpha-amylase protein, which breaks down starch (Janeček 1994). For a detailed description of each of gene and its impacts, please refer to the Human Health section above.

The FDA considers Pioneer's consultation on ZM-AA1 alpha-amylase protein in DP-32138-1 to be complete (Appendix B). Pioneer also submitted information on identity, function, characterization of genes, expression levels of gene products, as well as information on the potential allergenicity and toxicity of the expressed proteins to APHIS. APHIS' assessment of the safety of this product for animals focuses on plant pest risk (USDA-APHIS 2010) and effects on wildlife and threatened and endangered species (section on Animals and Threatened and Endangered Species), and those analyses are based on the comparison of the GE-corn to its non-GE counterpart. No new issues appear to be associated with the *zm-aa1* transgene in DP-32138-1 corn (Pioneer 2009).

The FDA considers Pioneer's consultation on DsRed2 protein in DP-32138-1 to be complete (Appendix A). Pioneer also submitted information on identity, function, characterization of genes, expression levels of gene products, as well as information on the potential allergenicity and toxicity of the expressed proteins to APHIS. APHIS' assessment of the safety of this product for animals focuses on plant pest risk (USDA-APHIS 2010) and effects on wildlife and threatened and endangered species (section on Animals and Threatened and Endangered Species), and those analyses are based on the comparison of the GE-corn to its non-GE counterpart. No new issues appear to be associated with the *DsRed2 (Alt1)* transgene in DP-32138-1 corn.

Based on the assessment of laboratory data provided by Pioneer in the submitted petition and an analysis of the scientific literature (USDA-APHIS 2010), along with the completion of the consultation process with FDA regarding DsRed2 color marker protein in DP-32138-1 (Appendix A), APHIS has concluded that under this alternative, a determination of nonregulated status of DP-32138-1 would have no significant impacts on animal feed, nor on animal health. Pioneer has communicated to APHIS (Weber, personal communication) that transgenic seed produced with the DP-32138-1 line is not intended for and is not expected to enter the general corn commodity supply. The procedures established by Pioneer will effectively prevent appearance of the SPT genes in corn commodities unless willful actions are taken to contradict company policy and practice.

Cumulative Effects: Animal Feed

There are no significant impacts on animal health related to the No Action Alternative or a determination of nonregulated status of DP-32138-1 corn, and no cumulative effects have been identified.

Socioeconomic Issues

Domestic Economic Environment at Risk

The U.S. domestic market for seed corn is estimated to be $4.6 billion in 2007 and $5.2 billion in 2008 based on the estimated seed costs per acre of $49 per acre in 2007 and $60 per acre in 2008 (USDA-ERS 2010b) and 93.5 million acres planted in 2007 and 86.0 million acres planted in 2008 (USDA-NASS 2010a).

The production of hybrid seed requires substantial control over the pollen sources. Detasseling is the most common procedure for pollen control. Tassels are removed by hand or by machine; both methods are expensive and result in major plant injury with accompanying lower seed yields. In 1988, detasseling costs ranged from $100 to $130 per acre (Wych 1988). Much of the cost of detasseling is personnel costs. Assuming that detasselers earn approximately minimum wages and based on minimum hourly wage rates of $3.35 in 1988 and $7.25 in 2009 (US-DOL 2010), it can be estimated that 2009 detasseling costs range from $216 to $281 per acre. Yield reduction due to detasseling has been estimated to range from 1.5% to 13.5% for hand detasseling and 2%-45% for mechanical detasseling (Wych 1988). To estimate cost of yield losses caused indirectly by detasseling, several assumptions can be made. For the production of any seed that requires detasseling, strict contracts are put in place with farmers who are willing to grow the low yielding inbred plants along with the challenges of growing corn to exacting planting, growing and harvesting protocols and schedules. In return for the complexity of growing seed corn, paying premiums and minimums are standard parts of the contract. The minimum standards are likely to be equivalent to the returns that would be expected from growing conventional corn, which would be based on the average yields in the area and the commodity corn price. For example, the average yield might be 170 bushels with a commodity price of $4.00 per acre [yields and prices based on approximate national averages for 2009 (USDA-NASS 2009a)] making the minimum standard $680 per acre. A 10% yield reduction from detasseling is approximately $68 per acre. Total costs of detasseling (cost of detasseling plus yield reduction) may be in the range of $280 to $350 per acre. The total industry wide cost of detasseling of up to 1 million acres is estimated at approximately $280-350 million. Spreading this total cost over 90 million acres of conventional corn production indicates that the detasseling costs are approximately $3 to $4 per planted acre of conventional corn production which is 6%-8% of the 2007 seed costs and 3%-7% of the 2008 seed costs.

<u>No Action: Domestic Economic Environment</u>

Under the No Action Alternative, DP-32138-1 would remain a regulated article and would require an APHIS permit or notification for release into the environment. Seed corn production methods would be the same as currently used under the No Action Alternative. Manual and mechanical detasseling are the major methods for controlling pollen in the hybrid seed corn production fields. No effects are anticipated in the commercial production of corn under the No Action Alternative.

<u>Preferred Alternative: Domestic Economic Environment</u>

Under the Preferred Alternative, the availability of seed corn would be determined by the market demand for commercial corn grain as it is under the No Action Alternative. However, under the Preferred Alternative, DP-32138-1 could be used to reduce the need for detasseling along with the associated cost of detasseling. This could lead to a yield increase due to a reduction of plant injury caused by mechanical detasseling. The yield increases would reduce the total number of acres devoted to seed corn production and would lower overall seed production costs. If all 1 million acres of seed increase used DP-32138-1 corn, the cost savings from avoiding detasseling operations and associated increased yields would most likely exceed $200 million. The estimated cost of detasseling is about $3-$4 per planted acre of conventional corn production which is equivalent to yield of about one bushel per acre in conventional corn production.

The cost savings from eliminating detasseling operations would provide seed companies with several options. One option is to keep their seed sales price unchanged from previous seasons to enhance their profit margin. Another option would be to pass along the entire cost savings by reducing the selling price of seed to the farmer. This option may help to gain company market share for the company. One other option is to keep the cost savings and invest the money in research to make better varieties. The method of dealing with these cost savings, whether it is a choice of one of these options or a combination is a business decision. The cost savings for DP-32138-1-produced seed corn will potentially be small ones for conventional corn growers, but the impact on conventional grain production levels or prices using DP-32138-1 would be smaller still and difficult to measure.

No estimate is available for the level of acceptance, if any, of using DP-32138-1 in seed corn production for the applicant or for the industry as a whole. Since 1995, three GE male sterility systems for corn have been proposed and deregulated by APHIS, but have not been used commercially (Pioneer 2009). Therefore the potential implementation of the commercial use of DP-32138-1 technology by the seed corn industry would be speculative.

The Seed Production Technology is a process that uses the DP-32138-1 SPT insertion to produce hybrid seed to contain no transgenes and without need for detasseling. To make male sterile inbreds (the female parent for hybrid production), the male fertile SPT maintainer line contributes pollen to a male sterile inbred line; virtually all SPT containing pollen grains are inviable, and thus will not be transferred to the seed for the male sterile female parent. 99.999% of seed from this cross is lacking the SPT insert. To eliminate any possible transgenic SPT seed, this seed is passed twice through a seed sorter (with an accuracy of detection of 99.95%). Two steps, non-transgenic pollen shed and seed sorting, result in a process that produces male sterile female inbreds that are predictably and reliably transgene free. These plants are then used by contract seed growers to produce commercial seed.

Production of the DP-32138-1 SPT maintainer line and production of the female inbred parent lines (Step I and II of Figure 1,) would be accomplished at Pioneer-controlled field sites or on those sites of other companies having rights to the technology (should Pioneer choose to license it). Thus, the transgenic lines would be under the continuous control of a seed technology provider. After each of these two steps, seed would be sorted by Pioneer into transgenic and non-transgenic seeds, with the discarding of all transgenic, marked seeds after Step II (Figure 1). Pioneer demonstrated that the efficiency of the SPT system in producing non transgenic seed was >99.999% (Pioneer 2009). The accuracy of the color-based sorting system for transgenic seed was >99.95%. Consequently, the likelihood that a transgenic seed would not be removed from the Step II selection would be the product of both the accuracy of detection and seed removal with the efficiency of the process, which would occur with a frequency approaching zero. One additional means of analyzing transgenic content was undertaken. When non transgenic machine-identified seed was sorted from marked transgenic seed, and the putative non transgenic seed was used to grow corn, leaf tissue was analyzed for the presence of transgenes. When DP-32138-1 was used as a pollen donor for male sterile female inbred production, seed was collected from this cross and sorted twice to remove any remaining DP-32138-1 corn seed. Plants grown from the 15,000 collected seed were analyzed using PCR techniques; none of the plants contained genes derived from the SPT insertion (Pioneer 2009).

The non-transgenic seed derived from the Step II increase (Figure 1) would be used to provide the male-sterile female plants for hybrid production. Although the DP-32138-1 line is genetically engineered, the progeny of DP-32138-1 that would be grown in Step III (last major increase before planting commercial grain fields) along with Step IV (commercial grain production) would reliably and predictably yield non-transgenic seed. The final phase of seed production, Step III, would typically be accomplished by contract growers, whose non-transgenic seed would be sold to seed suppliers for commercial production.

No information was found to determine whether growers and consumers of non-GE or organic corn would accept corn derived through GE technology, but now containing no transgenes. Therefore, for buyers who desire non-GE corn seed or commodity corn in the US, the marketing potential for seed produced from DP-32138-1 is unclear and speculative.

Cumulative Effects: Domestic Economic Environment

Assuming complete acceptance of the DP-32138-1 technology, cumulative domestic economic effects on seed corn producers would be cost savings of an estimated $200 million per year. Because the cost savings for DP-32138-1 seed corn are small with respect to all conventional corn production, using DP-32138-1 may have little measurable cumulative impact on conventional grain production levels or prices.

Trade Economic Environment at Risk

Seed corn and commercial corn grain are both major exports for the U.S. In 2005 (the most recent date with specific information on seed corn) seed corn exports totaled $136 million (USDA-FAS 2005). Corn is exported in bulk, as corn by-products, and feed. Since corn is used heavily as an animal feed for poultry, hogs and cattle, export of animal products could also be considered as related to corn production. Export of corn grain in bulk was $8.8 billion in 2009, $13.4 billion in 2008, and $9.8 billion in 2007 (USDA-ERS 2010c). Foreign customers in Europe and Japan may have concerns with a new variety of GE grain and new GE traits must undergo extensive regulatory assessment before they are approved for import (USGC 2006).

No Action: Trade Economic Environment

The availability of methods used to produce seed corn would be the same as currently used in seed corn production systems with detasseling being a major method for controlling pollen in the hybrid seed corn production fields. Since crop production practices are not anticipated to change, no effects are anticipated in the commercial production of corn under the No Action Alternative.

Preferred Alternative: Trade Economic Environment

Under the Preferred Alternative, the total supply of seed corn would be determined by the international market demand for commercial corn grain as it is under the No Action

Alternative. However, under the Preferred Alternative, DP-32138-1, savings would accrue to the seed producers in reducing resource outlays, and total cost of production and increasing seed crop yields (see discussion in preceding section, Preferred Alternative: Domestic Economic Environment). The level of acceptance of the technology is not easily predicted (see discussion in preceding section, Preferred Alternative: Domestic Economic Environment) which makes potential for impacts on trade unclear. Another difficulty is determining how international consumers and regulatory frameworks would categorize corn produced with GE technologies but now containing no transgenes. If DP-32138-1 is used to produce seed corn for export to those regions and countries with rigorous regulatory regimes, the potential impacts for international marketing of seed produced with DP-32138-1 is unclear and acceptance would be speculative. Because use of the DP-32138-1 cannot be detected or otherwise established in commercial corn seed, it is possible that use by seed providers would cause no impacts. Because export of seed corn is an important market for US companies, these providers would likely need to negotiate seed availability using the technology with international customers and regulatory systems well in advance of future sales abroad. Because use of the technology is elective, and seed providers could negotiate acceptance of the technology with these customers and regulatory systems, the potential impact of DP-32138-1 is not likely to be significant.

Cumulative Effects: Trade Economic Environment

There are no likely cumulative effects identified for this issue. Because the cost savings for DP-32138-1 seed corn are so small with respect to total conventional corn production, using DP-32138-1 may have little measurable impact on conventional grain production levels or prices for the international commodity markets.

Social Environment at Risk from Seed Production Activities

Conventional seed corn production relies heavily on detasseling as the method for pollen control and often rely heavily on thousands of field workers to accomplish this operation (Pioneer 2007). Detasseling jobs provide a source of income, but also has the potential to expose workers to safety risks associated with the use of heavy machinery used for seed corn detasseling operations.

No Action: Social Environment

The availability of agricultural methods used to produce seed corn would be the same as currently used in seed corn production systems under the No Action Alternative with detasseling being a major method for controlling pollen in the hybrid seed corn production fields. Detasseling operations would continue to primarily rely on thousands of field workers. Potential economic impacts would be unchanged.

Preferred Alternative: Social Environment

Under the preferred alternative, DP-32138-1 would be deregulated. If this technology is accepted commercially, all seed corn production acres that use this technology would no longer require detasseling as the method for pollen control. Depending on the level of acceptance by the seed corn production industry, field workers would no longer have detasseling as a possible source of income. The possible economic effects of losing these temporary jobs are unclear, but it is reasonable to assume that it would have a potential negative impact until another source of income could be secured.

Cumulative Effects: Social Environment

Depending on the level of acceptance by the seed corn production industry, thousands field workers would no longer have detasseling as a possible source of income. This impact is expected to be only a short term one as these workers secure other sources of income to replace those incomes lost.

Other Cumulative Effects

All potential cumulative effects regarding specific issues have been analyzed and addressed above. No further potential cumulative effects have been identified. Stacked varieties, those crop varieties that may contain more than one trait, are currently found in the marketplace and in agricultural production. In the event of a determination of nonregulated status of DP-32138-1, DP-32138-1 may be used to combine with non-GE and GE corn varieties by traditional breeding techniques, resulting in hybrids that, for example, may also be resistant to herbicides or insects. The resulting hybrids would not contain the SPT transgene.

APHIS' regulations at 7 CFR Part 340 do not provide for Agency oversight of GE corn varieties previously deregulated pursuant to Part 340 and the Plant Protection Act, nor over stacked varieties combining deregulated GE varieties unless it can be positively shown that such stacked varieties were to pose a likely plant pest risk. To date, none of the GE corn varieties that have been deregulated pursuant to Part 340 and the Plant Protection Act and used for commercial breeding program have been subsequently found to pose a plant pest risk.

There is no guarantee that DP-32138-1 will be stacked with any particular deregulated GE variety, as company plans and market demands play a significant role in those business decisions. Moreover, DP-32138-1 could even be combined with non-GE corn varieties. Thus, predicting all potential combinations of stacked varieties that could be created using both deregulated GE corn varieties and also non-GE corn varieties is hypothetical and purely speculative.

Threatened and Endangered Species

APHIS analyzed the potential for effects from cultivation of DP-32138-1 and its progeny on federally listed threatened and endangered species (TES) and species proposed for listing, as well as designated critical habitat and habitat proposed for designation, as required under Section 7 of the Endangered Species Act. Direct effects are analyzed by considering the response that TES could have if exposed to DP-32138-1 corn. Indirect effects are those that could result from the use of DP-32138-1 in corn production, would occur later in time, but are still reasonably certain to occur. Consideration is given for the potential of DP-32138-1 to change the baseline habitat of TES including critical habitat. If the analysis determines that a determination of nonregulated status of DP-32138-1 may affect listed species or critical habitat, consultation with the United States Fish and Wildlife Service (USFWS) or the National Marine Fisheries Service (NMFS) is required.

DP-32138-1 was developed solely to be used to produce male sterile inbred plants for the generation of hybrid corn seed that is not transgenic (Pioneer 2009). The carefully controlled expression of a seed color marker gene and pollen fertility and sterility genes allows for the generation of red transgenic seed for seed increase of male sterile-female inbred lines that are used in non transgenic hybrid commercial seed. DP-32138-1 contains a DNA insert with three genes- a male fertility gene, *Ms45*, from corn; an alpha-amylase gene, *zm-aa1*, from corn; and a fluorescent color marker protein gene, *DsRed2(Alt1)*, from a marine coral *Discosoma sp*., the only gene not native to corn.

DP-32138-1 is not genetically engineered to produce a toxin or pesticide, and is not genetically engineered to be tolerant to an herbicide. A NPC for the DsRed2 protein color marker was submitted to FDA on October 11, 2006 with the follow up letter of January 9, 2010 received from FDA (Appendix A). The FDA considers Pioneer's consultation on ZM-AA1 alpha-amylase protein to be complete (Appendix B).

If the seed corn industry completely converts to using this technology, the DP-32138-1 would be grown on a maximum of 20,000 acres (the total area currently used for corn seed production and 0.02% of all corn production acres in the U.S.). These 20,000 acres would likely be the same 20,000 acres used in conventional seed corn production that have been in agricultural production for many years. The same agronomic practices for conventional seed corn production would be used for DP-32138-1 production - including the same fertilizers, herbicides, fungicides, insecticides, irrigation, crop rotations, and tillage. Corn seed for planting produced using DP-32138-1 and the plants grown from these seed no longer have any of the transgenes. Therefore, the maximum exposed area is up to 20,000 acres in locations where agricultural seed corn production has historically occurred. There is no hazard associated with producing corn seed using the DP-32138-1 technology that would be different from production of conventional corn.

Corn is a feed commonly provided to many livestock and wildlife (e.g., birds, deer, and rodents). To identify the direct effects on listed animal species that could result from feeding, APHIS compared the composition and nutritional quality of DP-32138-1 corn, with a non-genetically engineered control corn line and the natural variation found in two commercial corn inbred lines. If the composition of DP-32138-1 inbred is similar to other commercial corn inbred plants, it is unlikely that DP-32138-1 would affect listed animal species, regardless of exposure.

The data presented in the petition suggests there is no difference in compositional and nutritional quality of DP-32138-1 compared to conventional corn, apart from the presence of DsRed2 protein (MS45 protein and ZM-AA1 alpha-amylase protein are both found in conventional corn). Although some of the variables measured by the applicant showed statistically significant differences between DP-32138-1 corn and the nontransgenic inbred controls (Pioneer 2009), none of the values for the grain composition characteristics were outside the range of natural variability of conventional corn as found in the International Life Sciences Institute Crop Composition Database (OECD 2003; Ridley, Shillito et al. 2004; ILSI 2008) or in the OECD consensus document on corn composition (OECD 2003). DP-32138-1 does not express additional proteins, natural toxicants, allelochemicals, pheromones, hormones, etc. that could directly or indirectly affect a listed TES or species proposed for listing. A toxicological safety assessment conducted on DsRed2 protein showed that it is unlikely to be a toxin or have any relevant similarities to known or putative allergens (Pioneer 2009). Thus, the composition and nutritional quality of DP-32138-1 is not biologically different than conventional corn, and would not be expected to affect TES differently.

Corn itself is not sexually compatible with any listed plant species; therefore there is no potential for a direct effect of DP-32138-1 on TES plants. Indirect effects of DP-32138-1 on listed plant species were also evaluated. Corn does not have sexually-compatible relatives found in 'natural' areas; corn is only able to reproduce with other corn plants in the U.S. (OECD 2003). As stated above, DP-32138-1 will have no effect on animals, including animals such as insects, bats or birds that may be pollinators of TES plants. Because corn cannot naturalize and would not affect pollinators, there are no expected indirect effects of DP-32138-1 on TES plants.

Cultivation of DP-32138-1 is not expected to differ from practices normally used for seed corn production or commercial corn production. DP-32138-1 corn plants with transgenes are expected to be grown only on a maximum of 20,000 acres (0.02% of all corn produced in the USA) and is expected to replace only conventional corn varieties currently grown for seed. The potential environmental impacts on TES from this product are those associated with typical corn seed production and commercial corn production, in areas where corn seed and commercial corn are typically produced, and therefore would not affect the baseline habitat of any listed species.

After reviewing possible effects of a determination of nonregulated status of DP-32138-1 corn, APHIS has not identified any stressor that could affect the reproduction, numbers, or distribution of a listed TES or species proposed for listing. As a result, a detailed exposure analysis for individual species is not necessary. APHIS has considered the effect of DP-32138-1 production on designated critical habitat or habitat proposed for designation and could identify no difference from effects that would occur from the seed production of other corn varieties. Therefore, APHIS has reached a conclusion that the release of DP-32138-1 corn, following a determination of nonregulated status, would have no effect on federally listed threatened or endangered species or species proposed for listing, nor would it affect designated critical habitat or habitat proposed for designation. Consequently, consultation with the USFWS or NMFS is not required for this action.

Consideration of Executive Orders, Standards and Treaties Relating to Environmental Impacts

Executive Order (EO) 12898 (US-NARA 2010), "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 or low-income communities from being subjected to disproportionately high and adverse human health or environmental effects. ***EO 13045 (US-NARA 2010), "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) required 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. Based on the information submitted by the applicant and assessed by APHIS, DP-32138-1 is not significantly different than conventional corn production. FDA has reviewed the introduced proteins (DsRed2 and ZM-AA1) in the unlikely case that the Event may inadvertently enter the commodity grain stream. Pioneer concluded that the proteins were safe for food and feed use (Pioneer 2009) and FDA agreed that the presence of the proteins would not lead to safety concerns when present in low levels in the food supply. Therefore, DP-32138-1 is not expected to have a disproportionate adverse effect on minorities, low-income populations, or children. Based on historical experience with corn production and the data submitted by the applicant and assessed by APHIS, DP-32138-1 should eliminate the need for detasseling, which in turn would increase worker safety by significantly reducing the number of seasonal workers exposed to the hazards of working around heavy farm machinery.

EO 13112 (US-NARA 2010), "Invasive Species", states that Federal agencies take action to prevent the introduction of invasive species, to provide for their control, and to minimize the economic, ecological, and human health impacts that invasive species cause. Both non-GE and GE corn varieties that have been previously deregulated pursuant to Part 340 and the Plant Protection Act are widely grown in the U.S. Based on historical experience with corn and the data submitted by the applicant and assessed by APHIS, DP-32138-1 plants are very similar in fitness characteristics to other corn varieties currently grown and are not expected to become weedy or invasive (USDA-APHIS 2010).

EO 13186 (US-NARA 2010), "Responsibilities of Federal Agencies to Protect Migratory Birds", states that Federal agencies taking actions that have, or are likely to have, a measurable negative effect on migratory bird populations are directed to develop and implement, within 2 years, a Memorandum of Understanding (MOU) with the Fish and Wildlife Service that shall promote the conservation of migratory bird populations. Data submitted by the applicant has shown no difference in compositional and nutritional quality of DP-32138-1 compared to conventional corn, apart from the presence of DsRed2 protein. The migratory birds that occasionally forage in corn fields are unlikely to contain high amounts of DP-32138-1 as corn grain availability is limited by seed germination and harvest. Based on APHIS' assessment of DP-32138-1 it is unlikely that a determination of nonregulated status of this corn variety will have a negative effect on migratory bird populations.

Impacts on Unique Characteristics of Geographic Areas.

There are no unique characteristics of geographic areas such as park lands, prime farm lands, wetlands, wild and scenic areas, or ecologically critical areas that would be adversely impacted by a determination of nonregulated status of DP-32138-1 corn. The common agricultural practices that would be carried out under the proposed action will not cause major ground disturbance, do not cause any physical destruction or damage to property, do not cause any alterations of property, wildlife habitat, or landscapes, and do not involve the sale, lease, or transfer of ownership of any property. This action is limited to a determination of nonregulated status of DP-32138-1 corn. The product will be deployed on a limited number of acres of agricultural farm land used for corn seed production which may be focused in a small number of sites where Pioneer and future potential licensees may produce seed corn, but potential for such production may exist where corn is grown in all US states, except Alaska. Progeny of this variety that express the identified traits of the DP-32138-1 corn will be retained by Pioneer or licensed users, and those progeny derived from production of hybrid commercial corn will be predictably and reliably without transgenes. This action would not convert land use to nonagricultural use and therefore would have no adverse impact on prime farm land. Standard agricultural practices for land preparation, planting, irrigation, and harvesting of plants would be used on agricultural lands planted to DP-32138-1 corn including the use of EPA registered herbicides. Applicant's adherence to EPA label use restrictions for all pesticides will mitigate potential impacts to the human environment. In the event of a determination of nonregulated status of DP-32138-1 corn, the action is not likely to affect historic or cultural resources, park lands, prime farmlands, wetlands, wild and scenic rivers, or ecologically critical areas that may be in close proximity to corn production sites.

International Implications

EO 12114 (US-NARA 2010), "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 U.S. in the event of a determination of nonregulated status of DP-32138-1 corn. 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 trade of DP-32138-1 subsequent to a determination of nonregulated status for the product would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed under the ***International Plant Protection Convention*** (IPPC 2010).

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" (IPPC 2010); the protection it affords extends to natural flora and plant products and includes both direct and indirect damage by pests, including weeds. The IPPC set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (172 countries as of March 2010). In April 2004, a standard for pest risk analysis (PRA) 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 genetically engineered 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.

Compliance with Clean Water Act and Clean Air Act

This EA evaluated the changes in corn production due to the unrestricted use of DP-32138-1 corn. DP-32138-1 will not lead to the increased production of corn in U.S. agriculture. There is no expected change in water use due to the production of DP-32138-1 compared to current corn seed production regimes, nor is it expected that air quality will change because of production of DP-32138-1.

National Historic Preservation Act (NHPA) of 1966 as Amended

The NHPA of 1966, and its implementing regulations (36 CFR 800), requires Federal agencies to: 1) determine whether activities they propose constitute "undertakings" that has the potential to cause effects on historic properties; and 2) if so, to evaluate the effects of such undertakings on such historic resources and consult with the Advisory Council on Historic Preservation (i.e., State Historic Preservation Office, Tribal Historic Preservation Officers), as appropriate.

A determination of nonregulated status of DP-32138-1 corn will not adversely impact cultural resources on tribal properties. Any farming activities that may be taken by farmers on tribal lands are only conducted at the tribe's request; thus, the tribes have control over any potential conflict with cultural resources on tribal properties.

A determination of nonregulated status of DP-32138-1 corn would have no impact on districts, sites, highways, structures, or objects listed in or eligible for listing in the National Register of Historic Places, nor would they likely cause any loss or destruction of significant scientific, cultural, or historical resources. This action is limited to a determination of non-regulated status of DP-32138-1 corn. Standard agricultural practices for land preparation, planting, irrigation, and harvesting of plants would be used on these agricultural lands including the use of EPA registered pesticides. Applicant's adherence to EPA label use restrictions for all pesticides will mitigate impacts to the human environment.

A determination of nonregulated status of DP-32138-1 corn is not an undertaking that may directly or indirectly cause alteration in the character or use of historic properties protected under the NHPA. In general, common agricultural activities conducted under this action do not have the potential to introduce visual, atmospheric, or audible elements to areas in which they are used that could result in effects on the character or use of historic properties. For example, there is potential for audible effects on the use and enjoyment of a historic property when common agricultural practices, such as the operation of tractors and other mechanical equipment, are conducted close to such sites. A built-in mitigating factor for this issue is that virtually all of the methods involved would only have temporary effects on the audible nature of a site and can be ended at any time to restore the audible qualities of such sites to their original condition with no further adverse effects. Additionally, these cultivation practices are already being conducted throughout the corn production regions. The cultivation of DP-32138-1 corn does not inherently change any of these agronomic practices so as to give rise to an impact under the NHPA.

V. References

Agarwala, S. C., P. N. Sharma, et al. (1981). "Development and enzymatic changes during pollen development in boron deficient maize plants." Journal of Plant Nutrition **3**: 329-336.

Albertsen, M. C., T. W. Fox, et al. (1993). "Tagging, cloning, and characterizing a male fertility gene in maize." American Journal of Botany Suppl **80**: 16.

Allen, R. L. and D. M. Lonsdale (1993). "Molecular characterization of one of the maize polygalacturonase gene family members which are expressed during late pollen germination." Plant Journal **3**(2): 261-271.

Aneja, V. P., W. H. Schlesinger, et al. (2009). "Effects of Agriculture upon the Air Quality and Climate: Research, Policy, and Regulations." Environ. Sci. Technol. **43**: 4234–4240.

AOSCA (2004). Association of Official Seed Certifying Agencies Operational Procedures, Association of Official Seed Certifying Agencies.

AOSCA (2008). "99% Non-GMO corn grain program requirements Association of Official Seed Certifying Agencies." Retrieved March 11, 2010, from http://www.identitypreserved.com/handbook/aosca-nongmocorn.htm.

AOSCA (2010). "General IP Protocols Standards.". Retrieved November 8, 2010, from http://www.identitypreserved.com/handbook/aosca-general.htm.

APHIS-BRS (2010). "Petitions of Nonregulated Status Granted or Pending by APHIS as of February 2, 2010." Retrieved February 26, 2010, from http://www.aphis.usda.gov/brs/not_reg.html.

Aylor, D. E., N. P. Schultes, et al. (2003). "An aerobiological framework for assessing cross-pollination in maize." Agricultural and Forestry Meteorology **119**: 111-129.

Bais, H. P., T. L. Weir, et al. (2006). "The role of root exudates in rhizosphere interactions with plants and other organisms." Annual Review of Plant Biology **57**: 233-266.

Baker, J., R. Southard, et al. (2005). "Agriculture dust production in standard and conservation tillage system in the San Joaquin Valley." J. Environ. Qual. **34**: 1260-1269.

Bastida, F., J. L. Moreno, et al. (2009). "Soil metaproteomics: a review of an emerging environmental science. Significance, methodology and perspectives." European Journal of Soil Science **60**: 845–859.

Bhadula, S. K. and V. K. Sawhney (1989). "Amylolytic activity and carbohydrate levels during the stamen ontogeny of a male-fertile, and a 'Gibberellin-Sensitive" male-sterile mutant of tomato
(Lycopersicon esculentum)." Journal of Experimental Botany **40**: 789-794.

Bitzer, R. J., M. E. Rice, et al. (2005). "Biodiversity and community structure of epadaphic and euedaphic springtails (Collembola) in transgenic rootworm Bt corn." Environmental Entomology **34**: 1346-1376.

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

Brewbaker, J. L. (1964). Agricultural Genetics. Englewood Cliffs, NJ, Prentice-Hall.

Brewbaker, J. L. (1971). Pollen enzymes and isozymes. . Pollen Development and Physiology. e. Heslop-Harrison. London, Butterwors**:** 156-170.

Brock, R. (2007). Plant Corn On CRP Land? Hay and Forage Grower. Minneapolis, MN

Brooks, D. R., D. A. Bohan, et al. (2003). "Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. I. Soil-surface-active invertebrates." Philosophical Transactions of the Royal Society of London B **358**: 1847-1862.

Byron, E. (2002) Detasseling, a Midwest Rite of Passage, Faces Extinction. Mindfully.org

Campos, F., J. Xavier-Filho, et al. (1989). "Resolution and partial characterization of proteinases and α-amylases from midguts of larvae of the bruchid beetle Callosobruchus maculatus (F.)." Comparative Biochemistry and Physiology B **92**: 51-57.

Casselman, J. (2010). "Evaluating Your Crop Plan." Retrieved March 11, 2010, from http://ccaontario.com/FCKEditor/File/Evaluating%20Your%20Crop%20Plan.pdf.

Castro, A. J. and C. Clement (2007). "Sucrose and starch catabolism in the anther of Lilium during its development: a comparative study among the anther wall, locular fluid and microspore/pollen fractions." Planta **225**(6): 1573-1582.

Chen, M., J.-Z. Zhao, et al. (2008). "A critical assessment of the effects of Bt transgenic plants on parasitoids." PLoS ONE **3**(5): e2284. doi:2210.1371/journal.pone.0002284.

Cole, C. V., J. Duxbury, et al. (1997). "Global estimates of potential mitigation of greenhouse gas emissions by agriculture." Nutrient Cycling in Agroecosystems **49**: 221–228.

Cristofoletti, P., A. Ribeiro, et al. (2001). "Apocrine secretion of amylase and exocytosis of trypsin along the midgut of Tenebrio molitor larvae." Journal of Insect Physiology **47**: 143-155.

Daum, D. R. (1996). Soil Compaction and Conservation Tillage. Penn State Conservation Tillage Series. University Park, PA, Pennsylvania State University College of Agricultural Sciences Cooperative Extension.

Di Giovanni, F., P. G. Kevan, et al. (1995). "The variability in settling velocities of some pollen and spores." Grana **34**: 39-44.

Dietrich, C. and E. Maiss (2002). "Red fluorescent protein DsRed from Discosoma sp. as a reporter protein in higher plants. ." Biotechniques **32**(2): 286-293.

Dolbeer, R. A. (1990). "Ornithology and integrated pest management: red-winged blackbirds Agleaius phoeniceus and corn." The International Journal of Avian Science **132**: 309-322.

Erickson, B. and J. Lowenberg-DeBoer (2005). Weighing the returns of rotated vs. continuous corn, Purdue University.

Farnham, D. (2001). Corn Planting Guide. D. Marks, Iowa State University University Extension.

Fausay, J. C., J. J. Kells, et al. (1997). "Giant foxtail interference in non-irrigated corn." Weed Science **45**: 256-260.

Fawcett, R. and S. Caruana (2001). Better Soils, Better Yields A Guidebook to Improving Soil Organic Matter and Infiltration. West Lafayette, IN. , Conservation Technology Information Center.

Fernandez-Cornejo, J. (2004). The Seed Industry in U.S. Agriculture: An Exploration of Data and Information on Crop Seed Markets, Regulation, Industry Structure, and Research and Development, United States Department of Agriculture, Economic Research Service.

Figueiredo, M., T. F. Fernandes, et al. (2008). "A novel viral vector for expressing DsRed2 fluorescent protein in noradrenergic rat brainstem neurones." Proceedings of The Physiological Society (Poster communication, pc32).

Flessa, H., U. Wild, et al. (1998). "Nitrous oxide and methane fluxes from organic soils under agriculture." European Journal of Soil Science **49**: 327–335.

Freibauer, A., M. D. A. Rounsevell, et al. (2004). "Carbon sequestration in the agricultural soils of Europe." Geoderma **122**: 1-23.

Fulton, M. and K. Giannakas (2001). "Agricultural Biotechnology and Industry Structure." AgBioForum **4**(2): 137-151.

Gelderman, R. (2009). Soil compaction probable in South Dakota fields this year. Brookings, SD, Agbio Communications Unit South Dakota State University.

Giannessi, L. P. (2005). "Economic and herbicide use impacts of glyphosate-resistant crops." Pest Management Science **61**: 241-245.

Greenland, A., P. Bell, et al. (1997). Reversible male sterility: A novel system for the production of hybrid corn. Control of plant development: Genes and signals. G. AJ, EM, Meyerowitz, M, Steers, eds. Cambridge, UK, Company of Biologists Ltd.**:** 141-147.

Gustafson, M. (2008). Detasseling. Flak Magazine

Hansen Jesse, L. C. and J. J. Obrycki (2000). "Field deposition of Bt transgenic corn pollen: lethal effects on the monarch butterfly." Oecologia **125**: 241-248.

Haughton, A. J., G. T. Champion, et al. (2003). "Invertebrate responses to the management of genetically modified herbicide-tolerant and conventional spring crops. II. Within-field epigeal and aerial arthropods. ." Philosophical Transactions of the Royal Society of London B **358**: 1863-1877.

Havey, M. J. (2004). The use of cytoplasmic male sterility for hybrid seed production. Molecular Biology and Biotechnology of Plant Organelles. . D. H, C, Chase, eds. The Netherlands, Springer Publisher**:** 617-628.

Hawes, C., A. J. Haughton, et al. (2003). "Responses of plants and invertebrate trophic groups to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide-tolerant crops." Philosophical Transactions of the Royal Society of London B **358**: 1899-1913.

Heap, I. (2008). "International survey of herbicide resistant weeds." Retrieved March 11, 2010, from www.weedscience.com.

Henry, C., D. Morgan, et al. (2003). Farm scale evaluations of GM crops: monitoring gene flow from GM crops to non-GM equivalent crops in the vicinity—Part I. Forage maize, Central Science Laboratory Sand Hutton, Centre for Ecology and Hydrology.

Hoeft, R. G., E. D. Nafziger, et al. (2000). Modern Corn and Soybean Production. Champaign, IL, MCSP Publications.

Holloway, M. (2010). "Holloway's Detasseling: How is Corn Detasseling done?". Retrieved March 30, 2010, from http://hollowaysdetasseling.com/index.htm.

ILSI (2008). "International Life Sciences Institue Crop Composition Database, 3.0.". Retrieved March 30, 2010, from http://www.cropcomposition.org/.

IPCC (2007). 14.4.4.4. Agriculture, forestry and fisheries IPPC Fourth Assessment Report Climate Change 2007: Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, 2007. M. L. Parry, O. F. Canziani, J. P. Palutikof, P. J. van der Linden and C. E. Hanson. Cambridge United Kingdom and New York, NY, USA Intergovernmental Panel on Climate Change.

IPPC (2010). "Official web site for the International Plant Protection Convention: International Phytosanitary Portal ". Retrieved March 30, 2010, from https://www.ippc.int/IPP/En/default.jsp.

Iserman, K. (1993). "Agriculture's share in the emission of trace gases affecting the climate and some cause-oriented proposals for sufficiently reducing this share." Environmental Pollution **83**: 95-111.

Jach, G., E. Binot, et al. (2001). "Use of red fluorescent protein from Discosoma sp. (DsRed) as a reporter for plant gene expression. ." Plant Journal **28**(4): 483-491.

Janeček, Š. (1994). "Sequence similarities and evolutionary relationships of microbial, plant and animal α-amylases." Eur. J. Biochem. **224**: 519-524.

Janeček, Š., E. Lévêque, et al. (1999). "Close evolutionary relatedness of α-amylases from archaea and plants." Journal of Molecular Evolution **48**: 421-426.

Jones, M. D. and J. S. Brooks (1950). Effectiveness of distance and border rows preventing outcrossing in corn. Stillwater, Okla, Oklahoma Agricultural Experimental Station.

Kalaitzandonakes, N., J. M. Alston, et al. (2007). "Compliance costs for regulatory approval of new biotech crops." Nature Biotechnology **25**(5).

Kalaitzandonakes, N. and M. L. Hayenga (1999). Structural Change in the Biotechnology and Seed Industrial Complex: Theory and Evidence. Transitions in AgBiotech: Economics of Strategy and Policy Conference. Washington, DC.

Kalaitzandonakes, N. and A. Magnier (2004). "Biotech Labeling Standards and Compliance Costs in Seed Production." Choices: A Publication of the American Agricultural Economics Association **2**.

Kalla, R., K. Shimamoto, et al. (1994). "The promoter of the barley aleurone-specific gene encoding a putative 7 kDa lipid transfer protein confers aleurone cell-specific expression in transgenic rice." Plant Journal **6**(6): 849-860.

Kamp, T., H. Steindl, et al. (2001). "Monitoring trace gas fluxes (N2O, CH4) from different soils under the same climatic conditions and the same agricultural management." Phyton - Annales Rei Botanicae **41**: 119-130.

Kegley, S. E., B. R. Hill, et al. (2010). "PAN Pesticide Database." Retrieved March 2010, 2010, from http:www.pesticideinfo.org.

Kok, H., R. K. Taylor, et al. (1996). AF-115 Soil Compaction Problems and Solutions. Manhatten, KS, Cooperative Extension Service.

Krueger, J. E. (2007). "If your farm is organic, must it be GMO free? Organic farmers, genetically modified organisms and the law ". Retrieved March 11, 2010, from http://www.flaginc.org/topics/pubs/arts/OrganicsAndGMOs2007.pdf.

Kuepper, G. (2002). "Organic field corn production. National Sustainable Agricultural Information Service." Retrieved March 11, 2010, from http://attra.ncat.org/attra-pub/fieldcorn.html.

Kuepper, G., H. Born, et al. (2007). "Organic systems plan (OSP) templates for certifiers." Retrieved March 11, 2010, from http://ww2.attra.ncat.org/index.php/OSPtemplates.html

Lal, R. (2003). "Soil erosion and the global carbon budget." Environment International **29**: 437-450.

Lal, R. and J. P. Bruce (1999). "The potential of world cropland soils to sequester C and mitigate the greenhouse effect." Environmental Science & Policy **2**: 177-185.

Lévêque, E., Š. Janeček, et al. (2000). "Thermophilic archaeal amylolytic enzymes." Enzyme and Microbial Technology **26**: 3-14.

Marshall, E. J. P., V. K. Brown, et al. (2003). "The role of weeds in supporting biological diversity within crop fields." European weed research society **43**: 77-89.

Marvier, M., C. McCreedy, et al. (2007). "A meta-analysis of effects of Bt cotton and maize on nontarget invertebrates." Science **316**: 1475-1477.

Matz, M. V., A. F. Fradkov, et al. (1999). "Fluorescent proteins from non-bioluminescent Anthozoa species." Nature Biotechnology **17**: 969–973.

Mirabella, R., C. Franken, et al. (2004). "Use of the fluorescent timer DsRED-E5 as reporter to monitor dynamics of gene activity in plants." Plant Physiology **135**: 1879-1887.

Monsanto (2010). "Innovation and the Competitive Seed Market." Retrieved April 22, 2010, from http://www.monsanto.com/monsanto_today/for_the_record/innovation_and_the_competitive_seed_market.asp.

Morris, M. L. (1998). Overview of the world maize economy. Maize seed industries in developing countries. . M. L. Morris. Boulder, Colo and Mexico, Lynne Rienner Publishers, Inc. and CIMMYT, Int.

NCAT (2003). "NCAT's organic crops workbook: a guide to sustainable and allowed practices." Retrieved March 30, 2010, from http://attra.ncat.org/attra-pub/PDF/cropsworkbook.pdf.

NCGA (2007). "Truths about Water Use, Corn, and Ethanol." Retrieved February 26, 2010, from http://www.ncga.com/files/GetTheFactsOnWaterUse.pdf.

NCGA (2010). "Know Before You Grow." Retrieved March 4, 2010, from http://www.ncga.com/know-you-grow.

Neilsen, B. (2005). "Symptoms of deer corn damage." Retrieved March 11, 2010, from http://www.ppdl.purdue.edu/PPDL/weeklypics/1-10-05.html.

Nielsen, R. L. and P. R. Thomison (2002). Late-Planted Corn and Seeding Rates, Purdue University Department of Agronomy.

NSFC-IPM (2010). "Crop Profiles and Timelines." Retrieved January 13, 2010, from http://www.ipmcenters.org/cropprofiles/index.cfm.

OECD (2003). "Consensus Document on the Biology of Zea Mays Subsp. Mays (Maize).". from http://www.olis.oecd.org/olis/2003doc.nsf/LinkTo/NT0000426E/$FILE/JT00147699.PDF.

Olempska-Beer, Z. S., R. I. Merker, et al. (2006). "Food-processing enzymes from recombinant microorganisms - a review." Regulatory Toxicology and Pharmacology **45**: 144-158.

Olson, R. A. and D. H. Sander (1988). Corn Production. Corn and corn improvement. G. F. S. a. J. W. Dudley. Madison, WI, American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc.**:** 639-686.

Ortiz, B. (2010). Weekly National Grain Market Review. St Joseph, MO, USDA-MO Dept of Ag Market News Service.

OTA (2010). "Organic Trade Association." Retrieved April 22, 2010, from http://www.ota.com/index.html.

Pariza, M. W. and E. A. Johnson (2001). "Evaluating the safety of microbial enzyme preparations used in food processing: update for a new century. ." Regulatory Toxicology and Pharmacology **33**: 173–186.

Patterson, M. P. and L. B. Best (1996). "Bird abundance and nesting success in Iowa CRP fields: the important of vegetation structure and composition." American Midland Naturalist **135**: 153-167.

Pilcher, C. D., M. E. Rice, et al. (2005). "Impact of transgenic Bacillus thuringiensis corn and crop phenology on five nontarget arthropods." Environmental Entomology **34**: 1302-1316.

Pioneer (2007). "Increased Demand for Corn Increases Demand for Field Workers." Retrieved March 30, 2010, from http://www.pioneer.com/web/site/portal/menuitem.e920f5adeba7ab243cda47b2d10093a0/.

Pioneer (2008). "DuPont Reaffirms Expectations for North America Seed Corn Market Share." Retrieved March 30, 2010, from http://www.pioneer.com/web/site/portal/menuitem.3838193e84e5d111828d9b26d10093a0/.

Pioneer (2009). Petition for the Determination of Nonregulated Status for Maize 32138 SPT Maintainer Used in the Pioneer Seed Production Technology (SPT) Process. Submitted by N. Weber, Registration Manager. Pioneer Hi-Bred International, Inc., Johnston, IA (See Table: http://www.aphis.usda.gov/biotechnology/not_reg.html)

Pioneer (2010). "Corn." Retrieved March 4, 2010, from http://www.pioneer.com/web/site/portal/products/corn/.

Pollak, L. M. and P. J. White (1995). "Corn as a food source in the United States. I. Historical and current perspectives." Cereal Foods World **40**: 749-754.

Ponsard, S., A. P. Gutierrez, et al. (2002). "Effect of Bt-toxin (Cry1Ac) in transgenic cotton on the adult longevity of four heteropteran predators." Environmental Entomology **31**: 1197-1205.

Raimbaud, E., A. Buleon, et al. (1989). "Hydrophobic cluster analysis of the primary sequences of α-amylases." International Journal of Biological Macromolecules **11**: 217-225.

Raynor, G. S., E. C. Ogden, et al. (1972). "Dispersion and deposition of corn pollen from experimental sources." Agronomy Journal **64**: 420-427.

Riddle, J. A. (2004). Best management practices for producers of GMO and non-GMO crops, University of Minnesota, School of Agriculture.

Ridley, W. P., R. D. Shillito, et al. (2004). "Development of the International Life Sciences Institue Crop Composition Database." Journal of Food Composition and Analysis **17**: 423-438.

Roegge, M. (2010). The Effect of Soil Compaction on the 2010 Crop. Urbana-Champaign, Illinois University of Illinois Press Release. **2010**.

Romeis, J., A. Dutton, et al. (2004). "Bacillus thuringiensis toxin (Cry1Ab) has no direct effect on larvae of the green lacewing Chrysoperla carnea (Stephens) (Neuroptera: Chrysopidae)." Journal of Insect Physiology **50**(2-3): 175-183.

Romeis, J., M. Meissle, et al. (2006). "Transgenic crops expressing Bacillus thuringensis toxins and biological control." Nature Biotechnology **24**: 63-71.

Rooney, L. W. and S. O. Serna-Saldivar (1987). Food uses of whole corn and dry-milled fractions. Corn: chemistry and technology. S. A. Watson and P. E. Ramstad. St. Paul, MN, American Association of Cereal Chemists, Inc. **:** 399-429.

Roy, D. B., D. A. Bohan, et al. (2003). "Invertebrates and vegetation of field margins adjacent to crops subject to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide-tolerant crops." Philosophical Transactions of the Royal Society of London B **358**: 1879-1898.

Sanvido, O., F. Widmer, et al. (2008). "Definition and feasibility of isolation distances for transgenic maize cultivation." Transgenic Research **17**: 317-335.

Sawyer, J. (2007) Nitrogen fertilization for corn following corn. Integrated Crop Management (IC) **498**

Schernikau, P. (2010). "Schernikau Detasseling." Retrieved March 30, 2010, from http://schernikaudetasseling.net/site2/.

Schnitkey, G. (2008). Late Planting and Crop Insurance Decisions, Farmdoc, University of Illinois.

Shaw, R. H. (1988). Climate requirement. Corn and corn improvement. G. F. Sprague, Dudley, J. W. Madison, Wisconsin, American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America**:** 609-638.

Silva, C., W. Terra, et al. (1999). "Digestion in larvae of Callosobruchus maculatus and Zabrotes subfasciatus (Coleoptera:Bruchidae) with emphasis on α-amylases and oligosaccaridases." Insect Biochemistry and Molecular Biology **29**: 355-366.

Sisterson, M. S., R. W. Biggs, et al. (2004). "Arthropod abundance and diversity in Bt and non-Bt cotton fields." Environmental Entomology **33**: 921-929.

Skibbe, D. S. and P. S. Schnable (2005). "Male sterility in maize." Maydica **50**: 367-367.

Sleper, D. A. and J. M. Poehlman (2006). Breeding Corn (Maize). Breeding Field Crops, Blackwell Publishing**:** 277-296.

Stallman, H. R. and L. B. Best (1996). "Small-mammal use of an experimental strip intercropping system in northeastern Iowa. ." American Midland Naturalist **135**: 226-273.

Steffey, K. L., M. E. Rice, et al. (1999). Handbook of corn insects. Lanham, Maryland, Entomological Society of America.

Sterner, R. T., B. E. Petersen, et al. (2003). "Impacts of small mammals and birds on low-tillage, dryland crops." Crop Protection **22**: 595-602.

Stuitje, A. R., E. C. Verbree, et al. (2003). "Seed-expressed fluorescent proteins as versatile tools for easy (co)transformation and highthroughput functional genomics in Arabidopsis. ." Plant Biotechnology Journal **1**: 301-309.

Sundstrom, F. J., J. Williams, et al. (2002). "Identity Preservation of Agricultural Commodities " Agricultural Biotechnology in California Series.

Thomas, T. (2007). "It's still all about yields: how can the seed sector respond to biofuels?" Seed World(February 2007): 26-27.

Tilghman, G. (2009). Wet harvest could cause soil compaction. Glasgow Daily Times. Glasgow, KY.

Torres, J. B. and J. R. Ruberson (2005). "Canopy- and ground-dwelling predatory arthropods in commerical Bt and non-Bt cotton fields: patterns and mechanisms." Environmental Entomology **34**: 1242-1256.

UK (2010). "The Corn/Soybean Planting Decision Aid." Retrieved April 26, 2010, from http://www.uky.edu/Ag/AgEcon/pubs/software/cornvsoybean.html.

Unger, E., S. Betz, et al. (2001). "Selection and orientation of adjacent genes influences DAM-mediated male-sterility in transformed maize." Transgenic Research **10**: 409-422.

Unger, E., M. A. Cigan, et al. (2002). "A chimeric ecdysone receptor facilitates methoxyfenozide-dependent restoration of male-fertility in ms45 maize." Transgenic Research **11**: 455-465.

US-DOL (2010). "History of Federal Minimum Wage Rates Under the Fair Labor Standards Act, 1938 - 2009." Retrieved March 30, 2010, from http://www.dol.gov/whd/minwage/chart.htm.

US-EPA (2010). "Draft 2010 Inventory of Greenhouse Gas Emissions and Sinks Executive Summary." Retrieved March 30, 2010, from http://www.epa.gov/climatechange/emissions/.

US-FDA (2010). "The FDA List of Completed Consultations on Bioengineered Foods." Retrieved January 4, 2010, from http://www.fda.gov/Food/Biotechnology/Submissions/default.htm.

US-NARA (2010). "Executive Orders disposition tables index." Retrieved March 11, 2010, from http://www.archives.gov/federal-register/executive-orders/disposition.html.

USDA-AMS (2010). "National Organic Program." Retrieved April 22, 2010, from http://www.ams.usda.gov/AMSv1.0/nop

USDA-APHIS (2010). Plant Pest Risk Assessment for DP-32138-1 Corn, USDA.

USDA-ERS (2005). "Agricultural chemicals and production technology: soil management." from http://www.ers.usda.gov/Briefing/AgChemicals/soilmangement.htm#importance.

USDA-ERS (2008). "Data sets: organic production." Retrieved March 30, 2010, from http://www.ers.usda.gov/Data/Organic/.

USDA-ERS (2010a). "USDA Long-Term Agricultural Projection Tables." Retrieved March 11, 2010, from http://usda.mannlib.cornell.edu/usda/ers/94005/2010/index.html.

USDA-ERS (2010b). "Commodity Costs and Returns: Data." Retrieved March 30, 2010, from http://www.ers.usda.gov/Data/CostsAndReturns/testpick.htm.

USDA-ERS (2010c). "Foreign Agricultural Trade of the United States." Retrieved April 2009, 2010, from http://www.ers.usda.gov/Data/FATUS/.

USDA-FAS (2005). "Planting Seed Trade Statistics & Analysis." Retrieved March 30, 2010, from http://www.fas.usda.gov/seeds/seedstats.html.

USDA-NASS (2008b). "Crops Harvested from Irrigated Farms: 2008 and 2003." 2008 Farm and Ranch Irrigation Survey. Retrieved March 16, 2010, from http://www.agcensus.usda.gov/Publications/2007/Online_Highlights/Farm_and_Ranch_Irrigation_Survey/index.asp.

USDA-NASS (2009a). "Acreage." Retrieved March 4, 2010, from http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1000.

USDA-NASS (2009b). "United States Summary and State Data." 2007 Census of Agriculture. Retrieved March 11, 2010, from http://www.agcensus.usda.gov/Publications/2007/Full_Report/Volume_1,_Chapter_1_US/usv1.pdf.

USDA-NASS (2010a). "Crop Production 2009 Summary." Retrieved March 4, 2010, from http://usda.mannlib.cornell.edu/usda/current/CropProdSu/CropProdSu-01-12-2010.pdf.

USDA-NASS (2010b). "Quick Stats." Retrieved March 30, 2010, from http://www.nass.usda.gov/QuickStats/indexbysubject.jsp?Pass_group=Crops+%26+Plants.

USDA-NASS (2010c). "Organic Production Survey (2008)." Retrieved March 11, 2010, from http://www.agcensus.usda.gov/Publications/2007/Online_Highlights/Organics/ORGANICS.pdf.

USDA-NASS (2010d). "The Census of Agriculture." Retrieved January 11, 2010, from http://www.agcensus.usda.gov/.

USGC (2006). "Value-enhanced corn report 2005/2006." Retrieved February 26, 2010, from http://www.agmrc.org/media/cms/USGC_Value_Enhanced_Corn_Report_200_08C7959C2B1E6.pdf.

USGC (2008). "Corn." Retrieved March 16, 2010, from http://www.grains.org/corn.

Vercauteren, K. C. and S. E. Hygnstrom (1993). White-tailed deer home range characteristics and impacts relative to field corn damage. Great Plains Wildlife Damage Control Workshop. Internet Center for Wildlife Management. Lincoln, University of Nebraska**:** 217-219.

Vogel, J. R., M. S. Majewski, et al. (2008). "Pesticides in rain in four agricultural watersheds in the United States." J Environ. Qual. **37**: 1101-1115.

Wakhle, D. M., R. P. Phadke, et al. (1983). "Study on enzyme activities of pollen and honey bees (Apis cerana Fab.)." Indian Bee Jounal **45**: 3-5.

Wasson-Blader, T. (2001). "Living Colors™ DsRed2: Improved red fluorescent protein for use in living cells." Clontechniques **16**(3): 2-3.

Weber, W. E., T. Bringezu, et al. (2007). "Coexistence between GM and non-GM maize crops—tested in 2004 at the field scale level (Erprobungsanbau 2004)." Journal of Agronomy and Crop Science **193**: 79-92.

Wenck, A., C. Pugieux, et al. (2003). "Reef-coral proteins as visual, non-destructive reporters for plant transformation. ." Plant Cell Reports **22**: 244-251.

White, P. J. and L. M. Pollak (1995). "Corn as a food source in the United States. II: Processes, products, composition, and nutritive values. ." Cereal Foods World **40**: 756-762.

Wolfenbarger, L. L., S. E. Naranjo, et al. (2008). "Bt crop effects on functional guilds of non-target arthropods: a meta-analysis." PLoS ONE **3**(5): e2118. doi:2110.1371/journal.pone.0002118.

Wych, R. D. (1988). Production of hybrid seed corn. Corn and Corn Improvement. G. F. a. D. Sprague, J. W. Madison, WI, American Society of Agronomy**:** 565-607.

VI. Appendix A

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
College Park, MD 20740

JAN 29 2010

Ms. Natalie Weber
Registration Manager
Pioneer Hi-Bred International, Inc., a DuPont Company
DuPont Agriculture and Nutrition
P.O. Box 80353
Wilmington, DE 19880-0353

Re: NPC 00004: red fluorescent protein, DsRed2

Dear Ms. Weber:

This letter is in response to Pioneer Hi-Bred International, Inc.'s (Pioneer) early food safety evaluation of the protein, a red fluorescent protein, DsRed2, expressed in a new plant variety under development for seed selection, which you submitted to the Food and Drug Administration (FDA) on October 11, 2006, under FDA's guidance to industry, "Recommendations for the Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use" (71 FR 35688; June 21, 2006, and available on the FDA home page at http://www.fda.gov - follow the hyperlinks from the "Food" topic to the "Biotechnology" program area). As used in the guidance and in this letter, the term "food" refers to both human food and animal feed. All materials relevant to this evaluation have been placed in a file designated NPC 00004. This file will be maintained in the Office of Food Additive Safety in the Center for Food Safety and Applied Nutrition.

In cases of inadvertent low level presence in the food supply of a new food plant variety, FDA believes that any food or feed safety concern would be limited to the safety of the new protein(s) in that plant (generally, the potential allergenicity and toxicity of the new protein(s)). Based on Pioneer's early food safety evaluation, it is our understanding that Pioneer has concluded that DsRed2 protein would not raise food safety concerns when it is in a new food plant variety that is present at low levels in the food supply. We have completed our evaluation of your submission, and we have no questions at this time regarding Pioneer's conclusion.

Sincerely yours,

Antonia Mattia

Antonia Mattia, Ph.D.
Division Director
Division of Biotechnology
and GRAS Notice Review
Center for Food Safety
and Applied Nutrition

VII. Appendix B

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
College Park, MD 20740

JAN 29 2010

Ms. Natalie Weber
Registration Manager
Pioneer Hi-Bred International, Inc., a DuPont Company
DuPont Agriculture and Nutrition
P.O. Box 80353
Wilmington, DE 19880-0353

Re: NPC 00011: modified maize alpha-amylase, ZM-AA1

Dear Ms. Weber:

This letter is in response to Pioneer Hi-Bred International, Inc.'s (Pioneer) early food safety evaluation of the protein, a modified maize alpha-amylase, ZM-AA1, expressed in a new plant variety under development for seed selection, which you submitted to the Food and Drug Administration (FDA) on June 23, 2009, under FDA's guidance to industry, "Recommendations for the Early Food Safety Evaluation of New Non-Pesticidal Proteins Produced by New Plant Varieties Intended for Food Use" (71 FR 35688; June 21, 2006, and available on the FDA home page at http://www.fda.gov - follow the hyperlinks from the "Food" topic to the "Biotechnology" program area). As used in the guidance and in this letter, the term "food" refers to both human food and animal feed. All materials relevant to this evaluation have been placed in a file designated NPC 00011. This file will be maintained in the Office of Food Additive Safety in the Center for Food Safety and Applied Nutrition.

In cases of inadvertent low level presence in the food supply of a new food plant variety, FDA believes that any food or feed safety concern would be limited to the safety of the new protein(s) in that plant (generally, the potential allergenicity and toxicity of the new protein(s)). Based on Pioneer's early food safety evaluation, it is our understanding that Pioneer has concluded that ZM-AA1 protein would not raise food safety concerns when it is in a new food plant variety that is present at low levels in the food supply. We have completed our evaluation of your submission, and we have no questions at this time regarding Pioneer's conclusion.

Sincerely yours,

Antonia Mattia, Ph.D.
Division Director
Division of Biotechnology
and GRAS Notice Review
Center for Food Safety
and Applied Nutrition

VIII. Appendix C

Description of Seed Production Technology (SPT)

(from **Pioneer 2009**; all figure and table references are to the Petition figures and tables)

I. Introduction to Seed Production Technology (SPT)

I-B. Maize Hybrid Seed Production

Hybrid maize (*Zea mays* L.) is grown on more than 80 million acres in the United States (USDA-National Agricultural Statistics Service (NASS), 2008), making it the largest producer of hybrid maize. The seed used to produce hybrid maize is generated by crossing two unique inbred parental lines referred to as the 'male inbred parent' (the plant that is the pollinator) and 'female inbred parent' (the plant on which the seed is produced).

For inbred parent seed increase, male and female inbred parental lines are grown separately and allowed to self-pollinate in Pioneer fields that are reproductively isolated from other maize plants in order to maintain genetic purity and increase the quantity of the inbred parents (Figure 1). In order to produce hybrid seed, male and female inbred parent lines are inter-planted in adjacent rows (*e.g.,* 2 rows male, 4 rows female, 2 rows male) in reproductively isolated contractor fields and allowed to open pollinate.

To produce pure hybrid seed, the male inbred parent must cross pollinate the female inbred parent and the female inbred parent must be prevented from self-pollinating. The most common practice to accomplish this is to cut off the pollen-bearing tassels (*i.e.,* detasseling) on the female inbred parent (Figure 1).

In the hybrid seed production fields, the ears of the detasseled female inbred parent can only be fertilized with pollen from the male inbred parent planted in adjacent rows. The male inbred parent is removed from the fields before the ears reach maturity. Thus, the seed produced on the female seed parent will be uniformly hybrid. The F1 hybrid seed is harvested, cleaned, conditioned, and bagged for sale to growers. When planted in growers' fields, the hybrid seed will produce a uniform F1 crop. The grain harvested from growers' fields is used for feed and fuel production, processed into food products and industrial materials, or exported.

I-C. Maize Fertility Control in Hybrid Maize Production

I-C.1. Detasseling

The physical removal of the pollen-producing tassels (detasseling) has been the most widely used method of preventing female inbred parent self-pollination since maize hybrids were first produced in the 1920s (Wych, 1988). Less common or experimental methods include the application of chemical sterilants, the use of biological systems based on cytoplasmic male-sterility, and systems to control male-fertility using biotechnology (Refer to Appendix 1 for additional information on these systems).

Detasseling can be accomplished manually or mechanically with tassel-cutting or tassel-pulling machines. Thousands of temporary workers are employed to detassel maize hybrid seed production fields. The detasseling process is not entirely reliable, and on occasion a female plant will escape complete detasseling. For example, natural variation in plant development can result in the emergence of tassels after the detasseling process is complete or a mechanical detasseler can fail to remove a tassel completely. Incomplete detasseling results in pollen shed by the female inbred parent plant and self-pollination. When this occurs, seed of the female inbred parent is harvested along with the hybrid seed which results in reduced seed quality, lowered trait purity, and lost yield potential. Mechanical detasseling is faster than hand detasseling, but causes more damage to the plants and results in up to 40% reduction in seed yield (Wych, 1988). Today, a combination of mechanical and hand detasseling is often used; no form of detasseling is entirely satisfactory. Therefore, a need exists for an alternative method of pollen control that is safe, efficient, and highly effective.

I-C.2. Genetic Male-Sterility

Male-sterility in maize refers to the failure to produce functional pollen. Male-sterile maize plants can neither self-pollinate, nor sib-pollinate neighboring plants and can serve as female inbred parents in the production of hybrid maize seed. Male-sterility traits display either nuclear (also known as genetic) or cytoplasmic inheritance (Refer to Appendix 1 for details).

Genetic male or pollen sterility in maize was first described by Eyster (1921) that it is due to a single recessive gene, designated as *ms*. Since then, many different genetic male-sterility loci have been identified in maize. The chromosome number and map position of nearly all presently identified male-sterility genes are well characterized (Neuffer *et al*., 1997). Sterility phenotype is determined by a single gene and the allele for male-sterility is recessive (*ms*), although a small number of dominant male-sterile alleles have been described (Neuffer *et al*., 1997). In maize, a male-sterility plant trait is expressed only by maintenance of a homozygous recessive (*ms/ms*) condition for the fertility gene.

Limitations to the Use of Homozygous Recessive (*ms/ms*) Lines as Male-Sterile Parents

Increase of male-sterile (*ms/ms*) inbred parent seed is not possible *via* self-pollination as the homozygosity of the recessive gene (*ms*) results in male-sterility.

One way to increase the seed of the male-sterile inbred parent is to cross-pollinate male-sterile plants (*ms/ms*) with male-fertile plants which are heterozygous for the male-sterility gene (*Ms/ms*). The progeny from such a cross-breeding are approximately fifty percent male-sterile (*ms/ms*) and fifty percent male-fertile (*Ms/ms)*. Since the male-sterile seed can not be separated from the male-fertile seed, this is not a practical way to increase the male-sterile parent seed.

Recovery and maintenance of pure male-sterile (*ms/ms*) inbred populations using traditional breeding methods requires: a) additional maize lines for the maintenance of the male-sterility trait, and b) several intermediate crosses and selection methods that are time consuming. Therefore, it is not practical for use in commercial hybrid maize production.

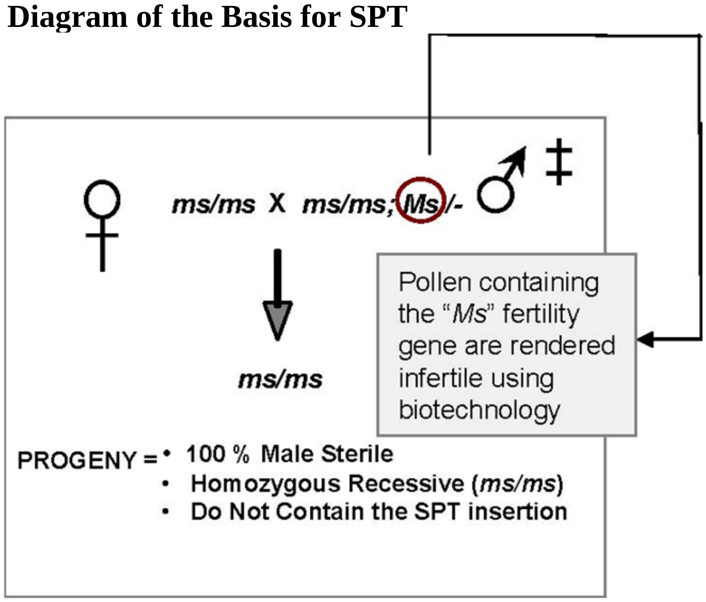
Thus, a need exists for a convenient and effective process for generating male-sterile inbred female parent lines.

I-C.3. Basis for the Development of Seed Production Technology

Section I-C.2 described how cross-pollinating the male-sterile (*ms/ms*) plants with heterozygous (*Ms/ms*) male-fertile plants yields a mix of male-sterile and male-fertile genotypes. However, if all pollen containing the fertility restoration gene (*Ms*) could be eliminated from the heterozygous male parent (*Ms/ms*) thereby ensuring pollination of male-sterile (*ms/ms*) parent only with pollen carrying the recessive *ms* allele, the male-sterile progeny would retain their homozygous (*ms/ms*) condition and could be used as male-sterile female inbred parent in hybrid seed production. This is the basis for the Pioneer Seed Production Technology (SPT) process utilizing a transgenic 32138 SPT maintainer (Figure 2).

Pioneer's novel SPT process generates male-sterile female inbred parent lines that maintain the homozygous recessive state (*ms/ms*) without any intermediate crosses. Male-sterile female inbred parent seed can be multiplied, while maintaining the homozygous recessive state of the male-sterility gene and providing for full restoration of fertility in the F1 hybrid plants grown in commercial fields.

Figure 2. Schematic Diagram of the Basis for SPT



‡ = Represents 32138 SPT maintainer.

The SPT construct containing three gene cassettes [*Ms45, zm-aa1, and DsRed2(Alt1)*] was inserted into a male-sterile maize line that is homozygous recessive for *ms45* (*ms45/ms45*) shown as *ms/ms* in Figure 2. The SPT insertion was integrated in the genome at a locus that segregates independently from the endogenous *ms45* locus (data not shown). Therefore, 32138 SPT maintainer is homozygous for the *ms45* recessive allele and hemizygous for the recombinant *Ms45* gene from the SPT insertion, indicated as *Ms/-* in Figure 2. A single copy of *Ms45* gene from the 32138 SPT insertion restores fertility in the male-sterile (*ms45/ms45*) line (Figure 7B). 32138 SPT maintainer sheds two different types of pollen in a 1:1 ratio: a) non-transgenic fertile pollen (with the *ms45* allele) that do not contain the SPT insertion, and b) transgenic infertile* pollen (with both the *ms45* allele and the transgenic *Ms45* allele) that contain the 32138 SPT insertion (Figure 5). The transgenic pollen carrying *Ms45* are rendered infertile due to the α-amylase gene (Refer to Sections 1-D and 1-H.2 for more details) in the SPT insertion (Figure 9).

*= Pollen infertility refers to the inability of pollen to germinate and fertilize the ovule.

Therefore, homozygous recessive (*ms45/ms45*) male-sterile female inbred parent seed can be increased by utilizing the pollen from an isogenic SPT maintainer (Figure 3, Step II and Figure 5).

I-D. Description of the SPT Process

Seed Production Technology is a process rather than a trait or a product. The SPT process works across all germplasm, requires no detasseling, and produces hybrid seed that are fully fertile. The male-sterile female inbred parent increase process is entirely controlled by Pioneer and the total acreage in the US planted to the transgenic 32138 SPT maintainer and SPT derived male-sterile female inbred parent seed increase each year will be less than 5,000 acres.

32138 SPT Maintainer

The SPT process uses a transgenic 32138 SPT maintainer in an *ms45/ms45* genetic background (Figure 3, Step I). The 32138 insertion in SPT maintainer contains three gene expression cassettes essential for the functioning of the SPT system: *Ms45*, *zm-aa1*, and *DsRed2(Alt1)* (Figure 6).

The 32138 SPT maintainer is homozygous for the endogenous *ms45* recessive mutant allele and hemizygous for the SPT insertion (*i.e.*, *ms45/ms45; SPT/-*) and therefore contains a single copy of the *Ms45* gene. Expression of a single copy of *Ms45* gene in the *ms45/ms45* genetic background restores male-fertility and enables pollen production in the 32138 SPT maintainer.

The 32138 SPT maintainer sheds two different types of pollen in a 1:1 ratio: a) fertile, does not contain SPT insertion, and b) infertile, does contain 32138 SPT insertion (Figure 5). Pollen containing the 32138 SPT insertion is rendered infertile due to the action of ZM-AA1 α-amylase protein. Expression of ZM-AA1 α-amylase in 32138 SPT maintainer pollen results in the depletion of starch (Figure 9) and depriving the SPT pollen of the energy reserves required for successful pollen germination and fertilization. Hence, any pollen containing the 32138 SPT insertion is made infertile.

A fluorescent color marker (*DsRed2(Alt1)*) gene is linked to the other two SPT genes (*Ms45 and zm-aa1*) and it confers a pinkish red phenotype to any seed expressing the 32138 SPT insertion (Figures 5 and 10). The DsRed2 protein exhibits a high fluorescent intensity and emits a strong red fluorescence under appropriate illumination so seeds containing the SPT insertion can be readily detected and separated from the progeny seeds that do not contain the 32138 SPT insertion in a highly efficient manner using mechanical color sorting (Figure 3, Color Sort A and B; Refer to Appendix 3).

Step I: 32138 SPT Maintainer Seed Increase

32138 SPT maintainer seed is replicated *via* self-pollination (Figure 3 Step I and Figure 4). The SPT insertion is transmitted maternally. Upon self-pollination, 32138 SPT maintainer produces two different types of seed (yellow: pinkish red) in a 1:1 ratio (Figures 5 and 11A). Yellow seed is non-transgenic for SPT (does not contain the 32138 SPT insertion). Pinkish red seed that fluoresces a bright red color under appropriate illumination is transgenic for SPT (contains the 32138 SPT insertion).

Color Sort A

Self-pollinated 32138 SPT maintainer seed containing a mixture of yellow and pinkish red (SPT maintainer) seed in a 1:1 ratio is passed thru mechanical color sorters twice (Refer to Appendix 3 for details) to separate the 32138 SPT maintainer seed from the yellow seed (Figure 3, Color Sort A and Figure 4). Color sorters detect the fluorescent red 32138 SPT maintainer seed and separate it from the yellow/non-transgenic for SPT seed. Pure seed of 32138 SPT maintainer is then available for the propagation of non-transgenic male-sterile female inbred parent seed (Figure 3, Step I). Therefore, color sort A is for the purification of 32138 SPT maintainer seed. Yellow seed collected from color sort A will be discarded (Refer to Appendix 3 for more details).

Step II: Male-Sterile Female Inbred Parent Seed Increase

For seed increase of male-sterile (*ms45/ms45*) female inbred parent, 32138 SPT maintainer will be planted in either a 2:2 or 4:4 or 4:2 planting pattern (male-sterile female: 32138 SPT maintainer rows). Fertile pollen (that does not contain the 32138 SPT insertion and carry the *ms45* recessive allele) from the 32138 SPT maintainer plants will cross-pollinate and fertilize nearly isogenic male-sterile (*ms45/ms45*) female inbred parent plants (Figure 3, Step II and Figure 5). The inbred seed produced on the male-sterile female inbred plants will maintain their homozygous recessive (*ms45/ms45*) state and genetic purity. All male-sterile progeny seed harvested will be yellow in color and will not contain the 32138 SPT insertion (Figure 5 and Refer to Appendix 4 for more details).

Color Sort B

The SPT system is highly genetically efficient (Refer to Appendix 4 and 5) and virtually all of the fertile pollen from 32138 SPT maintainer should be non-transgenic for SPT and therefore all of the male-sterile inbred progeny seed made using the pollen from 32138 SPT maintainer should also be non-transgenic for SPT. The purpose of the color sort B is to ensure the purity of the male-sterile female inbred parent by removing any seeds of 32138 SPT maintainer. Each batch of male-sterile female inbred parent seed is passed twice through the color sorter (Figure 3, Color Sort B and Figure 5). This step helps to assure that no seed of the 32138 SPT maintainer will be planted in the F1 hybrid seed fields that produce seed sold to growers (Figure 3, Step III).

Steps III. Commercial Maize Hybrid Seed Production

Commercial maize hybrid seed production (Figure 3, Steps III) using the male-sterile progeny seed derived using SPT technology will take place normally. The purity of the male-sterile female inbred progeny derived from the SPT process is insured by passing the seed twice through the mechanical color sorters (Refer to Appendix 3 for details on the color sorter). Therefore, the F1 hybrid seed harvested and sold to growers for commercial grain production is non-transgenic for SPT and the resulting F1 hybrid plants are fully fertile.

Steps IV. Commercial Maize Grain Production

Commercial maize grain production using the F1seed generated using the male-sterile female inbred parent will take place normally. The F1 hybrid plants grown in the growers' fields are non-transgenic for SPT and therefore, the grain harvested from growers' fields is also non-transgenic for SPT (Figure 3, Step IV).