Pioneer Hi-Bred International, Inc.
High Oleic 305423 Soybean

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Draft Environmental Assessment

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I. Purpose & Need

"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 varieties) can provide benefits to the environment, consumers, and farm income.

In 1986, the Federal Government's Office of Science and Technology Policy (OSTP) published a policy document known as the Coordinated Framework for the Regulation of Biotechnology. This document specifies three Federal agencies that are responsible for regulating biotechnology in the U.S.: USDA's APHIS, the U.S. Department of Health and Human Services' Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA). APHIS regulates genetically engineered (GE) organisms under the Plant Protection Act of 2000. FDA regulates GE organisms under the authority of the Federal Food, Drug, and Cosmetic Act. 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. Products are regulated according to their intended use and some products are regulated by more than one agency. 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). Together, these agencies ensure that the products of modern biotechnology are safe to grow, safe to eat, and safe for the environment. USDA, EPA, and FDA enforce agency-specific regulations to products of biotechnology that are based on the specific nature of each GE organism.

The APHIS Biotechnology Research 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 genetically engineered 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 genetically engineered (GE) organisms and products. A GE organism is no longer subject 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 sufficient information to determine if the GE organism is unlikely to pose a plant pest risk.

A person may petition the agency to evaluate submitted data and determine that a particular regulated article is unlikely to pose a plant pest risk, and, therefore, should no longer be regulated under 7 CFR 340.6 entitled "Petition for determination of nonregulated Status." 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. If the agency determines that the regulated article is unlikely to pose a plant pest risk, the GE organism will be granted deregulated status.

Pioneer Hi-Bred International, Inc. (Pioneer) of Johnston, IA submitted a petition to APHIS seeking a determination of non-regulated status for their transgenic high oleic acid soybean event DP-3Ø5423-1 (hereafter referred to as Pioneer 305423 soybean) (Pavely, 2007). According to Pioneer, their 305423 soybean is engineered to produce increased amounts of monounsaturated fatty acid (oleic) and decreased amounts of polyunsaturated fatty acids (linoleic and linolenic) and to lesser extent, saturated fatty acid (palmitic acid). This soybean is also engineered to express a new protein, a modified soybean acetolactate synthase. The modified soybean acetolactate synthase was used as a selectable marker for transformation. The Pioneer 305423 soybean is currently regulated under 7 CFR part 340. Interstate movements and field trials of Pioneer 305423 soybean have been conducted under permits issued or notifications acknowledged by APHIS.

Under the authority of 7 CFR part 340, APHIS has the responsibility for the safe development and use of genetically engineered organisms under the provisions of the Plant Protection Act. APHIS must respond to petitioners that request a determination of the regulated status of genetically engineered organisms, including genetically engineered crop plants such as Pioneer 305423 soybean. If a petition for nonregulated status is submitted, APHIS must make a determination if the genetically engineered organism is unlikely to pose a plant pest risk.

As a Federal agency subject to compliance with the National Environmental Policy Act (NEPA) (42 U.S.C. 4321 *et seq.*), APHIS has prepared this environmental assessment (EA) to consider the potential environmental effects of this proposed action (granting nonregulated status) and the reasonable alternatives to that action consistent with NEPA regulations (40 CFR parts 1500-1508, 7 CFR 1b, and 7 CFR part 372). This EA has been prepared in order to specifically evaluate the effects on the quality of the human environment[1] that may result from the deregulation of Pioneer 305423 soybean.

The Pioneer 305423 soybean is designed for human and animal consumption and as such, may also be subject to regulation by Food and Drug Administration (FDA). FDA policy 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 biotechnology-derived food. Pioneer submitted a summary of its safety and nutritional assessment to FDA for Pioneer 305423 soybean in 2007. Pioneer

[1] Under NEPA regulations, the "human environment" includes "the natural and physical environment and the relationship of people with that environment" (40 CFR §1508.14)

concluded that, with the exception of the intended change in fatty acid composition, the 305423 soybean and the foods and feeds derived from it are not materially different in composition, safety, or any other relevant parameter from soybeans now grown, marketed, and consumed. In January 2009, FDA considered Pioneer's consultation on the 305423 soybean to be completed regarding the safety and nutritional assessment for Pioneer 305423 soybean and had no further questions regarding the safety of Pioneer 305423 soybean (FDA, 2009). To view the text of the FDA's scientific and regulatory assessment response for Pioneer 305423 soybean refer to http://www.fda.gov/Food/Biotechnology/Submissions/ucm155595.htm.

Public Involvement

APHIS-BRS routinely seeks public comment on draft environmental assessments prepared in response to petitions to deregulate GE organisms. APHIS-BRS does this through a notice published in the Federal Register. 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. Comments received by the end of the 60-day period will be analyzed and used by APHIS to determine if the petition to deregulate the Pioneer 305423 soybean should be granted.

II. Introduction

Pioneer has developed a transgenic soybean line "Pioneer 305423 Soybean" that produces soybean seeds with increased levels of monounsaturated (oleic) fatty acid, decreased levels of polyunsaturated fatty acids (linoleic and linolenic) and decreased levels of palmitic acid (Pavely, 2007). In addition, Pioneer 305423 soybean also contains a slightly modified version of a soybean acetolactate synthase gene. The expression of the modified version of a soybean acetolactate synthase protein can increase the inherent tolerance level to the ALS-inhibiting class of herbicides. This trait is intended for selecting and identifying this high oleic soybean bioengineered event, rather than as a separate commercial trait as this version of the gene does not confer commercial levels of herbicide tolerance in Pioneer 305423 soybean. Pioneer indicated there is no plan to commercially promote Pioneer 305423 soybean as tolerant to sulfonylurea herbicides (Pavely, 2007).

Genetic modification of the fatty acid composition of soybean oil has been one of the major goals of many soybean breeders over the last 50 years. The levels of polyunsaturated fatty acids are one of the major factors influencing the quality of vegetable oils. Soybean oils rich in monounsaturated fatty acids could provide improved commercial value.

Unmodified soybean oil has poor oxidative stability due to its unstable chemical structure and naturally occurring levels of polyunsaturated fatty acids. Polyunsaturated fatty acids increase rancidity compared with saturated and monounsaturated fatty acids, especially

after prolonged contact with oxygen, light or heat. This characteristic reduces product stability and shelf life. Hydrogenation is a chemical process that improves stability and shelf life necessary for food application; however, hydrogenation has the undesirable consequences of creating *trans*-fatty acids.

In recent years, *trans*-fatty acid have come under considerable scrutiny because of their negative affects on human health. On July 9, 2003, the Food and Drug Administration (FDA) issued a regulation requiring manufacturers to list *trans*-fatty acids, or *trans* fat, on the Nutrition Facts panel of foods and some dietary supplements (FDA, 2003). With this rule, consumers have more information to make food choices that could lower their consumption of *trans*-fat as part of a heart-healthy diet.

USDA APHIS has previously granted nonregulated status to a high oleic soybean variety (USDA-APHIS, 1997) developed by DuPont in which the high oleic phenotype was conferred by introduction of the soybean omega-6 desaturase gene 1 (*fad2-1* gene). Those DuPont high oleic soybean varieties received regulatory approval in Australia, Canada, Japan, New Zealand, and the United States.

Pioneer 305423 soybean has been field tested in the United States since 2005 as authorized by APHIS. Associated notifications acknowledged and permits issued by APHIS are listed in Figure 23 (Pavely, 2007, p.67). The list compiles a total of 13 test sites in diverse regions of the U.S. and Canada including the major soybean growing area of the Midwest and winter nurseries in Hawaii. 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 as the agency assesses 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, 2009).

III. Affected Environment

A. Soybean

The soybean (*Glycine max* (L.) Merr.) is an economically important leguminous crop, providing oil and protein. Soybean plants are grown for their seed, which is further processed to yield oil and meal. Soybean is ranked number one in oil production (56%) among the major oil seed crops production in the world (Soy Stats, 2008). Other expanding uses for soybeans in the U.S. include soy biodiesel, animal agriculture, exports, and edible soybean oil (USB, 2007). Increased public focus on data supporting the human health benefits of soybeans could create more consumer demand, and will be examined further in the Environmental Consequences section of this EA.

The OECD Consensus Document (OECD, 2000) provides detailed information about the crop biology of soybean. The genus *Glycine* is divided into two subgenera, *glycine* and

soja. The subgenus *soja* consists of three annual species: *G. soja* Sieb. and Zucc., the wild form of soybean, *G. gracilis* Skvortz., the weedy form of soybean and *G. max*, which is the cultivated soybean. These species do not exist naturally in the United States (USDA-NRCS, 2008). Soybean lacks sexually compatible wild relatives in the United States and its territories. Therefore, there is no potential for gene flow from cultivated soybean plants to wild soybean relatives in the U.S.

Transgenes in crops have the potential to move within a population. The potential for outcrossing can be defined as the ability of gene escape to other soybean fields. Soybean is a highly self-pollinating species with a cross-pollination rate of less than one percent in plants grown in close proximity (OECD, 2000; Caviness, 1966). Cross-pollination greater than 4.6 m from a pollen source has been rarely observed although it has been reported that insects can sometimes transfer the pollen that distance or more (Caviness, 1996). Even if gene flow occurred, the nature of this trait (oil composition changes) does not confer a selective advantage. The only known propagation method for soybean is through seed germination (i.e., there are no reports of vegetative propagation under field conditions in the United States). Mature soybean seeds have no innate dormancy, are sensitive to cold, and are not expected to survive in freezing winter conditions (Raper et al., 1987). Volunteer plants that might grow under certain environmental conditions can be easily controlled mechanically or with herbicides (Zollinger, 2005). Soybean is not weedy (Holm et al., 1977), is not found outside of cultivated areas, and does not compete well with other cultivated plants (Hymowitz et al., 1987).

B. Agricultural Production of Soybean

Soybean is grown as a commercial crop in over 35 countries. In the United States, soybeans are grown on over 70 million acres as an annual crop in at least 31 states. Over one million acres are grown in each of the following states: IA, IL, MN, IN, MO, NE, OH, SD, AR, ND, KS, MI, MS, WI, NC, KY, TN (USDA-NASS, 2008). The U.S. soybeans were harvested on 72.1 million acres in 2008 (USDA-NASS, 2008). This area for harvest was only one percent below the record high acreage in 2006. It is unlikely that "new," previously uncultivated land will be brought into soybean production.

Processed soybeans are the largest source of protein feed and the second largest source of vegetable oil in the world. Soybeans are about 90 percent of U.S. total oilseed production, while other oilseeds—such as cottonseed, sunflower seed, canola, and peanuts—account for the remainder (USDA-ERS, 2008).

Agricultural production of genetically engineered herbicide tolerant soybean

Based on USDA survey data, adoption of genetically engineered herbicide-tolerant (HT) soybeans increased from 17 percent of U.S. soybean acreage in 1997 to 68 percent in 2001 and 92 percent in 2008 (Fernandez-Cornejo, 2008). Use of herbicide-resistant crops is a major change in agriculture. Weed control had been one of the biggest challenges for soybean growers. Infestation with weeds during an entire growing season can result in soybean yield losses ranging from 12 to 80% (Barrentine, 1989). By the early 1990's, there were over 70 individual herbicides or combination products registered for weed control in soybean (Gianessi et al., 2002). Along with the increased use of herbicides,

biotypes of various plant species developed resistance to certain herbicide modes of action (Heap, 2007). With the 1996 commercial introduction of glyphosate tolerant soybean, a major shift occurred with an increased use of glyphosate concurrent with the increased planting of glyphosate tolerant soybean (92% of all soybean planted in the United States in 2008) and a decrease in use of other soybean herbicides(Gianessi et al., 2002). According to USDA's Agricultural and Resource Management Surveys (ARMS) in 2001-03, growers who used glyphosate-tolerant soybean technology cited the simplicity in weed control as the most common reason for growing HT soybean varieties. The popularity of glyphosate-tolerant soybean is due to advantages of the technology over conventional weed control practice.

Agricultural production of conventional soybean and genetically-engineered, non herbicide tolerant soybean

According to the report, Adoption of Genetically Engineered Crops in the U.S., "approximately 8% of total soybean acres in 2008 were planted with non-herbicide tolerant varieties (Fernandez-Cornejo, 2008)." A portion of this area (about 0.1% of U.S. soybean production) was devoted to the production of organic soybean (USDA-ERS, 2005).

Weed control is one of the biggest challenges for all soybean farmers. United States soybean farmers began switching from the use of tillage to control weeds to herbicides in the late 1950s. Herbicides were estimated to be used on more than 97 percent of the total soybean acreage in 1997 in the U.S. (Fernandez-Cornejo et al., 2002). Soybean is major market for pesticides in general and for herbicides in particular.

For Pioneer 305423 soybean (a non-herbicide tolerant soybean), there are no anticipated changes in pesticide use for weed management compared to conventional soybean varieties. The potential herbicide use in growing conventional soybean or Pioneer 305423 soybean should be very similar. Currently, there are at least 70 registrations for herbicides for weed management in soybean, plus numerous mixtures (Crop Protection Reference, 2009).

Organic soybean production

The production of organic soybeans represents about 0.1% of U.S. soybean production (USDA-ERS, 2005). In 2005, 122,217 acres of soybean in United States were certified organic. Under the USDA National Organic Program guideline, the use of synthetic pesticides, fertilizers, and genetically engineered crops is strictly limited. Pioneer 305423 soybean is not approved for use in organic systems because it is genetically engineered.

Maintaining the integrity of the organic production process is important to producers of organic soybeans. There are many practices organic producers use to prevent movement of GE soybean or the pollen from GE soybean into their organic production fields. Growers may use plant only organic seed, reducing the potential of GE soybean seeds enter their fields. Organic farmers may plant earlier or later than neighboring farmers who may be using GE crops, ensuring that the flowering times between GE and organically produced crops will differ, thus minimizing the change of pollen movement

between fields. Organic producers may also employ adequate isolation distances between the organic field and the fields of neighbors to minimize the chance that pollen will be carried between the fields. Organic growers must also maintain records to show that production and handling procedures comply with USDA organic standards.

C. Soybean Composition

Generally, soybean seed consists of oil (about 20 %), protein (about 40%), carbohydrate (about 35%) and ash (about 5%) (Liu, 1997). Soybean oil is currently the predominant plant oil produced in the world, and is used in a wide variety of food applications. After the oil is extracted the remaining solid materials in the form of flakes are toasted and ground to produce soybean meal.

Oil

Soybean is ranked number one in oil production (56%) among the major oil seed crops (Soy Stats, 2008). Conventional soybean oil is composed of a mixture of several fatty acids. The major unsaturated fatty acids in conventional soybean oil are 7% linolenic acid; 51% linoleic acid; and 23% oleic acid. It also contains saturated fatty acids 4% stearic acid and 10% palmitic acid (Codex standard for edible fats and oil, 1996). Soybean oil has a relatively high proportion of oxidation prone linolenic acid, which reduces product stability and shelf life and which is an undesirable property for the food industry. The hydrogenation process (chemical addition of hydrogen) is used to enhance the oil's stability by reducing its polyunsaturated fatty acid content. But this process has undesirable consequences including the formation of *trans* fatty acid isomers and a characteristic "hydrogenated flavor" (Fernandez, 1995). Partially hydrogenated oils are used by the food industry because they extend the shelf life and have a desirable taste and texture.

Meal

Soybean meal, which contains about 50% protein by dry weight, remains the primary product for soybean, with 95% of domestic soybean meal consumed by the livestock industry. Only a small proportion of the soybean crop is consumed directly by humans. Soybeans are considered to be a source of complete protein. A complete protein is one that contains significant amounts of all the essential amino acids that must be provided to the human body because of the body's inability to synthesize them. The ten essential amino acids are arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (Kuiken et al., 1949). Cystine is also an important amino acid as it can partially substitute for methionine. Protein Digestibility Corrected Amino Acids Score (PDCAAS) is considered as the gold standard for measuring protein quality for humans since 1990. The PDCAAS rating has been adopted by the FDA and FAO/WHO as the preferred method to determine protein quality (FAO/WHO, 1990). By this criterion, soybean protein was reported to be equivalent to animal protein with the highest rating of 1.0 (Hasler, 2002). Humans can produce 10 of the 20 amino acids. Soybean contains the other ten essential amino acids (Kuiken, et al., 1949) that are necessary for human nutrition and are not produced naturally in the body. The essential amino acid composition of soybean is included in the USDA Nutrition database (USDA-ARS, 2006).

Isoflavones
Soybeans naturally contain isoflavone compounds which are reported to possess biochemical activity, including estrogenic and hypocholesterolemic effects. The major isoflavones in soybeans include genistein and daidzein. Isoflavones are polyphenol compounds, produced primarily by beans and other legumes, including peanuts and chickpeas. Isoflavones are closely related to the antioxidant flavonoids found in other plants, vegetables and flowers.

Antinutrients
Soybean grain contains several key antinutrients, such as oligosaccharides, lectins, phytic acid and protease inhibitors (OECD, 2001). These ingredients could be toxic at high concentrations. Others bind nutrients, preventing their digestion of proteins resulting in decreased animal growth. The activity of these inhibitors is destroyed during the heat treatment processing of the soybean products.

Lectins are sugar-binding proteins and can be rapidly degraded upon heating also. The low molecular weight carbohydrates (e.g. stachyose, raffinose) can cause flatulence when consumed (Rackis, 1974). Phytic acid binds most of the phosphorus in soybean. It is common practice to add a phytic acid degrading enzymes to the animal feed formula.

IV. Alternatives

This EA analyzes the potential environmental consequences of a proposal to grant nonregulated status to Pioneer 305423 soybean. In order for Pioneer 305423 soybean to be granted nonregulated status, APHIS must determine that Pioneer 305423 soybean is unlikely to pose a plant pest risk. The analysis by APHIS in its plant pest risk assessment (USDA-APHIS, 2009) demonstrates that there was sufficient data to determine that Pioneer 305423 soybean is unlikely to pose a plant pest risk and therefore is eligible for nonregulated status.

The regulations at 7 CFR 340.6(d)(3)(i) state that APHIS may "approve the petition in whole or in part." Because APHIS has found that Pioneer 305423 soybean is unlikely to pose a plant pest risk, the only action alternative considered in this EA is to grant nonregulated status “in whole” to Pioneer 305423 soybean. An “in part” deregulation can be given if there is a plant pest risk associated with some, but not all lines requested in a petition. The petition for Pioneer 305423 soybean only requested APHIS to grant nonregulated status to one soybean event, therefore, an “in part” determination is not an appropriate consideration. Under another “in part” determination option, the petition may be considered with geographic restrictions if there is a geographic variation in plant pest risk. There are no geographic differences in plant pest risks for Pioneer 305423 soybean (USDA-APHIS, 2009). Thus, only two alternatives will be considered in this EA: (1) no action and (2) to grant nonregulated status to Pioneer 305423 soybean, “in whole.”

A. No Action: Continuation as a regulated article

Under the Federal "no action" alternative, APHIS would deny the petition. Under this alternative, Pioneer 305423 soybeans and progeny derived from them 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 Pioneer 305423 soybeans 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 Pioneer 305423 soybeans.

Soybean breeders have achieved soybean oil compositional changes by both conventional breeding and genetic engineering (Fehr 2007). Under this no action alternative, growers and other parties who are involved in production, handling, processing or consumption of soybean would continue to have access to existing deregulated GE high oleic acid soybean products as well as conventional high or mid level oleic soybean varieties. However, growers would not have widespread access to soybean varieties based on Pioneer 305423 soybean since it would continue to be regulated under Part 340. There is no potential for human consumption of Pioneer 305423 soybean high oleic acid soybean under this alternative. This alternative is not the preferred alternative because APHIS has already determined through a plant pest risk assessment (USDA-APHIS, 2009) that Pioneer 305423 soybean is unlikely to pose a plant pest risk. Choosing this alternative would hinder the purpose and need of APHIS to allow for the safe development and use of GE organisms given that Pioneer 305423 soybean is unlikely to pose a plant pest risk

B. Preferred Alternative: Determination that Pioneer 305423 soybean is no longer a regulated article

Under this alternative, Pioneer 305423 soybeans and progeny derived from them would no longer be regulated articles under the regulations at 7 CFR part 340. Pioneer 305423 soybean is eligible for nonregulated status because APHIS has determined that this GE organism is unlikely to pose a plant pest risk (USDA-APHIS, 2009). Permits issued or notifications acknowledged by APHIS would no longer be required for introductions of high oleic acid soybeans derived from this event. APHIS might choose this alternative if there was sufficient evidence to demonstrate the lack of plant pest risk from the unconfined cultivation of high oleic acid soybeans derived from this event.

Under this alternative, growers may have future access to Pioneer 305423 soybean and progeny derived from this variety if the developer decides to commercialize Pioneer 305423. In addition, growers and other parties that are involved in production, handling, processing or consumption of soybean would continue to be able to use the current high or mid level oleic soybean products by conventional breeding as well as the genetically engineered soybean variety. Consumers may benefit by having access to a greater range of potentially healthful food products. By granting nonregulated status to Pioneer 305423 soybean, the purpose and need to allow the safe development and use of GE organisms is

met.

APHIS has chosen Alternative B as the preferred alternative for the proposed action because APHIS has determined that Pioneer 305423 soybean is unlikely to pose a plant pest risk (USDA-APHIS, 2009). APHIS has assessed the potential for environmental impacts for each alternative in the "Potential Environmental Consequences" sections below.

V. Potential Environmental Consequences

According to APHIS regulations at 7 CFR part 340, an organism is no longer subject to regulatory requirements when it is demonstrated not to present a plant pest risk (USDA-APHIS, 2009).

Under the regulations, APHIS is required to render a determination on a petition for nonregulated status. 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 from unconfined cultivation of Pioneer 305423 soybean.

A. No Action

Under the Federal "no action" alternative, Pioneer 305423 would continue to be a regulated article and so growers would not be able to plant Pioneer 305423 soybean, which has been developed as an alternative to products available in the market today.

A. Soybean

Under the 'no action' alternative, flowering and reproduction in soybean production fields will remain unchanged. DuPont's GE variety remains eligible for production; however, Pioneer 305423 will remain a regulated article.

B. Agricultural Production of Soybean

Most of the soybean acreage in the U.S. is planted to GE varieties. Of the total soybean acres planted in 2008, 92% were GE glyphosate tolerant soybean varieties (USDA-NASS, 2008). Conventional production practices that use GE varieties will still dominate in terms of acreage, or perhaps increase in acreage, without granting nonregulated status to Pioneer 305423 soybean under the "no action" alternative. Currently available seed for conventional varieties will remain the same under the "no action" alternative, except Pioneer 305423 soybean variety will be unavailable. Soybean is currently produced in more than 30 states according to the 2007 State Soy crop Statistics, and under the "no action" alternative, this range of production will be unchanged.

Weed control is one of the biggest challenges for conventional soybean farmers because poorly controlled weeds drastically decrease crop yield and quality. Full season infestation of weeds can result in soybean yield losses ranging from 12 to 80% (Barrentine, 1989).

Under this "no action" alternative, herbicides will still be used alone or in combination and selected on the basis of their effectiveness on the different weed species in the soybean field. Different herbicides have different modes of action; the correct herbicide rate must be used for each in order to obtain good weed control results and to minimize soybean plant injury.

The large scale commercial cultivation of glyphosate tolerant soybean crop acreage has steadily increased from 1996, accounting for 92 % of soybean acreage in 2008. The primary reason that farmers have switched to glyphosate-tolerant soybean is the simplicity of the weed management programs. Glyphosate is a highly effective, nonselective, broad-spectrum herbicide and in general, considered "environmentally friendly" when compared to other herbicides. There would not be any affect on the availability or use of glyphosate tolerant soybean under the 'no action' alternative. These GE varieties will remain non-regulated GE soybean varieties. Thus, it is also likely that under the 'no action' alternative, greater than 90% of the soybean acres produced in the U.S. will be GE varieties.

C. Soybean Composition

Soybean production for modified fatty acid composition

The genetic changes in soybeans that resulted in soybean oil composition changes have been achieved by both conventional and genetic engineering techniques. Mutagenesis was used to develop the major genes for reduced palmitic and linolenic acids that are in the cultivars currently grown for commercial production (Fehr, 2007). Conventional soybean breeders have obtained varieties with greater than 70% oleic acid by intercrossing (Alt et al., 2005). As described in this EA, DuPont (USDA-APHIS, 1997) has used genetic engineering techniques to increase oleic acid composition in its soybean to near 80%. Therefore, under the 'no action' alternative, soybean varieties that have modified oil content, either through conventional breeding techniques or through genetic engineering, will still be available and on the market, except for Pioneer 305423 soybean. Additionally, other seed high in oleic oil (e.g. GE sunflower oil) is in commerce.

B. Preferred Alternative

Under this alternative, Pioneer 305423 soybean would no longer be a regulated article under 7 CFR part 340. Permits issued and/or notifications acknowledged by APHIS would no longer be required for introductions of Pioneer 305423 soybean. APHIS has chosen the preferred alternative for the proposed action because the Pioneer 305423 soybean lacks plant pest characteristics, as determined in APHIS' Plant Pest Risk Assessment (USDA-APHIS, 2009). APHIS' assessment of environmental consequences under the preferred alternative is described below.

A. Soybean

Under this alternative, Pioneer 305423 soybean would be available to growers. A potential environmental impact to consider as a result of planting this soybean variety, as with any other commercially-available variety, is the potential for gene flow.

Based on the plant pest risk assessment, APHIS has determined that Pioneer 305423 soybean is not a plant pest and that gene flow between this product and weedy and wild relatives is not going to occur in the United States. APHIS does note that gene flow can take place between a field planted with Pioneer 305423 soybean and a neighboring soybean crop. Although the biology of the soybean crop limits the amount of gene flow that may occur between two soybean fields, certain measures can be taken to minimized this issue (e.g. isolation distance to avoid the mechanical mixing).

The food/feed nutritional and safety assessment for Pioneer 305423 soybean has been reviewed by the FDA. Under 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 Pioneer 305423 soybean must be in compliance with all applicable legal and regulatory requirements. In January 2009, FDA completed the safety and nutritional assessment for this product and had no further questions regarding the safety of Pioneer 305423 soybean (FDA, 2009). APHIS assessment of the safety of this product focuses on its potential to pose a plant pest risk, and that analysis, is based on the comparison of the GE-soybean to its non-GE counterpart (USDA-APHIS, 2009).

Based on the assessment of the laboratory evidence provided by Pioneer (Pavely, 2007) and scientific literature (Reference section), APHIS has concluded that under this alternative, the proposed action to deregulate Pioneer 305423 soybean would have no significant impacts on human or animal health.

B. Agricultural Production of Soybean

In 2008, GE soybean was planted on 92% of all soybean acres in the US, and the use of GE soybean has been steadily increasing over the last 3 years (USDA-NASS 2006, 2007, 2008). Conventional and GE soybean production occurs on land that is dedicated to crop production. Most soybean is planted in fields that have been in crop production for years. Pioneer 305423 soybean has been field tested in the United States since 2002. The field test sites included the major soybean growing areas of the Midwest and the winter nursery in Hawaii. Agronomic and phenotypic data were collected to assess agronomic comparability to conventional soybean. To evaluate the agronomic and phenotypic characteristics of Pioneer 305423 soybean, the data were collected to address specific characteristics that influence reproductive and survival biology. Based on these data, agronomists evaluated the potential for weediness as compared to conventional soybean. There were no biological significant differences in weediness potential between Pioneer 305423 soybean and other soybean varieties. These results show that the agronomic and phenotypic characteristics of Pioneer 305423 soybean were not altered when compared to nontransgenic soybean. Granting nonregulated status to Pioneer 305423 soybean under the “preferred” alternative is not expected to significantly alter the range of soybean cultivation as the new GE trait (high oleic acid) does not change the growth habits of GE soybean varieties compared to conventional varieties (USDA-APHIS, 2009).

Additionally, Pioneer 305423 soybean oil will be marketed as "TREUS™[2]" high oleic soybean oil. This soybean variety will likely be introduced to areas where soybean is currently grown for oil production as a replacement product to other varieties (conventional and GE) already available in the market.

On July 9, 2003, the Food and Drug Administration (FDA) announced that it would require the labeling of all food products for their *trans*-fat content beginning January 1, 2006. As a result of the federal regulation, the food industry in the United States has been actively pursuing alternatives to hydrogenated oils so that their products can be labeled as containing 0 g of *trans*-fat. The oil from Pioneer 305423 soybean with high oleic acid could be adopted as one of the alternatives to hydrogenated oil.

Weeds growing with soybeans compete with the crop for light, moisture, and nutrients. Uncontrolled weeds reduce soybean yields and interfere with harvest. Soybeans are very competitive with weeds once they develop a canopy, but early emerging weeds can cause serious problems. Thus, early-season weed control is the key to providing the soybeans with a competitive advantage and minimizing the effect of weeds. Crop rotation is one of the most effective ways to manage certain weed problems specific to a crop. Herbicides, if used properly, are a safe and effective method to control certain weeds in soybeans.

Pioneer 305423 soybean was genetically engineered to express a modified soybean acetolactate synthase. However, this modified soybean acetolactate synthase was used as a selectable marker for transformation and does not confer commercial levels of herbicide tolerance in this event. Also, Pioneer 305423 soybean is not genetically engineered for tolerance to glyphosate. Because of the herbicide usage similarity of Pioneer 305423 soybean to conventional soybean, there is no change in herbicide use with Pioneer 305423 soybean under the preferred alternative.

Organic Soybean Production

Organic farming operations as described by the National Organic Program, which is administered by USDA's Agricultural Marketing Service, requires organic production operations to have distinct, defined boundaries and buffer zones to prevent unintended contact with 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.

Organic certification involves oversight by an accredited certifying agent of the materials and practices used to produce or handle an organic agricultural product. This oversight includes an annual review of the certified operation's organic system plan and on-site inspections of the certified operation and its records. Although the National Organic

[2] TREUS™ is a trademark of Pioneer Hi-Bred International, Inc.

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, 2007). 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 (such as isolation zones, use of buffer rows surrounding the organic crops or adjusting planting dates and appropriate cleaning of planting and harvesting equipment) to avoid contact with the products of excluded methods as detailed in their approved organic system plan. Organic certification of a production or handling operation is a process claim, not a product claim.

It is not likely that farmers, including organic and conventional farmers, who choose not to plant transgenic soybean varieties or sell transgenic soybeans varieties, will be significantly impacted by the commercial use of this product. Non-transgenic soybean will likely still be sold and will be readily available to those who wish to plant it. An internet search of "Soybean Seed Company" identifies vendors that offer all types of soybean seeds for purchase including conventional and transgenic. A few of the many searchable sites available include www.lathamseeds.com, http://www.stineseed.com/ and http://www.bo-jac.com/soybean.php. If Pioneer receives regulatory approval from all appropriate agencies, it will make Pioneer 305423 soybean available to growers and breeders. It is not likely that other farmers who choose not to plant or sell Pioneer 305423 soybean or other transgenic soybeans will be significantly impacted by the expected commercial use of this product as (a) non-transgenic soybeans will likely still be sold and will be readily available to those who wish to plant it; (b) soybean is a highly self-pollinated plant and therefore buffer requirements would be minimal; and (c) 92% of the 2008 soybean acreage in the United States is already planted to transgenic herbicide tolerant varieties; and (d) APHIS expects that Pioneer 305423 soybean may replace some of the presently available GE soybean varieties without significantly affecting the overall total soybean acreage so organic farmers will be able to coexist with biotech soybean producers as they do now.

If APHIS choose the no action alternative there would be no direct impact on organic or other non-transgenic soybean farmers. The current cultivation practices are unlikely to change and 92% of the soybeans produced would likely be planted with the current herbicide tolerant biotech soybean varieties. If Pioneer 305423 soybean is granted nonregulated status, there also would be no direct impact on organic or other non-transgenic soybean farmers as the market share of transgenic soybean are unlikely to change by the introduction of Pioneer 305423 soybean.

C. Soybean Composition

Soybean is one of the leading agricultural products in the United States. Soybean is classified as an oilseed and is characterized by its high (38–45%) protein content as well as its high (20%) oil content. The molecular analysis data supplied in the Petition show that Pioneer 305423 soybean contains multiple copies of soybean fatty acid desaturase gene (*Gm--fad2-1*) and one modified version of the soybean acetolactate synthase gene

(*Gm-hra*). Pioneer 305423 soybean is a transgenic soybean product that provides soybean seeds with increased levels of monounsaturated (oleic) fatty acids and decreased levels of polyunsaturated fatty acids (linoleic and linolenic).

The compositional assessment was conducted in accordance with the Organization of Economic Co-operation and Development (OECD) consensus document on compositional considerations for new varieties of soybean (OECD, 2001) (Pavely, 2007, p 76).

Oil Composition

The Pioneer 305423 soybean was generated by the insertion of a second copy of a soybean fatty acid desaturase gene (*Gm-fad2-1*) into a publicly available soybean cultivar "Jack". The fatty acid desaturase gene is responsible for the synthesis of linoleic acid, which is the major polyunsaturated fatty acid present in soybean oil. By silencing[3] the fatty acid desaturase gene, it prevents linoleic acid from being synthesized and leads to the accumulation of oleic acid in the seeds. An overview of fatty acid biosynthesis in soybean is illustrated in Figure 11, page 41 of the Petition (Pavely, 2007). The intended change to Pioneer 305423 soybeans is to greatly increase oleic acid content in the seed (Heppard, 1996). Multiple copies of the *Gm-fad2-1* gene appear to be necessary for effective co-suppression of the endogenous fatty acid desaturase gene 1 (El-Shemy et al., 2004; Mishra et al., 2005).

APHIS has previously granted nonregulated status to high oleic soybean (APHIS Petition # 97-008-01p) (USDA-APHIS, 1997) developed by DuPont in which high oleic phenotype was conferred by introduction of the soybean omega-6 desaturase gene 1 (*fad2-1* gene). DuPont high oleic soybean varieties received regulatory approval in the Australia, Canada, Japan, New Zealand, and the United States. Australia New Zealand Food Authority (ANZFA) developed a draft "risk analysis report" (ANZFA, 2000) for the DuPont high oleic product. ANZFA concluded that high oleic acid soybeans do not raise any public health and safety concerns. Health Canada gave DuPont a "no objection to the food use of high oleic soybeans" in 2000. Data that support the safety of *GmFad2-1* gene was reviewed by FDA resulting in their issuing a FDA Biotech Consultation BNF #000039 (FDA, 1996_b). FDA has evaluated the food safety of the new proteins in bioengineered plants since 1992 and provided recommendations concerning its food safety (FDA, 2004). In January, 2009, FDA has completed the voluntary consultation of the Pioneer 305423 soybean for "Food and Feed Safety and Nutritional Assessment". FDA has no further questions concerning Pioneer 305423 soybean based on the information Pioneer has provided (FDA, 2009).

Fatty acid compositional data was collected on Pioneer 305423 soybean and comparisons were made to conventional control lines and a set of reference soybean varieties (OECD, 2001). A total of 25 fatty acids were analyzed in Pioneer 305423 soybean and control lines. Eleven fatty acid concentrations were near or below the detection limits of the assay. These fatty acids are listed in Petition (Pavely, 2007, page 79). The analyses of

[3] Gene silencing refers to a technique for selectively turn off specific genes within a cell.

other 14 fatty acids are presented in Table 5, Petition (Pavely, 2007, page 81-82). Six of the 14 fatty acids were statistically significantly different from control soybean lines: myristic acid, palmitoleic acid, stearic acid, arachidic acid, eicosenoic acid and lignoceric acid (Pavely, 2007, page 79-82). However, the levels of these fatty acids are not biologically significant; they are relatively minor fatty acids (together comprising less than 6% of fatty acid content) and are common fatty acids in vegetable oils at similar levels (USDA-ARS, 2006). Two of the 14 fatty acids were not statistically significantly different: behenic acid and linoleic acid isomer (9,15). The remaining six fatty acids (oleic acid, linoleic acid, linolenic acid, palmitic acid, heptadecanoic acid, and heptadecenoic acid) are discussed below.

Fatty acids analyses confirmed that Pioneer 305423 soybean has significantly more oleic acid (from an average of 21% to 76% of total fatty acid content) and less in linoleic acid (from an average of 52% down to 3.6%) and palmitic acid (from an average of 10.3% down to 6.28%) than conventional soybeans. High oleic acid levels are found in other commonly consumed premium edible oils (e.g., olive oil and high oleic sunflower and canola oil). The consumption of high levels of oleic acid is not considered to pose any safety concerns. Linolenic acid is produced directly from conversion of linoleic acid and therefore the decrease (from an average of 9.3% down to 5.4%) in the linolenic acid was directly related to decreased linoleic acid levels. Linoleic acid and alpha-linolenic acid are fatty acids the human body requires, and cannot be constructed from other components by known chemical pathways. They therefore must be obtained from the diet. These two fatty acids are widely distributed in plant oils at (e.g. safflower oil, poppy seed oil, walnut oil, olive oil) and other food sources (USDA-ARS, 2006).

The decrease in palmitic acid content in Pioneer 305423 soybean is one of the intended effects. Palmitic acid is one of the most common saturated fatty acids found in animals and plants. Palmitic acid constitutes between 20 and 30 percent of most animal fats and is also an important constituent of most vegetable fats (35 - 45 percent of palm oil).

Pioneer also noted increased levels of two minor fatty acids, heptadecanoic acid and heptadecenoic acid (Pavely, 2007, pp. 80-82). These increases likely result from changes in the GM-HRA protein that shifts a metabolic pathway leading to production of the C17[4] fatty acids. Pioneer describes this complex oil biosynthesis in the Petition (Pavely, 2007, Appendix7, pp. 171-172). The combined value of heptadecanoic and heptadecenoic fatty acids in control soybean line is less than 0.2% of total fatty acids. In Pioneer 305423 soybean, the combined value of these two fatty acids is about 2% of total fatty acids. Heptadecanoic acid is commonly found in meat, tofu and butter (USDA-ARS, 2006), and heptadecenoic acid is commonly found in meat (Senaratne, 2009) and tofu (USDA-ARS, 2006). The levels of these two fatty acids in Pioneer 305423 soybean are comparable to those already found in the diet and there is no evidence to indicate that exposure to either fatty acid through dietary sources would have adverse effects in humans.

[4] C17 fatty acids are fatty acids containing 17 carbon atoms (e.g. heptadecanoic acid and heptadecenoic acid).

Soybean Meal Composition

Amino Acids

Soybean meal is fed to animals primarily as a source of protein. Amino acid content in Pioneer 305423 soybean was determined for 18 amino acids. APHIS reviewed and analyzed the data presented in the petition, and concludes that there are no significant differences in amino acid composition between Pioneer 305423 soybean and control soybean lines (Pavely, 2007).

Novel protein

The inserted *Gm-hra* gene is a slightly modified version of the soybean acetolactate synthase gene (*als* gene) that is responsible for tolerance to ALS inhibiting herbicides. The *Gm-hra* gene encodes the GM-HRA protein with two amino acid residues modified from the endogenous enzyme. In Pioneer 305423 soybean, GM-HRA is the only novel protein expressed and was solely used as a selectable marker during the transformation procedure. The HRA fragment in Pioneer 305423 soybean does not confer commercial levels of sulfonylurea or other ALS (acetolactate synthase) inhibitor herbicide tolerance. Pioneer has no plan to commercially market Pioneer 305423 as a sulfonylurea herbicide tolerant variety.

ALS (acetolactate synthase) proteins are present in nature, as *als* genes have been isolated from bacteria, fungi, algae and plants (Friden et al., 1985; Falco et al., 1985; Reith et al., 1995; Mazur et al., 1987). Several commercialized non-GE crops (Clearfield trade mark) with the herbicide tolerant *als* gene are available in the current market. Data that support the safety of modified soybean acetolactate synthase proteins have been reviewed by FDA in the FDA Biotech Consultation BNF #000110 (FDA, 2009) for GE Pioneer soybeans Petition #06-354-01p (Pavely, 2007). FDA Biotech Consultation BNF #000050 (FDA, 1998) for GE flax, FDA Biotech Consultation BNF #000030 (FDA, 1996_a) for GE cotton, FDA Biotech Consultation BNF #000108 (FDA, 2007) for GE Pioneer soybeans Petition #06-271-01p (USDA-APHIS, 2006).

Pioneer also assessed the acute toxicity in mice of a dose of 2000 mg purified GM-HRA protein per kilogram of body weight (Pavely 2007, pp62-63). Even at this high dosage, no clear threshold of acute toxicity in mice was reached. Since GM-HRA protein is expressed at such a low level in Pioneer 305423 soybean, the amount of these soybeans that would need to be consumed to cause harm in mammals would be outside the limits of any realistic scenario for consumption. It is clear there is a wide margin of safety for GM-HRA.

Based on Pioneer's data and information in Petition, the recently published Environmental Assessment for APHIS Petition # 06-271-01p for GE Pioneer soybeans (USDA-APHIS 2006) and results of multiple previous FDA consultations, there is no indication of risk from GM-HRA protein due to exposure or other environmental safety concerns under the preferred alternative.

GM-HRA protein was also assessed by the applicant for possible allergenicity and toxicity using internationally accepted guidance from the Codex Alimentarius Commission. APHIS reviewed this information (Pavely, 2007, p62) and concludes that GM-HRA proteins are unlikely to be either allergenic or toxic to humans or animals.

Proximate, isoflavones and antinutrients

Data on proximate and fiber in soybean grain was provided to APHIS in the petition (Pavely, 2007). Proximate analysis is a chemical method of assessing and expressing the nutritional value of a feed. Soybeans are occasionally used as an alternative forage source when alfalfa or clovers are in short supply. In 2001, an OECD consensus document on compositional considerations for new soybean varieties, suggested parameters that soybean developers should measure. The proximate nutrient content, including crude protein, crude fat, fiber, and ash content of soybean meal is one of the parameters.

APHIS reviewed this information and concludes that the ranges for crude protein, fat, ash, neutral detergent fiber (NDF) and acid detergent fiber (ADF) are comparable to nontransgenic soybean lines or reference soybean lines. APHIS also concludes that proximate and fiber analysis of soybean forage and grain samples demonstrate that there is no unexpected difference between Pioneer 305423 soybean and control soybean lines.

Antinutrients are compounds which decrease the nutritional value, usually by making an essential nutrient unavailable or indigestible when consumed by humans/animals. Soybean seeds are known to contain different compounds displaying antinutrient effects. Soybean grain contains several key antinutrients, such as oligosaccharides, trypsin inhibitors, lectins and phytic acid. APHIS reviewed the information on the key antinutrients stachyose, raffinose, lectins, phytic acid and trypsin inhibitor presented in the petition for Pioneer 305423 soybean and the control lines and concludes there were no statistically significant differences were seen between Pioneer 305423 soybean and control soybean lines.

APHIS also reviewed the information presented on isoflavones and concludes there were no statistically significant differences observed between Pioneer 305423 soybean and control soybean lines.

APHIS has concluded that Pioneer 305423 soybean, and the foods and feeds derived from it are not materially different in safety, composition, or any other relevant parameter from soybeans now grown, marketed, and consumed. Results of these comparisons indicate that Pioneer 305423 soybean is compositionally and nutritionally equivalent to conventional soybean varieties currently in commerce except for the intended changes in the fatty acid profiles.

APHIS concludes that there is no apparent potential for significant impact to the human environment by Pioneer 305423 soybean through unconfined cultivation, if APHIS implements the preferred alternative granting the petition for nonregulated status.

Soybean seeds both genetically engineered and conventionally breed for altered fatty acid composition of the soybean oil have been sold commercially and have a history of safe use. The commercialization of Pioneer 305423 soybean could be beneficial for the consumer's health. Increased intake of oils high in monounsaturated fatty acids, such as oleic acid have been shown to have positive effects on total cholesterol levels when compared to equal intakes of hydrogenated oils (Lichtenstein et al., 2006). Likewise, increased intake of oils high in oleic acid can decrease LDL-cholesterol levels compared to equal intakes of saturated oils (Mensink et al., 1989) and increased HDL-cholesterol levels compared to an equal intake of polyunsaturated oil (Mata et al., 1992). Moderate consumption of oil high in oleic acid has also demonstrated decreases in systolic blood pressure (Bondia-Pons et al., 2006).

Cumulative effects

APHIS considered whether the proposed action could lead to significant cumulative impacts, when considered in light of other past, present, and reasonably foreseeable future actions, regardless of what agency or person initiated such actions. As mentioned above, Pioneer 305423 soybean is not the first high oleic soybean product to be granted nonregulated status. APHIS has previously made determinations of nonregulated status for DuPont high oleic soybean in the market currently.

APHIS has evaluated the potential cumulative impacts of granting nonregulated status to Pioneer 305423 soybean. In 2008, GE soybean was planted on 92% of all soybean acres in the US, and the use of GE soybean has been steadily increasing over the last 3 years (USDA-NASS, 2006, 2007, 2008). Conventional and GE soybean production occurs on land that is dedicated to crop production. Most soybean is planted in fields that have been in crop production for years. Pioneer 305423 soybean will not affect the amount of acreage devoted to GE varieties, nor will Pioneer 305423 soybean alter the growing regions available for soybean production. The Pioneer 305423 is not herbicide tolerant at commercial, herbicide application rates. Therefore, APHIS has no reason to believe that there will be any substantive change in current herbicide use rates.

In 2003, several thousand acres of DuPont high oleic soybeans were grown under contract in Iowa for DuPont Protein Technologies. There were no known or reported environmental impacts due to the production of a high oleic soybean variety. Additionally, the DuPont high oleic soybean variety has not been planted since 2005 due to changes in market strategy for DuPont.

Based on this information, APHIS has determined that there are no 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 Pioneer 305423 soybean is planted.

Potential impact on non-target organisms, including beneficial organisms and threatened or endangered species

Unintended effects on non-target organisms are one of the safety concerns considered for genetically engineered foods, including the potential for altered expression of plant produced toxicants and anti-nutrients or the formation of novel toxins. Generally, unintended effects can be predicted or explained through our current knowledge of plant biology and metabolic pathways. Molecular and biochemical analysis data can also help the risk assessors to determine the levels of transcriptional and translational changes. APHIS reviewed and analyzed the data presented for Pioneer 305423 soybean and evaluated the potential of this GE soybean variety to cause damage or have toxic effects directly or indirectly on non-target organisms.

The only novel protein expressed in Pioneer 305423 soybean is GM-HRA. The potential toxicity of the GM-HRA protein was assessed by comparing the amino acid sequence of the GM-HRA protein with known protein toxins in the bioinformative database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=protein) as well as in an acute toxicity feeding study in mice (Pavely, 2007, p 63). The study found no evidence of acute toxicity in mice fed with high doses of purified GM-HRA protein (Pavely, 2007, p. 63). Since Pioneer 305423 soybean was proposed for use as a food/feed product, there is a wide safety margin (the dosage) taken into account in the acute toxicity feeding study. APHIS concludes, after reviewing the data presented, including FDA's food safety and nutritional assessment, that there will not be any toxic effect at any feasible level of consumption.

The information on the possible allergenicity of Pioneer 305423 soybean was reviewed and analyzed by APHIS. GM-HRA protein is not known to be a protein toxin/allergen based on the information in the database of the Food Allergy Research and Resource Program (FARRP), University of Nebraska, Allergen Database (Version 6.0, January 2006). Likewise, the rapid proteolytic degradation (less than 30 seconds) under simulated mammalian digestion conditions provides further evidence to confirm the safety of the protein (FAO, 1996). In addition, Pioneer provided a detailed assessment of human and animal safety assessment of the GM-HRA protein to FDA as part of the consultation on food and feed safety for Pioneer 305423 soybean (FDA, 2009). FDA had no further questions on the safety of Pioneer 305423 soybean.

Nutritional studies on targeted animals are performed in cases where the composition of the GE plant differs significantly from the non-GE counterpart. Pioneer conducted a 42-day feeding study on broiler chickens comparing Pioneer 305423 soybean and non-transgenic soybean as the main diets. Broilers are the choice animal for feeding studies because they are fast growing and are particularly sensitive to the presence of toxic elements in their feed. APHIS evaluated the results of feeding tests on broilers (Pavely, 2007) (and concluded that there is no difference between Pioneer 305423 soybean and traditional soybean variety counterparts, and Pioneer 305423 soybean can be considered as nutritionally equal to the non-GE counterparts (Kuiper et al., 1999). Therefore, when

compared to conventional soybean varieties, Pioneer 305423 soybean is not expected to have any adverse effects on birds and animals feeding in the field.

Plant protease inhibitors have been determined to play a potent defensive role against certain pests and pathogens of soybeans (Boulter, 1993; Hilder et al., 1987; Williamson et al., 1996; Joshi et al. 1998) as described in the Plant Pest Risk Assessment (USDA-APHIS, 2009, p 3-5). The reduced protease activity could potentially result in Pioneer 305423 soybean being more susceptible to insect damage or fungal disease than the non-GE control lines. Pioneer provided data (Pavely, 2007, pp158-160) to support the conclusion that Pioneer 305423 soybean is comparable to non-GE control lines in environmental fitness and defense against pests and diseases. The mean value for trypsin inhibitor was statistically significantly different (lower) in Pioneer 305423 soybean as compared to the control line although remaining within the statistical tolerance interval. APHIS reviewed the information and concludes that reducing the protease activities of the Pioneer 305423 soybean is not expected to have any effect on insects or threatened and endangered insect species feeding in a soybean field (Pavely, 2007, pp158-160).

APHIS also evaluated the effects of production of Pioneer 305423 soybean on soil communities in agricultural settings. In this case the genetic modification does not confer herbicide tolerance or insect resistance. Therefore, the potential pesticide (herbicide and insecticide) usage should be similar as that for conventional soybean plantings. Currently, there are over 70 registrations for herbicides for weed management in soybean (Gianessi et al., 2002), plus numerous mixtures (Crop Protection Reference, 2009). In planting Pioneer 305423 soybean, the pesticide or herbicide runoff into ground and surface water should have no more deleterious effects on non-target organisms, including aquatic animals or aquatic invertebrates in ponds and streams than conventional plantings. Reports issued by the OECD (OECD, 1986) and a working group coordinated by the Royal Society of London (Royal Society, 2000) have indicated that environmental risks of biotechnology-derived crops are not fundamentally different from risks of conventionally derived products. Therefore, cultivation of Pioneer 305423 soybean should have no effects on either the microbial or invertebrate communities of soil ecosystems when compared to current agricultural practices for soybean.

Using the information pertaining to the potential impacts to non-target organisms described above, APHIS also considered the potential impact of deregulating Pioneer 305423 soybean on federally listed threatened or endangered species (TES) and species proposed for listing [http://ecos.fws.gov/tess_public/SpeciesReport.do (accessed April 10, 2009)], as well as designated critical habitat and habitat proposed for designation, as required under Section 7 of the Endangered Species Act. In this analysis, APHIS considered the biology of Pioneer 305423, and how its production would differ from typical agricultural practices associated with cultivation of soybean.

To identify negative effects or significant impacts on TES animal species, APHIS evaluated the risks to TES animals from consuming Pioneer 305423 soybean. Risk is a combination of hazard and exposure. APHIS first conducted hazard identification for Pioneer 305423 soybean. APHIS reviewed and analyzed the composition and nutritional quality of Pioneer 305423 soybean submitted in the petition, and compared the composition of Pioneer 305423

to the composition of a non-genetically engineered control soybean line and the natural variation found in commercial soybean varieties (Pavely, 2007). If the composition of Pioneer 305423 soybean is similar to other commercial soybean plants, it is unlikely that Pioneer 305423 poses a hazard to TES animal species. If no hazards are identified, then the risk of Pioneer 305423 soybean harming TES animal species is also unlikely, regardless of exposure.

As discussed in this EA, the proteins produced by the inserted genes and the changes in fatty acid composition do not raise safety issues. As noted above in this section, consumption of GM-HRA protein has shown no toxicity in lab testing with mice, and no measurable change in nutritional response on broiler chickens. The Pioneer 305423 soybeans do not express additional proteins, natural toxicants, allelochemicals, pheromones, hormones, etc. that could directly or indirectly affect a listed TES or species proposed for listing. Data submitted on the composition of the Pioneer 305423 soybeans indicate that these soybeans are not significantly different from non-transgenic soybeans and would not be expected to have any effect on TES that would be different from non-transgenic soybeans. The Pioneer 305423 soybean is not sexually compatible with a federally listed TES or a species proposed for listing. APHIS has not identified any stressor caused by the production of Pioneer 305423 that could affect the reproduction, numbers, or distribution of a listed TES or species proposed for listing. Consequently, an exposure analysis for individual species is not necessary.

Soybeans do not grow and persist in unmanaged habitats and would not be expected to invade and/or persist in the natural environment. Soybean fields are typically highly managed agricultural areas that can be expected to be dedicated to crop production for many years and cultivation of Pioneer 305423 soybean is not expected to differ from typical soybean cultivation. After reviewing possible effects of deregulating Pioneer 305423 soybean, APHIS expects Pioneer 305423 soybean to replace some of the presently available soybean varieties, but APHIS does not expect that Pioneer 305423 soybean will cause new soybean acres to be planted in areas that are not already devoted to agriculture. APHIS has considered the effect of Pioneer 305423 soybean production on habitat that could be used by TES, including critical habitat, and could identify no difference from affects that would occur from the production of other soybean varieties.

Based on the above information, APHIS has determined that the preferred alternative, deregulating Pioneer 305423 soybean would have no effect on federally listed threatened or endangered species and species proposed for listing, or on designated critical habitat or habitat proposed for designation. Consequently, a written concurrence or formal consultation with the USFWS is not required for this action.

Consideration of Executive Orders, Standards and Treaties relating to environmental impacts

Executive Order (EO) 12898 (US-NARA, 2008), “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, 2008), "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, Pioneer 305423 soybean is not significantly different than conventional soybean and has successfully completed the FDA voluntary consultation for food and feed use. Therefore, Pioneer 305423 soybean is not expected to have a disproportionate adverse effect on minorities, low-income populations, or children.

EO 13112 (US-NARA, 2008), "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 soybean varieties that have been granted nonregulated status are widely grown in the U.S. Based on historical experience with soybean and the data submitted by the applicant and assessed by APHIS, Pioneer 305423 soybean plants are very similar in fitness characteristics to other soybean varieties currently grown and are not expected to become weedy or invasive [see (USDA-APHIS, 2009) for the plant pest risk assessment of Pioneer 305423 soybean].

EO 13186 (US-NARA, 2008), "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 Pioneer 305423 soybean compared to conventional soybean, apart from the presence of Pioneer 305423 soybean. Pioneer also conducted feeding experiments on broiler chickens to evaluate the effects of Pioneer 305423 soybean on birds (page 15 of EA). The applicant reported no harmful effects to chickens from Pioneer 305423 soybean. The migratory birds that occasionally forage in soybean fields are unlikely to contain high amounts of Pioneer 305423 soybean as soybean availability is limited by seed germination and harvest. Based on APHIS' assessment of Pioneer 305423 soybean it is unlikely that granting nonregulated status to this soybean variety will have a negative effect on migratory bird populations.

INTERNATIONAL IMPLICATIONS

EO 12114 (US-NARA, 2008), "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. should nonregulated status be granted to Pioneer 305423 soybean. It should be noted that all the considerable, existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new soybean cultivars internationally, apply equally to those covered by an APHIS determination of nonregulated status under 7 CFR part 340. Any international trade of Pioneer 305423 soybean 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, 2008).

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, 2008); 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 (169 countries as of September 2008). 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.

The Cartagena Protocol on Biosafety is a treaty under the United Nations Convention on Biological Diversity (CBD) that established a framework for the safe transboundary movement, with respect to the environment and biodiversity, of LMOs, which includes those modified through biotechnology. The Protocol came into force on September 11, 2003, and 150 countries are Parties to it as of January, 2009 (CBD-UN, 2008). Although the U.S. is not a party to the CBD, and thus not a party to the Cartagena Protocol on Biosafety, U.S. exporters will still need to comply with domestic regulations that importing countries that are Parties to the Protocol have put in place to comply with their obligations. The first intentional transboundary movement of LMOs intended for environmental release (field trials or commercial planting) will require consent from the importing country under an advanced informed agreement (AIA) provision, which includes a requirement for a risk assessment consistent with Annex III of the Protocol, and the required documentation.

LMOs imported for food, feed, or processing (FFP) are exempt from the AIA procedure, and are covered under Article 11 and Annex II of the Protocol. Under Article 11 Parties must post decisions to the Biosafety Clearinghouse database on domestic use of LMOs

for FFP that may be subject to transboundary movement. To facilitate compliance with obligations to this protocol, the U.S. Government has developed a website that provides the status of all regulatory reviews completed for different uses of bioengineered products (NBII, 2008). These data will be available to the Biosafety Clearinghouse. APHIS continues to work toward harmonization of biosafety and biotechnology consensus documents, guidelines, and regulations, including within the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the U.S., and within the Organization for Economic Cooperation and Development. NAPPO has completed three modules of a standard for the *Importation and Release into the Environment of Transgenic Plants in NAPPO Member Countries* (NAPPO, 2008). APHIS also participates in the North American Biotechnology Initiative (NABI), a forum for information exchange and cooperation on agricultural biotechnology issues for the U.S., Mexico and Canada. In addition, bilateral discussions on biotechnology regulatory issues are held regularly with other countries including: Argentina, Brazil, Japan, China, and Korea.

COMPLIANCE WITH CLEAN WATER ACT AND CLEAN AIR ACT

This Environmental Assessment evaluated the changes in soybean production due to the unrestricted use of Pioneer 305423 soybean. Pioneer 305423 soybean will not lead to the increased production of soybean in U.S. agriculture. There is no expected change in water use due to the production of Pioneer 305423 soybean compared to current soybean production regimes, nor is it expected that air quality will change due to do the production of Pioneer 305423 soybean.

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