NATIONAL ENVIRONMENTAL POLICY ACT DECISION
AND
FINDING OF NO SIGNIFICANT IMPACT

Pioneer Hi-Bred International
High Oleic Soybean
DP-305423-1

United States Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology Regulatory Services

United States Department of Agriculture (USDA) and the Animal and Plant Health Inspection Service (APHIS), have developed a decision document to comply with the requirements of the National Environmental Policy Act of 1969, as amended, the Council of Environmental Quality's (CEQ) regulations implementing NEPA, and the USDA and APHIS' NEPA implementing regulations and procedures. This NEPA decision document is intended to state APHIS' NEPA decision and present the rationale for its selection.

In accordance with APHIS procedures implementing the NEPA Regulations (7 CFR part 372), APHIS has prepared an Environmental Assessment (EA) to evaluate and determine if there are any potentially significant impacts to the human environment from a determination on the regulated status of a petition request (APHIS number 06-354-01p) by Pioneer HiBred International, Inc. for DP-305423-1 (hereafter referred to as Pioneer 305423 soybean). This *Glycine max* (soybean) variety was genetically engineered to produce increased amounts of monounsaturated fatty acid (oleic) and decreased amounts of polyunsaturated fatty acids (linoleic and linolenic) and to lesser extent, decreased saturated fatty acid (palmitic acid). Pioneer 305423 contains the *gm-fad2-1* gene that is responsible for the unique oil profile. Pioneer 305423 also expresses a second gene, gm-hra, conferring tolerance to sulfonylurea herbicides which was used as a selective agent following transformation. APHIS has evaluated the plant pest risks posed by the production of Pioneer 305423 and prepared an EA to identify and evaluate any environmental impacts resulting from the approval of the petition for nonregulated status. The EA assesses alternatives to granting nonregulated status to Pioneer 305423 and analyzes the potential environmental and social effects that result from the proposed action and the alternatives. The proposed action of USDA APHIS, Biotechnology Regulatory Services (BRS) is to grant nonregulated status to Pioneer 305423 and remove this GE soybean variety from APHIS' regulatory oversight in accordance with 7 CFR part 340. Comments from the public involvement process were reviewed for substantive issues which were considered in developing this NEPA decision.

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 United States: USDA-APHIS, the U.S. Department of Health and Human Services' Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA). Products are regulated according to their intended use and some products are regulated by more than one agency. USDA-APHIS, FDA, and EPA enforce agency-specific regulations on products of biotechnology that are based on the specific nature of each GE organism. Together, these agencies ensure that the products of modern biotechnology are safe to grow, safe to eat, and safe for the environment.

APHIS regulates GE organisms under the Plant Protection Act of 2000. USDA APHIS-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 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 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 "Petition for Determination of Nonregulated Status." The petitioner is required to provide information (§ 340.6(c)(4)) related to plant pest risk that the agency uses to determine whether the regulated article is unlikely to present a greater plant pest risk than the unmodified organism. After receipt of a petition, as per the requirements of § 340.6, BRS makes a determination on whether an organism is not likely to pose a plant pest risk and is therefore no longer subject to the regulatory requirements of 7 CFR part 340. A GE organism is no longer subject to the regulatory requirements of 7 CFR part 340 when APHIS determines that it is not likely to pose a plant pest risk.

FDA regulates 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. 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).

Pioneer 305423 soybean has successfully completed the consultation process with the FDA concerning food and feed safety (BNF No. 000110). FDA has no more questions on nutritional or safety issues, and has provided a summary response indicating that "the food and feed derived from the 305423 soybean are as safe and nutritious as food and feed derived from conventional soybean varieties currently being marketed." Because Pioneer 305423 soybean does not contain any genetically engineered pesticides or tolerance to herbicides, EPA consultation is not required.

Document History

On December 20, 2006 APHIS BRS received a petition from Pioneer HiBred International seeking a determination of nonregulated status for DP-305423 soybean. A revised version of the petition was received on October 10, 2007. Upon receipt of the petition, BRS reviewed the information submitted and deemed the petition complete on October 22, 2007. Based upon information provided in the petition, BRS prepared a draft EA and Plant Pest Risk Assessment (PPRA) (USDA-APHIS 2009).

Public Involvement

On September 2, 2009, APHIS published a notice in the Federal Register (74 *FR* 45413-45415, Docket no. 2007-0156) announcing the availability of the Pioneer petition requesting non regulated status for 305423 soybean, a draft plant pest risk assessment and a draft EA for a 60 day public comment period. Because the original docket was not the authorized APHIS version, a subsequent 60 day comment period was published on October 26, 2009 in the Federal Register (74 FR 54950-54951, Docket no. 2007-0156) announcing the availability of the corrected EA. This comment period ended on December 28, 2009. In total, 40 comments were received from the public during the two comment periods. All comments were analyzed to identify new issues, alternatives, or information. Responses to the substantive comments are attached to the docket submitted to the Federal Register with this Finding of No Significant Impact.

Major Issues Addressed in the EA

The EA describes the alternatives considered and evaluated using the identified issues. Issues considered in the EA were developed based on APHIS' determination to grant nonregulated status for certain genetically engineered organisms and for this particular EA, the specific deregulation of Pioneer Event DP-305423 for production of high oleic fatty acid. The following issues were identified as important to the scope of the analysis (40 CFR 1508.25):

Soybean

- Gene Movement (Pollen Flow)
- Weediness
- Human Health
- Animal Feed

Agricultural Production of Soybean

- Growing Regions and Acreage
- Organic and Conventional Soybean Production
- Herbicide Use

Soybean Composition

- Oil Composition
- Soybean Meal Composition
- Proximate, Isoflavones and Antinutrients

Impacts on Non-target Organisms

- Toxicity and Allergencity
- Nutrition
- Pest and Disease
- Soil Communities
- Threatened and Endangered Species

Affected Environment:
Although the preferred alternative would allow for plantings of Pioneer 305423 soybean to occur anywhere in the U.S., APHIS limited the environmental analysis to those areas that currently support soybean production. To determine areas of soybean production, APHIS used data from the National Agricultural Statistics Service (NASS) 2002 Census of Agriculture to determine where soybean is produced in the United States (www.nass.usda.gov, accessed 2/19/2010). Only 17 states in 2008 produced more than 1 million acres among the 31 that produced soybean according to the 2002 Census of Agriculture. Because the oil derived from this Pioneer 305423 soybean will likely replace that from existing soybean varieties, it is not likely that new land beyond that currently or historically used for soybean production will be planted to soybean by growers.

Alternatives that were fully analyzed:
The EA analyzes the potential environmental consequences of a proposal to grant nonregulated status to 305423 soybean. In order for 305423 soybean to be granted nonregulated status, APHIS must determine that 305423 soybean is not likely to pose a plant pest risk. The analysis provided in the plant pest risk assessment (USDA-APHIS 2009) demonstrates that there is sufficient data to determine that 305423 soybean is not likely 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 305423 soybean is not likely to pose a plant pest risk, the only alternative considered in the EA is granting nonregulated status "in whole" to 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. Thus, there are two alternatives that are considered in this EA: (1) no action and (2) to grant nonregulated status to 305423 soybean, "in whole."

<u>Alternative A. No Action</u>: Continuation as a Regulated Article
Under the "no action" alternative, APHIS would deny the petition. 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.

Alternative B. Preferred Alternative: Grant nonregulated status to Pioneer 305423 soybean, "in whole"- ***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).

Alternatives Considered but Rejected from Further Consideration:

Geographic restrictions -APHIS considered geographic restrictions based upon geographic variation in plant pest risk. As presented in APHIS plant pest risk assessment for Pioneer 305423 soybean, there is no geographic differences in the plant pest risks for Pioneer 305423 soybean (USDA-APHIS 2009). This alternative was rejected and not analyzed in detail because Pioneer 305423 soybean is unlikely to pose a plant pest risk and therefore, APHIS will have no regulatory authority over Pioneer 305423 soybean and will be unable to impose regulatory restrictions on this GE soybean variety.

Environmental Consequences of APHIS' Selected Action

The EA contains a full analysis of the alternatives to which we refer the reader for specific details. The following table briefly summarizes the results for each of the issues fully analyzed in the Environmental Consequences section of the EA.

Table 1.

Attribute/Measure	***Alternative A No Action***	***Alternative B Deregulation in Whole (Preferred Alternative)***
Meets APHIS Purpose and Need and Objectives	No	Yes
Unlikely to pose a plant pest risk	Satisfied through use of regulated field trials	Satisfied—risk assessment (USDA-APHIS 2009)
Farmer choice	Not available commercially	No restrictions
Soybean		
Gene Movement (Pollen Flow)	Minimal	Minimal
Weediness	None	None
Human Health	Unchanged	Unchanged
Animal Feed	Unchanged	Unchanged
Agricultural Production of Soybean		
Growing Region and Acreage	Unchanged	Unchanged
Organic and Conventional Soybean Production	Unchanged	Unchanged
Herbicide Use	Unchanged	Unchanged
Soybean Composition		
Oil Composition	Unchanged	FDA approved safety of changes

Soybean Meal Composition	Unchanged	Unchanged
Proximate, Isoflavones and Antinutrients	Unchanged	Unchanged
Impacts on Non Target Organisms		
Toxicity and Allergenicity	Unchanged	Unchanged
Nutrition	Unchanged	Unchanged
Pest and Disease	Unchanged	Unchanged
Soil Communities	Unchanged	Unchanged
Threatened and Endangered Species	Unchanged	Unchanged
Other Regulatory Approvals		
U. S.	Completion of FDA consultation	Completion of FDA consultation
Foreign Trade	Approvals from Canada, Mexico	Approvals from Canada, Mexico
Compliance with Other Laws		
CWW, CAA. EOs	Fully compliant	Fully compliant

Finding of No Significant Impact

The analysis in the EA indicates that there will not be a significant impact, individually or cumulatively, on the quality of the human environment as a result of this proposed action. I agree with this conclusion and therefore find that an EIS need not be prepared. This NEPA determination is based on the following context and intensity factors (40 CFR 1508.27):

Context – The term "context" recognizes potentially affected resources, as well as the location and setting in which the environmental impact would occur. This action has potential to affect conventional and organic soybean production systems, including surrounding environments and agricultural workers; human food and animal feed production systems; and foreign and domestic commodity markets. As identified in the Affected Environment section above, although the preferred alternative would allow for new plantings of DP-305423 soybean to occur anywhere in the U.S., the environmental analysis is limited to those areas that currently support soybean production, predominantly focused in 17 states. Users of soybean products, both food and industrial products could be potentially impacted by this action.

Intensity – Intensity is a measure of the degree or severity of an impact based upon the ten factors. The following factors were used as a basis for this decision*:*

1. *Impacts that may be both beneficial and adverse.*
The advantageous and healthful properties of oil derived from the DP-305423 soybean and properties of the fatty acid ratios are in demand by food producers to replace to some extent oil products from existing lines of soybean. 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). As identified in the response to comments, concern has been expressed that if a sufficient amount of Pioneer 305423 soybeans inadvertently enters the commodity soybean supply stream and not the appropriate identity preserved food chain, the Nutrition Facts Panel of some products may not correctly reflect the fatty acid ratios. As described in Chapter 5 of the EA, Pioneer 305423 soybean is expected to be adopted as an adjunct to conventional commodity soybean oil, but will be marketed as a specialty soybean and grown under an identity preserved process thereby reducing the potential of inadvertent mixing. Granting nonregulated status to DP 305423 soybean will not impact agricultural acreage devoted to soybean production, and will likely only displace production of existing varieties. If this line is given non regulated status, there are no foreseeable changes to the availability of GE, conventional, organic or specialty soybean varieties on the market.

2. *The degree to which the proposed action affects public health or safety.*

The proposed action to grant nonregulated status to DP-305423 soybean would have no significant impacts on human or animal health. High oleic acid event DP 305423 is not materially different in composition, safety, or any other relevant parameter from soybean now grown, marketed, and consumed, except for the desired increases in oleic acid, and decreases in linoleic and linolenic acid. Information presented in the petition suggests that because alterations of these common fatty acids resemble ratios of fatty acids that are derived from other oilseed crops, no impacts were expected from the commercial sale or use of the derived oils in foods. As described in Chapter 5 of the EA, soybean varieties with alterations of fatty acid ratios are currently available to growers, including those with elevated oleic acid. These crops have been used safely in the marketplace. FDA completed the safety and nutritional assessment for this product and had no further questions regarding the safety of Pioneer 305423 soybean (FDA 2009). Based on the assessment of the laboratory evidence provided in the petition and accompanying scientific literature, APHIS has concluded that Pioneer 305423 soybean would have no significant impacts on human or animal health.

3. *Unique characteristics of the geographic area such as proximity to historic or cultural resources, park lands, prime farmlands, wetlands, wild and scenic rivers, or ecologically critical areas.*

There are no unique characteristics of geographic area such as park lands, prime farm lands, wetlands, wild and scenic areas, or ecologically critical areas that would be significantly affected. DP-305423 will only be grown in areas suitable for the production of soybean and those historically used for soybean production.

There is no significant difference in performance or agricultural practices for the growth of DP-305423 soybean compared to other soybean varieties, and no natural resources or land usage will be significantly altered through the production of DP-305423 soybean.

4. *The degree to which the effects on the quality of the human environment are likely to be highly controversial.*

The effects on the quality of the human environment are not highly controversial. Although there is some opposition to the granting of nonregulated status to DP-305423 soybean, this action is not highly controversial in terms of size, nature or effect. Other than objections to all genetically engineered crops, the public comments did not register any specific factual concerns with the data provided APHIS for this crop and which were presented in the EA. Interest in maintaining product identity and separation was expressed, because some products might be affected by differences of DP-305423 soy oil from commodity soy oil. The importance of company sponsored stewardship plans and manufacturer surveillance of purchased oil is discussed in the APHIS response to comments.

5. *The degree to which the possible effects on the human environment are highly uncertain or involve unique or unknown risks.*

The effects of the proposed non regulated status for DP 305423 are not highly uncertain and do not involve unique or unknown risks. Based on the analysis documented in the EA, the effects on the human environment would not be significant. APHIS has no evidence for any unknown risks of this product when released for commercial production. As described in Chapter 5 of the EA and response to comments, well established management practices, production controls, and production practices (GE, conventional, and organic) are currently being used in soybean production systems in the US. Therefore, it is reasonable to assume that farmers, who produce conventional soybean, DP-305423 soybean, or produce soybean using organic methods, will continue to use these reasonable, commonly accepted best management practices for their chosen system and varieties during agricultural soybean production. Additionally, 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). The availability of DP 305423 soybean would offer growers and manufacturers another choice of modified fatty acids in addition to the options already available.

6. *The degree to which the action may establish a precedent for future actions with significant effects or represents a decision in principle about a future consideration.*

The proposed action would not establish a precedent for future actions with significant effects or represent a decision in principle about a future decision. Similar to past petitions for nonregulated status (http://www.aphis.usda.gov/brs/not_reg.html), the APHIS decision on the regulatory status of DP 305423 soybean will be based upon information provided in the applicant submitted petition. APHIS regulations at 7 CFR part 340, regulate the introduction (importation, interstate movement, or release into the environment) of certain GE organisms and products. 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 "Petition for Determination of Nonregulated Status." After receipt of a petition, BRS makes an independent determination on whether an organism is unlikely to pose a plant pest risk and is therefore no longer subject to the regulatory requirements of 7 CFR part 340. Each petition that APHIS receives undergoes this independent review to determine if the regulated article poses a plant pest risk.

7. *Whether the action is related to other actions with individually insignificant but cumulatively significant impacts.*
No significant cumulative effects were identified through this assessment. The EA discussed cumulative effects on soybean management practices, human and animal health, and the environment and concluded that such impacts were not significant. A cumulative effects analysis is included in Chapter 5 of the EA.

8. *The degree to which the action may adversely affect districts, sites, highways, structures, or objects listed in or eligible for listing in the National Register of Historic Places or may cause loss or destruction of significant scientific, cultural, or historical resources.*
DP 305423 soybean 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. Granting nonregulated status to DP 305423 soybean will not cause an increase in agricultural acreage devoted to soybean cultivation, or to acres devoted to GE soybean cultivation because the product oil will replace some existing soybean oil, both conventional commodity soybean oil and specialty oils such as low linolenic oil. DP-305423 soybean will not alter geographic locations of future soybean production in the U.S. because the crop has production needs that are the same as those for conventional soybean.

9. *The degree to which the action may adversely affect an endangered or threatened species or its habitat that has been determined to be critical under the Endangered Species Act of 1973.*
APHIS evaluated the potential for negative effects on federal threatened and endangered species as listed by the U.S. Fish and Wildlife Service from cultivation of 305423 soybean and its progeny and determined that the release of 305423 soybean, following a determination of nonregulated status, would have no effect on federally listed threatened or endangered species or species proposed for listing, or on designated critical habitat or habitat proposed for designation (see section on Threatened and Endangered Species, pages 25-28 of the EA).

10. *Whether the action threatens a violation of Federal, State, or local law or requirements imposed for the protection of the environment.*
The proposed action would be in compliance with all federal, state, and local laws. The proposed action to grant nonregulated status to DP-305423 and remove this GE soybean variety from APHIS' regulatory oversight would be carried out in accordance with 7 CFR part 340. DP-305423 soybean has successfully completed the consultation process with the FDA concerning food and feed safety

(Appendix 1 of the EA). DP-305423 soybean does not express any genetically engineered pesticides or tolerance to herbicides; thus EPA consultation is not required for this product. There are no other Federal, state, or local permits that are needed prior to the implementation of this action. A list of the current status of U.S. and international approvals is found in Table 1 of this Decision Document.

Literature Cited:

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USDA-NASS. (2008) Acreage. United States Department of Agriculture, National Agricultural Statistics Service, Washington, D.C. http://usda.mannlib.cornell.edu/usda/current/Acre/Acre-06-30-2008.txt

NEPA Decision and Rationale

I have carefully reviewed the EA prepared for this NEPA determination and the input from the public involvement process. I believe that the issues identified in the EA are best addressed by selecting Alternative B - Grant nonregulated status to Pioneer 305423 soybean, "in whole".

As stated in the CEQ regulations, "the agency's preferred alternative is the alternative which the agency believes would fulfill its statutory mission and responsibilities, giving consideration to economic, environmental, technical and other factors." The preferred alternative has been selected for implementation based on consideration of a number of environmental, regulatory, and social factors. Based upon our evaluation and analysis, Alternative B is selected because (1) it allows APHIS to fulfill its statutory mission 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; and (2) it allows APHIS to fulfill its regulatory obligations. Since APHIS has concluded that that Pioneer DP 305423 soybean is unlikely to pose a plant pest risk, APHIS has no authority to continue to regulate a GE organism once it has determined that the GE organism does not pose a plant pest risk. The comments identified from public involvement did not change the results of the analysis. Therefore, it is my decision to implement the preferred alternative as described in the EA.

Sidney W. Abel Eco

for Michael C. Gregoire
Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Services
U.S. Department of Agriculture

JUN 7 2010

Date

Response to Comments
Petition 06-354-01

APHIS reviews a petition for nonregulated status to determine if the genetically engineered (GE) organism should no longer be considered a regulated article under APHIS biotechnology regulations (7 Code of Federal Regulations (CFR) part 340). Prior to reaching a decision, APHIS prepared a plant pest risk assessment to evaluate whether Pioneer 305423 soybean is likely to pose a plant pest risk. After finding that Pioneer high oleic soybean is not likely to pose a plant pest risk, and is eligible for nonregulated status, APHIS prepared a draft environmental assessment (EA) to evaluate whether there could be significant impacts on the environment arising from a decision to grant a determination of nonregulated status to Pioneer 305423 soybean. APHIS prepared the EA as part of its obligation to meet the statutory requirements of the National Environmental Policy Act (NEPA) of 1969. As part of this process, APHIS considered public comments received on the petition for deregulation and associated draft EA. This document provides APHIS' response to these comments.

On September 2, 2009, APHIS published a notice in the Federal Register (74 *FR* 45413-45415, Docket no. 2007-0156) announcing the availability of the Pioneer petition requesting nonregulated status for 305423 soybean and associated draft EA for public review and comment for a 60-day period. This comment period was scheduled to end on November 2, 2009. Because the original docket was not the correct APHIS version, a subsequent 60 day comment period was published on October 26, 2009 in the Federal Register (74 FR 54950-54951, Docket no. 2007-0156) announcing the availability of the corrected EA. This comment period ended on December 28, 2009. APHIS received a total of 40 comments from various groups and individuals during the two comment periods. Twenty-two comments supported deregulation, eighteen comments generally opposed the development and use of GE foods (twelve from one individual), one comment emphasized a need for strict regulation, and one comment generally disagreed about the overall need for this soybean crop.

Those in favor of non regulated status included five state senators or representatives, five departments of agriculture, three food or industrial companies, three soybean associations, and two soy or food institutes. Those in opposition to granting non regulated status were individual consumers, who as noted, mostly did not offer opposition to this specific product, but were opposed to all genetically modified plants. Supporters of nonregulated status cited a number of benefits if this product were available for commercial production including: (1) the product's fatty acid ratios are more healthful to the public than conventional soy oil; (2) oil derived from the product would be useful to the food and non foods industry; and (3) the growers will be able to market a new product that expands the potential commercial possibilities for soybean because of an improved fatty acid balance. Several comments from various industry associations focused on Pioneer/Dupont's statement of support for adequate risk assessment and risk management programs sponsored by the technology provider, and Pioneer's acceptance of a total life cycle approach for the product in domestic and export markets. Of those opposed, none provided specific disagreement with the analysis provided by the environmental assessment, but were focused primarily on opposition to control of the seed supply by the federal government, or control by large corporations that ostensibly desire to increase the cost of seed. Some were concerned about consequences of gene splicing, such as creating plant pests, introducing dangers from cross-pollination of wild plants, generating unintended consequences in the GE plants, producing plants that were poisonous or otherwise hazardous, causing GE plants to become mutagenic, or causing organ toxicity, or otherwise generating unknown, long-term risks. Still other comments stated that an EIS needs to be done for this proposed product.

1. Ecological Effects

Two comments suggest that genetically engineered crops will cause chemical harm to the environment or that these GE crops endanger wild and native plants near where they are planted.

APHIS response: Based upon information and analysis presented in the petition, plant pest risk assessment (USDA 2009), and EA, APHIS has not identified any potential for chemical harm to the environment deriving from Pioneer 305423 soybean. Aside from the percentages of poly- and mono-unsaturated fatty acids, the chemical composition of high oleic soybean does not differ significantly from conventionally bred soybeans. Oleic acid is a naturally occurring, nontoxic substance present in soy and many other edible oils, canola and sunflower oils, especially those enhanced for high oleic acid expression (see EA Section V, C). Soybean is not a plant native to North America, and as a result, does not have any known wild or weedy relatives in the United States with which it could interbreed (see EA Section III, A). In addition, soybean is primarily self-pollinating, so gene flow beyond modest isolation distances to surrounding cultivated soybean (beyond 4.6 m) would be rare and not likely (see EA Section III, A).

2. Agricultural Impacts

Some comments suggested that inadvertent commingling of the high oleic soybean with commodity soybean will impact the accuracy of data on the Nutrition Facts Panel for food products, because the percentage of saturated fatty acids, mono- and poly-unsaturates would be altered if the product contained as little as 5% of the specialty product.

APHIS response: Pioneer 305423 soybean contains reduced levels of palmitic acid, a saturated fat, and elevated levels of high oleic acid, a monounsatured fat (see Section I of the EA). Because of the health benefits of an increase in specific fatty acids in Pioneer 305423 soybean as compared to commodity soybean, manufacturers may preferentially select these oils for manufacturing many food products. FDA requires that product fatty acid ratios be reflected in the Nutrition Facts Panel of the product label. If sufficient amounts of Pioneer 305423 soybeans inadvertently enter the commodity soybean supply stream and not the appropriate identity preserved food chain, the Nutrition Facts Panel of some products may not correctly reflect the fatty acid ratios. In public comments submitted to APHIS for this product, Pioneer suggests that commingling of above 5% content of high oleic acid soybean oil from Pioneer 305423 in commodity oils could be sufficient to require alteration of the labeled fatty acid content.

Because of the value-added traits of this soybean, growers and processers will preserve product identity to the point of sale, thus keeping Pioneer 305423 soybean separate from commodity soybean (see Pioneer 305423 petition, amended Section X-XF-7, Potential impact on organic and conventional farming). Pioneer proposes to use "an identity preserved (IP) system …from seed development through refined oil produced for delivery to end-use customers" (Pioneer Public Comment APHIS Docket 2007-0156-0045; Regulations.gov (2009). The details would resemble "similar systems … throughout the vegetable oil industry." Other IP oilseed management systems include sales of the seed only to growers who sign a contract with the purchaser to produce the oilseed to specified standards, and to sell their soybeans only to the contracted party and to its designated elevator or crushing facility. Oilseed processors would likely be required to sign contracts with refiners so that end users would have a trackable product that derived only from identified transgenic seed. In the same letter submitted as a public comment, Pioneer notes that analytical techniques are available to qualitatively identify fatty acid

content in delivered oilseed using NIR (Near infrared) technology. For analysis of fatty acids in derived oils, highly accurate gas chromatographic analytical techniques are available.

Oils derived from Pioneer 305423 soybean will be separately marketed from conventional soybean oils, because these oils contain healthy fatty acids (such as the monounsaturated oil, oleic acid) when consumed in manufactured food products such as salad oils (See EA Section I. and II.). Oleic acid is a natural constituent of a variety of foods, notably for example, olive oil (55-83% oleic acid, Vossen, 2010). Olive oil has been shown to be a health-promoting dietary constituent, and the oleic acid component has been directly shown to be responsible for lowering blood pressure (Terés et al., 2008).

To monitor product integrity, manufacturers of food products may need to continuously assay fatty acid ratios of constituent soybean oil. If manufacturers are vigilant and employ good quality control techniques, it is not likely that high oleic soybean oil would be inadvertently incorporated in sufficient amounts that would change the accuracy of the fatty acid ratio reflected on the Nutrition Facts Panel. In cases of a manufacturer receiving shipment of an ingredient oil (ie., high oleic soybean oil) different from that typically used in a specific product, the manufacturer could appropriately blend and dilute the oils before commercial sale and release, and the Nutrition Facts Panels would accurately reflect fatty acid content.

Regulations.gov (2009). Public Comment from Pioneer Hi-Bred (Natalie Hubbard, Director, Biotechnology, North America).
http://www.regulations.gov/search/Regs/home.html#documentDetail?R=0900006480a6ec3a

Terés, S., Barceló-Coblijn, G., Benet, M., Álvarez, R., Bressani, R., Halver, J.E. and Escribá, P. V. (2008). Oleic acid content is responsible for the reduction in blood pressure induced by olive oil. Proc Natl Acad Sci U S A 105, 13811–13816.

Vossen, P. (2007). International Olive Council (IOC) and California Trade Standards for Olive Oil. University of California, Cooperative Extension.
http://ucce.ucdavis.edu/files/filelibrary/2161/34496.pdf

Two comments state that GE soybeans are "dangerous" and that they are "noxious weeds and not food"

APHIS response: As summarized in the EA (Section V. B. A.), the products of these GE soybeans are as safe and nutritious as those currently available in the marketplace. Soybean as a crop is not weedy (EA Section III A) and the modified Pioneer 305423 soybean according to APHIS' assessment is also not weedy (EA Section V. B.B.). The 305423 soybean has been field tested since 2002 in the major soybean growing regions of the continental United States. Under field permits granted by USDA APHIS, Pioneer conducted comprehensive agronomic performance and ecological observations for 305423 soybean in replicated, multi-site field studies. In 2005 and 2006 growing seasons, Pioneer made observations and measurements of the soybean variety in a total of 13 North American locations. Characteristics such as emergence, seedling vigor, plant height, lodging, days to maturity, shattering, seed weight, yield, disease incidence and insect damage were measured. Data for seed germination and dormancy were also collected in laboratory experiments. From these observations and data submitted in the applicant's petition, APHIS has concluded in its plant pest risk assessment (USDA 2009) that there are no weed risk issues associated with Pioneer 305423 (See EA Section V B.B. of the EA).

USDA (2009). APHIS BRS Website. Petitions for Non-regulated Status. http://www.aphis.usda.gov/biotechnology/not_reg.html

Two comments stated that genetic engineering methods are less precise and differ from conventional breeding methods, and thus may have unintended harmful effects; one stated he had concerns about genetically modified organisms and that they should be treated differently than those plants altered through traditional breeding methods.

APHIS response. The insertion of transgenes is an imprecise process, in the sense that one cannot predict the insertion site within the host genome. Some unintended changes to the plant genome may result and may lead to undesired consequences, but plant breeders place considerable importance in avoiding negative effects on agronomic performance and overall health of the transformed plants. Potential unintended changes that might affect plant phenotype include insertions of the transgene into active host genes, insertion of multiple tandem copies of the transgene, and rearrangements of genetic material at the insertion site. Other genetic changes may occur at locations distant from the insertion site of the transgene of interest, either as a result of the insertion of transgene fragments or deriving from the culture process accompanying the transformation process.

As Pioneer described within the petition (p. 23, Pavely, 2007, Petition for the Determination of Nonregulated Status for High Oleic 305423 Soybean), agronomic properties of the plants were assessed over several generations, then backcrossed to elite, well-performing soybean lines a total of six times. Backcrossing with elite lines dilutes the presence of incidental genetic changes not closely linked to the transgene within the transgenic soybean and confers improved agronomic properties to the newly transformed line. As a result of backcrosses, the expected transgene is obtained within a final stable and predictable soybean line that has a reduced likelihood of additional, unexpected genes and traits (see page 20, Figure 2, Pavely, 2007).

Inadvertent, genetic changes closely linked to the site of insertion for the gene of interest would not be so easily removed by backcrossing. For instance, an inserted transgene could conceivably interrupt a gene directing a plant function ("insertional mutagenesis"). In many cases however, plants have multiple genes that provide the same function, such as the multiple FAD-2 genes of soybean that form linoleic acid (Graef et al., 2009). Likewise, plants also may have multiple structural proteins that contribute to the same plant parts (Barker, et al., 2008). If insertion sites do interrupt essential genes, the resulting plants would most likely be unhealthy and consequently be eliminated by developers.

The process of transformation could potentially lead to new genetic changes by somatic mutation, which may occur spontaneously during a procedure for regenerating plant tissues, which is typically part of the transgenesis process. However, the developer's normal process of selection, and then hybridization with conventional, non transgenic lines, along with final product development may also eliminate most of these unintended mutations. For those genetic changes that result in noticeable traits, breeders can assure removal of undesirable traits from future product releases.

APHIS requires that any notable changes in the expected plant phenotype, or its agronomic properties, and modifications of pest susceptibility be identified, both after it is released under APHIS permit and later, as a condition of its deregulation. Finally, developers conduct repeated tests to ensure the reproducibility of the trait, as well as its negligible impact on overall plant health. APHIS regulatory actions provide oversight of new genetically modified plants, and the oversight is extensive compared to those for new traits in conventionally bred plants.

Conventional breeding techniques to gain new traits are not necessarily more precise than biotechnological methods that transpose novel genes into the plant genome. Conventional seed breeders may gain new material for the host plant by crossing with other species or even genera and by subjecting plants to chemical or radiation mutagenesis. Through much trial and error, the host may acquire genes from the introgressed plant genus or species, or by mutation, but these may incorporate regulatory, structural or functional genes not identified or completely understood. The genetic material deriving from "wide crosses" may include large segments of chromosomes, or be associated with translocations or inversions (Osborn *et al.* 2007)). The unexpected random genetic changes from these crosses and also from mutations may be more extensive than those changes introduced by transgenic technology (Batista *et al.* 2008).

References.

Barker, M.S., Kane, N.C., Matvienko, M., Kozik, A., Michelmore, R.W., Knapp, S.J., and Rieseberg, L.H. (2008). Multiple paleopolyploidizations during the evolution of the compositae reveal parallel patterns of duplicate gene retention after millions of years. Molecular Biology and Evolution, 25, 2445-2455

Batista, R., Saibo, N., Lourenco, T., and Oliveira, M.M. (2008). Microarray analyses reveal that plant mutagenesis may induce more transcriptomic changes than transgene insertion. Proceedings of the National Academy of Sciences. 105: 3640-3645

Graef, G., LaVallee, B.J., Tenopir, P., Tat, M., Schweiger, B., Kinney, A.J., Van Gerpen, J.H., and Clemente, T.E. (2009). A high-oleic-acid and low-palmitic-acid soybean: agronomic performance and evaluation as a feedstock for biodiesel. Plant Biotechnology Journal 7, 411–421.

Osborn, T.C., Kramer, C., Graham, E., and Braun, C.J. (2007). Insights and Innovations from Wide Crosses: Examples from Canola and Tomato. Crop Science. 47: S-228-S-237.

Pavely, C. (2007). Petition for the Determination of Nonregulated Status for High Oleic 305423 Soybean, revised version. Pioneer Hi-Bred International, Inc., Johnston, IA. Available USDA BRS website.

One commenter from a food company asserted that these soybeans will reduce use of pesticides.

APHIS response: Pioneer 305423 is not genetically engineered to be glyphosate tolerant (see EA Section V. B.B.). Weed management practices for Pioneer soybean will not differ from those used in conventional soybean (see EA Section III.B.). Although sulfonylurea tolerant traits are expressed in this Pioneer soybean, these will not be promoted for this Pioneer line; the tolerance was only used for trait selection during product development (see EA Section V).

3. Human Health Impacts

Foods derived from biotechnology should not require a different or higher standard of safety than that of conventional foods, but should be examined if appropriate on a case-by-case basis for safety before commercialization.

APHIS response: Under the Coordinated Framework for biotechnology products, the FDA will continue to assess the safety and quality of GE foods, and the EPA will assess the safety of plant incorporated protectants and herbicide tolerant crop varieties (EA Section I). The coordinated deliberative process should continue to reduce concerns by the public about the healthfulness and safety of these genetically engineered products.

One comment stated that because long-term environmental and health risks of GE crops have not been studied, and because soybean is a commodity crop and ubiquitous in many food products, GE varieties should be strictly regulated, especially soybean.

APHIS response: GE varieties, including this Pioneer 305423 soybean are subject to considerable scrutiny by the FDA for food safety and by the USDA for agricultural safety. The Coordinated Framework for Products of Biotechnology intends that both health risks and environmental impacts will be thoroughly analyzed by the appropriate federal agencies. Products with undesirable properties causing extensive impacts greater than those from non-transgenic products of the same type could be identified by this assessment process. If these products are not acceptable under the regulations of an agency within the USDA, they will not be granted non regulated status. The FDA is confident that Pioneer 305423 soybean is not materially different from soybean already grown, except for the altered fatty acid content (see FDA Consultation Document and Section V.B.A.). Based upon information and analysis presented in the petition, plant pest risk assessment (USDA 2009), and EA, USDA-APHIS has concluded that Pioneer 305423 soybean will have no impacts on the agricultural or natural environment beyond those already arising from other commercial soybean varieties. Pioneer 305423 soybean has received extensive assessment from APHIS and FDA and based upon their assessments, will likely have only beneficial impacts on stakeholders and the public.

Some comments stated that GE soybeans are harmful because they may have as yet unrecognized adverse long term effects on human health.

APHIS response: Beginning in December 2006, Pioneer provided data to the FDA establishing the food safety and nutritional properties of this product. The FDA concurred with Pioneer's determination that the 305423 soybean and downstream products from it are not materially different from commercial soybeans in composition, safety or any other relevant parameter (FDA Consultation Document). In animal feeding tests using mice and broiler chickens (See studies cited in EA Section V. B.C.) no short term impacts could be observed from consumption of Pioneer 305423 soybean. No evidence of nutritional deficiencies were found after analysis of proteins, fats (except for the intentionally changed fatty acids) or fiber (EA Section V. B.C.). No differences were found in antinutrient content, when compared with average antinutrient content of similar commercial soybean varieties.

Under FDA supervision of tests of food ingredients, the chemical structure of a proposed additive and its expected concentration within foods must be considered in order to assign a concern level; the level will determine which types of toxicity tests will be done (NRC, 2006; FDA, 2000). For additives of low concern, short term feeding studies are required, and if of highest level of concern then lifetime studies and even some multi-generation studies, or studies contingent on certain results based on tests results from lower levels of concern are required (NRC, 2006). If new food components are rated as high risk to consumers, then successively more lengthy and demanding tests are required. FDA's level of concern is related to hazard and risk and FDA has required of Pioneer the types and terms of analyses appropriate to risk for 305423 soybean.

References.

FDA (2000). Redbook 2000. Revised July 2007. Guidance for Industry and Other Stakeholders Toxicological Principles for the Safety Assessment of Food Ingredients. http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/FoodIngredientsandPackaging/Redbook/default.htm

NRC (2006). Toxicity Testing for Assessment of Environmental Agents. Interim Report. Committee on Toxicity Testing and Assessment of Environmental Agents National Research Council of the National Academies. The National Academies Press. Washington, D.C.

One commenter asserts that GE crops have decreased nutritional value. Another comment states that GE food should be separated from "natural", non-genetically modified food.

APHIS response: Under the Federal Food, Drug, and Cosmetic Act, the FDA issued a policy statement concerning genetically engineered food and animal feed. Following the announced procedure, FDA employs a consultation process for products derived from biotechnology to ensure that regulatory issues such as food and feed safety and labeling concerns are addressed and resolved (see EA Section I.). Based upon this FDA consultation process, comparisons with commercial soybean show that Pioneer 305423 is equally safe and nutritious as other varieties currently marketed (FDA Premarketing Consultation: see attachment to EA) From FDA's consultation on foods derived from this soybean, and APHIS' assessment of the laboratory evidence provided by Pioneer (Pavely, 2007) and accompanying scientific literature, APHIS concludes that there is no scientific basis to require separate storage, marketing or handling of this variety and commodity soybeans.

Because of the value-added traits of this soybean, growers and processers will preserve product identity to the point of sale (Pioneer, see Summary in Pavely, 2007) as is commonly done for many specialty oils (Lee and Herbek, 2004), thus keeping Pioneer 305423 soybean separate from commodity soybean (see Petition amendment X-XF-7, Potential impact on organic and conventional farming). The operational details for Identity Preserved programs with oilseed crops are discussed in this Response to Public Comments under "2. Agricultural Impacts." Oils derived from Pioneer 305423 soybean will also be separately marketed from conventional soybean oils (Pioneer HiBred, See Summary in Pavely, 2007), because these oils contain healthy fatty acids (such as the monounsaturated oil, oleic acid) for manufactured food products such as salad oils (See EA Section I. and II.). Oleic acid is a natural constituent of a variety of foods, notably for example, olive oil (55-83% oleic acid, Vossen, 2010). Olive oil has been shown to be a health-promoting dietary constituent, and the oleic acid component has been directly shown to be responsible for lowering blood pressure (Terés 2008).

Other non transgenic varieties of soybean also express increased oleic acid from extracted oils (Fehr, 2007; Clemente and Calhoon, 2009). Some of these have been in commercial production (J.J.G. Minot, Iowa State University Research Foundation, personal communication). Soybean expressing a nontransgenic trait to decrease linolenic acid has also been combined with a trait for increased oleic acids (>50% oleic; i.e., IA2028 available from Iowa State University Research Foundation). These non-transgenic lines of soybean with altered fatty acids have been derived from mutagenized soybean and, highly selected lines. Pioneer's high oleic soybean varieties are not significantly different from these varieties derived by conventional means, and the non-transgenic soybeans have already been used commercially without reported environmental impacts (J.J.G. Minot, Iowa State University Research Foundation, personal communication)

References.

Clemente, T.E. and Calhoon, E.B. (2009). Soybean oil: Genetic approaches for modification of functionality and total content. Plant Physiology 11: 1030-1040.

Fehr, W.R. (2007). Breeding for modified fatty acid composition in soybean. Crop Science, 47, (SUPPL. DEC.) S72-S87

Lee, C and Herbek, J. (2004). Specialty Soybean Production and Management in Kentucky, University of Kentucky, Cooperative Agricultural Extension Service. AGR-182. http://www.ca.uky.edu/agc/pubs/agr/agr182/agr182.pdf

Terés, S., Barceló-Coblijn, G., Benet, M., Alvarez, R., Bressani, R., Halver, J.E., and Escribá, P.V. (2008). Oleic acid content is responsible for the reduction in blood pressure induced by olive oil. Proceedings of the National Academy of Sciences 105 (37): 13811.

Vossen, C. (2010 download). International Olive Oil Council (IOOC) Trade Standard for Olive Oil. University of Californian Cooperative Extension, UC Davis, Sonoma County Extension. http://cesonoma.ucdavis.edu/hortic/pdf/iocc_standards_purity_grade.pdf

One commenter says that GE has caused greater amounts of diseases in consumers since the introduction of GE crops.

APHIS response: Although the rate of certain diseases or conditions in consumers may have indeed increased since the introduction of GE crops in the mid-1990's (for example, see Branum and Lukacs, 2009), a trend cannot be attributed directly to use of any food item, including GE crops or their products. An apparent increase may often result from increases in incidence reporting (Branum and Lukacs, 2009). Increased incidence of disease could also be attributed to any number of environmental or medical causes. The Pioneer 305423 soybean does not contain any other constituent likely to enhance disease incidence. For example, measured content of antinutrients is not greater than conventional soybean (see EA Section V. B.C.), nor is the constitutive GM-HRA protein a known human allergen (see EA Section V. Potential impact on nontarget organisms). Pioneer submitted this data to the FDA who reviewed the conclusions to complete the FDA consultation. No consequences for other animals consuming products from this soybean line are expected on the basis of these tests, and none would be expected from the nature of the transformation in Pioneer 305423.

References:

Branum A.M., and Lukacs S.L. (2009). Food allergy among children in the United States. Pediatrics. 124, 1549-55.

4. Animal Impacts

One comment suggests that gene transformation is highly mutagenic and could lead to multi-organ toxicity and impacts on blood and immune systems. One commenter states that there are many animal studies that show a link between adverse health risks and

ingestion of GE foods and asks for a moratorium on GE food approvals. One commenter says that animals fed with GE crops show acute signs of aging, and decreased fertility.

APHIS response: Based upon information and analysis presented in the petition, plant pest risk assessment (USDA 2009), an EA and an FDA consultation process, 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.

Pioneer 305423 soybean contains a modified acetolactate synthase gene, which confers herbicide resistance for the purpose of selection during transformation (see EA Section I.). As part of the petition, Pioneer submitted studies showing that mice fed with 2000 mg of purified GM-HRA protein (derived from the *Gm-hra* gene, a modified version of the soybean acetolactate synthase gene) per kilogram of body weight showed no acute toxicity. Expected concentrations of ingested GM-HRA protein soybean grain are far lower, at 2.5 ng/mg, than the purified dosage used in the mice trial (See Section VII C., Taveley, 2007). It is not likely that Pioneer 305423 soybean will attain the high concentration to which these mice were exposed (see EA Section V.B.C.). A second transformation in Pioneer 305423 soybean does not produce a novel protein, but suppresses the conversion of oleic acid into linoleic acid by an endogenous gene normally present in soybean (see Summary in Tavely, 2007). The sequence gm-fad2-1 derived from an existing soybean gene, if expressed, would not have any novel nutritional impacts because it does not express a novel protein (Tavely, 2007).

In addition, APHIS evaluated evidence submitted by Pioneer of a 42-day study showing that broiler chickens, when fed 305423 soybean, did not have statistically significant differences in mortality, weight gain, mortality-adjusted feed efficiency, or carcass yields from those fed control (non-GE) soybean (see Petition, page 8 to 9). As a result, Pioneer 305423 soybean should not adversely affect wildlife feeding in soybean fields planted to 305423 (see EA Section V. Potential Impact on Nontarget Organisms). As far as the general assertion of transgenesis causing unhealthy mutations, the process of tissue culture used to regenerate a plant from an undifferentiated single cell to a mature organism after transformation may cause some somatic mutations (see discussion above). Selection and removal of plants with undesirable agronomic characteristics after transformation will exclude such mutations in the final commercialized product (Filipecki and Malepszy 2006). The issue of mutagenesis by gene disruption (insertional mutagenesis) was discussed in the response to a comment above about the precision of transgenic methods used to improve crop plants. M.W. Ho and J. Cummins have asserted that certain types of promoters used in transgenesis may be mutagenic but this opinion has received no confirmation or support from molecular biologists (Hodgson, 2000).

Evidence for impacts of transgenic foods on tissues, organs or blood, while asserted by a limited number of authors, is unlikely. For example, an initial report was shown to be inadequate, poorly controlled and further evidence was contradictory (Royal Society, 1999; Chassy, 2002). Although Ewen and Pusztai 1999b endeavored to show pathology of rat intestinal tissue in rats fed lectin transformed potatoes, (a line never seriously considered for commercialization or release) there are several later studies that contradict these findings of toxicity or pathology for a substantial number of histological benchmarks (Hashimoto et al. 1999; Pusztai et al., 1999a; Teshima et al., 2002; El-Sanhoty et al., 2004). APHIS asserts from considerable evidence that GE plants in general do not have any general toxic properties (as noted above, and in the detailed studies submitted in support of all petitions to grant nonregulated status for GE crop varieties). Lectins are known to have toxicities when animal cells are exposed to them (Vasconcellos and Oliveira

2004) and if expressed in potatoes could possibly induce pathologies, although even these were not substantiated in the case of Ewen and Puszai (1999; see Royal Society, 1999).

References.

Chassy, B.M. (2002). Food Safety Evaluation of Crops Produced through Biotechnology Journal of the American College of Nutrition 21, 166S-173S.

El-Sanhoty, R., El-Rahman, A.A.A., and Bögl, K.W. (2004). Quality and safety evaluation of genetically modified potatoes Spunta with Cry V gene: compositional analysis, determination of some toxins, antinutrients compounds and feeding study in rats. Nahrung: Food,48, 13-18.

Ewen, S.W.B. and Pusztai, A. (1999b) Effects of diets containing genetically modified potatoes expressing Galanthus nivalis lectin on rat small intestine. The Lancet 354, 1353-1354

Filipecki, M. and Malepszy, S. (2006). Unintended consequences of plant transformation:a molecular insight. J Appl Genet 47:277–286.

Hashimoto, W., Momma, K., Yoon, H-J, Ozawa, S., Ohkawa, Y., Ishige, T., Kito, M., Utsumi, S, and Murata, K. (1999). Safety Assessment of Transgenic Potatoes with Soybean Glycinin by Feeding Studies in Rats. Biosci. Biotechnol. Biochem. 63, 1942-1946.

John Hodgson (2000). Scientists avert new GMO crisis. Nature Biotechnology 18, 13 doi:10.1038/71838.

Palombo, J.D., De Michele, S.J., Liu, J.W., Bistrian, B.R., and Huang, Y.S. (2000). Comparison of growth and fatty acid metabolism in rats fed diets containing equal levels of gamma-linolenic acid from high gamma-linolenic acid canola oil or borage oil. Lipids 9, 975-81.

Pusztai, A., Grant, G., Bardocz, S., Alonso, R., Chrispeels, M.J., Schroeder, H.E., Tabe, L.M., and Higgins, T.J.V. (1999a). Expression of the Insecticidal Bean α-Amylase Inhibitor Transgene Has Minimal Detrimental Effect on the Nutritional Value of Peas Fed to Rats at 30% of the Diet. Journal of Nutrition. 129, 1597-1603.

Royal Society (1999) Review of Data on Possible Toxicity of GM Potatoes (Ref. 11/99). London: The Royal Society. http://royalsociety.org/WorkArea/DownloadAsset.aspx?id=5890

Teshima, R., Watanabe, T., Okunuki, H., Isuzugawa, K., Akiyama, H., Onodera, H., Imai, T, Toyoda, M., and Sawada, J. (2002). Effect of subchronic feeding of genetically modified corn (CBH351) on immune system in BN rats and B10A mice. Shokuhin Eiseigaku Zasshi. 43, 273-9.

5. Economic Impacts

One comment asked whether nonregulated status for Pioneer 305423 was necessary if naturally occurring oleic acid can be found in other sources, such as grapeseed oil.

APHIS response: 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.

For economic reasons, modified soybeans may provide the best solution for a food manufacturer's needs as well as for growers' crop choices. High oleic oils can be produced in other crops, such as sunflower ("NuSun"), safflower and canola (EA, Section V.B.B.) However, soybean oil is highly economical for use in foods, and soybean production may be more profitable for growers than either sunflower or canola in permissive growing regions such as eastern North Dakota (Metzger, 2009).

Reference:

Metzger, S. (2009) ND2010 Projected farm cash flow by crop, NDSU Carrington Research Extension Center, http://www.ndfarmmanagement.com/2010BEP121509.xls

One trade organization said that APHIS has a responsibility to consider scientific data assessing the risks to food functionality, risk mitigation and risk responsibility in its review of all special use traits like 305423.

APHIS response: APHIS' pest risk assessment for non-regulated status does not require that risks to food functionality or risk mitigation for inappropriate product use be considered (See 7 CFR part 340.6). For products developed using nonregulated GE organisms, APHIS does not have the statutory authority to mandate that product developers be assigned risk responsibility or provide compensation for adverse consequences to injured parties resulting from the use of a nonregulated GE organism (See Title 4. Plant Protection Act, June 20, 2000) . 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.

As appropriate, APHIS does consider potential food and industrial use impacts in their NEPA documents. The EA prepared for this petition and response to comments above, notes that the ratios of the typical soybean fatty acids have been altered by this Pioneer 305423 line, and that inadvertent entry of Pioneer 305423 into the commodity soybean supply stream is possible. However, because of the value-added traits of this soybean, growers and processers will preserve product identity to the point of sale (See Petition Amendment: X-XF-7, Potential impact on organic and conventional farming), thus keeping Pioneer 305423 soybean separate from commodity soybean. The methods that are chosen to prevent oilseed mixing into the commodity stream of soybean seed is discussed in Section 2, Agricultural Impacts, of this Response to Comments document.

APHIS supports the initiatives of industry associations in addressing issues of risk mitigation and responsibility. APHIS understands that the biotechnology industry consortium, BIO, is currently discussing stewardship plans with their constituent biotech companies that would address policies on these types of issues. When complete, the stewardship plans would likely assist the industry in addressing issues associated with risks to food functionality, risk mitigation and risk responsibility.

One food processing trade organization said that any precommercial release must include commercial assurances for downstream stakeholders relative to comingling of the product into the supply chain.

APHIS response: APHIS does not have regulatory authority or responsibility for validating the specific properties of a commodity nor mandating commercial assurances. Pioneer in public comments to APHIS (2007-0156-0045) plans to establish industry standard oilseed IP management procedures, and encourage use of analytic techniques to substantiate oil identity Oil derived from this variety may be beneficial for many downstream users, but may not be optimal for all users (See Pioneer public comment Docket No. APHIS-2007-0156, December 21, 2009, "Identity Preserved Systems" and "Comingling Implications). Marketing agreements put in place by product developers may influence acceptance and use of these oils by soybean industry stakeholders. Although many adverse impacts following widespread use of oil deriving from Pioneer 305423 soybean are not likely, increased surveillance may be warranted in specific instances (See Pioneer public comment Docket No. APHIS-2007-0156, December 21, 2009, "Comingling Implications") to detect novel fatty acid ratios of commodity soybeans and in soy oil. For example, characteristics of product stability, taste, and texture depend on fatty acid ratios (Warner et al., 1988). Distinction between input soybean varieties would be a necessary part of manufacturer procedure when multiple types of soybean fatty acid contents are in the marketplace. Soybean varieties can be distinguished by scanning cotyledons for differences in certain lipid classes using scanning spectroscopy (with Fourier Transformed Infra Red techniques (FTIR)), a fast and straightforward assessment (Caires, 2008).

To alleviate concerns surrounding inadvertent mixing of Pioneer 305423 high oleic soybean with soybeans in the commodity stream, the Grocery Manufacturers Association (GMA; Jeff Barach, personal communication, 2009) has indicated that its constituent members desire more information about use and functionality of the high oleic oil. The GMA expects that some of the technology providers of seeds with altered fatty acid oils will commit to extensively comparing physical properties of inadvertent oil mixtures deriving from the altered product and the commodity oil. By informing producers of the oil's compatibility with existing food processing and manufacturing procedures, this testing could avert potential economic losses sustained by producers.

References:

Caires, A.R.L., Teixeira, M.R.O., Suarez, Y.R., Andrade, L.H.C. and Lima, S.M. (2008). Discrimination of transgenic and conventional soybean seeds by Fourier transform infrared photoacoustic spectroscopy. Applied Spectroscopy, 62, 1044-1047.

Warner, K., Orr, P. and Glynn, M. (1988). Effect of altered fatty acid composition on soybean oil stability. Journal of the American Oil Chemists' Society 65, 624-628.

Some comments expressed concerns that without rigorous marketing agreements, or stewardship plans from technology companies, and a "higher level of commercial responsibility," commercial disruptions in the export market are possible, and these concerns need to be addressed if 'pre-commercial release" is the chosen production and marketing pattern. Precommercial release is the commercial production of a product which does not have international regulatory acceptance as a novel GE crop, such as within EU countries or Japan. Since some countries maintain a zero threshold for unapproved products, a high level of marketing policy measures, and strong enforcement and compliance with the policy is necessary for a pre-commercial release program to be successful.

APHIS response: APHIS does not have regulatory authority or responsibility for validating the specific properties of a commodity nor mandating commercial assurances for purchasers or

distributors of nonregulated products. However, because this soybean variety expresses value-added traits, growers and processers will voluntarily preserve product identity to the point of sale, thus keeping Pioneer 305423 soybean separate from commodity soybean (See Pavely, 2007, Pioneer Petition Amendment: X-XF-7). While undesired mixing of this specialty oilseed with standard commodity soybean oil is possible, several market forces will minimize its occurrence. Pioneer has committed itself to using mechanisms similar to industry standards to maintain product identity, which have "established effectiveness" for maintaining the integrity of the export and domestic market for soybean oils (Pioneer Public Comment APHIS 2007-0156-0045). Furthermore, APHIS supports the initiatives of industry associations in addressing issues of risk mitigation and responsibility as part of corporate stewardship of special crop production.

Some comments noted that Pioneer has proposed taking responsibility for the product beyond point of sale for seed, but that "further dialogue" and commitment to sharing of information for all stakeholders will be necessary throughout the lifecycle of the product. Three trade associations expressed concerns about the details of a Pioneer management plan to mitigate the risk of GE soybeans comingling with commodity soybeans and being sold abroad where GE is not accepted.

APHIS response: APHIS understands that the industry association, Biotechnology Industry Organization (BIO; http://www.bio.org/), has encouraged a common stewardship approach by all companies producing GE crop varieties, especially when properties of a standard food commodity are altered by a new trait. Pioneer may choose to participate in this stewardship approach for Pioneer 305423 soybean. APHIS has no role in these discussions, and advises stakeholders to work closely with these providers and BIO, who are both committed to the success of these products in domestic and export markets.

Another comment addressed the possible unintended impacts on crop uses or processing streams because of higher oleic acid, lower linoleic and linolenic acid content in the modified soybean oil deriving from this soybean variety.

APHIS response: As described in the EA, this product improves the qualities of soybean oil, decreasing the need to hydrogenate the soybean oil, which produces unhealthy *trans* fats. Pioneer has indicated that the primary use of Pioneer 305423 soybean will be for food oil as well as for industrial oil products (See Pavely, 2007, Section I.B., Benefits of 305423 Soybean). Because of the value-added traits of this soybean, growers and processers will preserve product identity to the point of sale, thus keeping Pioneer 305423 soybean separate from commodity soybean (See Taveley, 2007, Pioneer Petition Amendment: X-XF-7, and Section I.B.,Pavely, 2007). Pioneer in public comments to APHIS (2007-0156-0045) plans to establish industry standard oilseed IP management procedures, and encourage use of analytic techniques to substantiate identity of constituent fatty acids. The high oleic soybean would also be useful for deriving environmentally friendly, renewable, and cost effective industrial oils, such as lubricants and biodiesel oils (see Summary and I.B. in Taveley, 2007). Increased oleic acid at the expense of linoleic and linolenic would improve cetane numbers (time between injection into a cylinder and autoignition; Refaat, 2009), and improve lubricity because of higher numbers of unsaturated fatty acids and other factors (see I-B. Benefits of 305423 Soybean in Pavely, 2007 and Refaat, 2009). To determine which fatty acid ratios are useful for specific products, a use-by-use analysis would be required for each industry employing soybean oils. Pioneer notes that "Oil functionality considerations for [high oleic soybean] are the same as those for existing modified fatty acid oilseed crops, such as high oleic canola and mid/high oleic sunflower oil (Pioneer public comment Docket No. APHIS-2007-0156, December 21, 2009)."

Reference:

Refaat, A.A. (2009). Correlation between the chemical structure of biodiesel and its physical properties. Int. J. Environ. Sci. Tech. 6, 677-694.

Three commenters desired Pioneer to establish whether it will cover the costs of regulatory interceptions if these soybeans were comingled and then exported to countries in which the events were not approved.

APHIS responds: No requirement exists that APHIS should enforce mitigation standards for users of the technology, nor provide for consequences of their failure. This type of action is outside APHIS regulatory authority. Although some commenters suggest that Pioneer will cover costs of trade disruption caused by the possible presence of the new fatty acid soybean in export supplies, APHIS makes no interpretation of intention and takes no position on appropriateness of this policy. If Pioneer agrees to comprehensive product liability, they will do so without APHIS guarantees or support for the guarantee.

One commenter suggests that large agribusinesses monopolize seed production, and force farmers to use GE seeds.

APHIS response. As far as APHIS can determine, while large seed companies sell limited lines of non-transgenic soybean seed, some nontransgenic lines are available and can also be provided by smaller suppliers and state university foundations. Despite buyers' displeasure at the high technology fees of GE seed, they continue to purchase such GE seed, because growers benefit from reduced production expenses and require fewer farm operations for growing 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). As Traxler and Falck-Zepeda show (1999; for GE cotton), there are benefits to both producers and technology providers alike. Substantial competition exists for soybean seed and for differing oil products, including conventional soybean varieties that may serve many of the marketplace needs.

References.

Fernandez-Cornejo, J. (2008) Adoption of Genetically Engineered Crops in the U.S. (Data Sets). USDA-Economic Research Service. http://www.ers.usda.gov/Data/BiotechCrops/ (Accessed 1/4/2009).

Traxler, G., and Falck-Zepeda, J. (1999). The distribution of benefits from the introduction of transgenic cotton varieties. AgBioForum 2, 94-98.

Several commenters said that US growers of soybean have been losing extensive soybean acreage to foreign vegetable oil production because US manufacturers have needed to replace hydrogenated soybean oil in food products, following imposition of FDA labeling requirements. The proposed soybean line would provide an economic benefit to US soybean producers.

Pioneer 305423 will help soybean growers produce a desirable oil for food manufacturers (See EA Section I. Introduction), and potentially recapture market share that was previously lost (Balvanz, C., Iowa Soybean Association, public comment on APHIS 2007-0156-0018). When

FDA deemed the desired functionality of hydrogenated soybean oil a health risk, food manufacturers turned to foreign food grade oils (for example, increased use of palm oils: (Unnevehr and Jagmanaite, 2008; Soyatech, 2010)). High oleic oils such as Pioneer 305423 will have features of increased oil stability and useful blending properties (Warner and Gupta, 2005) and could replace imported oils such as palm oils for the necessary functional and quality properties needed by manufacturers.

References:

Soyatech (2010 download). Transfat facts. Soyatech LLC, Maine. http://www.soyatech.com/trans_fats.htm

Unnevehr, L.J. and Jagmanaite, E. (2008) Getting rid of trans fats in the US diet: Policies, incentives and progress. Food Policy 33, 497–503.

Warner, K. and Gupta, M. (2005). Potato chip quality and frying oil stability of high oleic acid soybean oil. J. Food Sci. 70. Supplement. Sensory and Nutritive Qualities of Food. S395-S400.

6. Process Comments

One comment stated that USDA is not adequately assessing the environmental impact of the genetically modified soybean.

APHIS response. APHIS carefully considered the possible environmental impacts of the proposed product, and is satisfied that the EA developed for Pioneer 305423 soybean is adequate and sufficient. The EA follows all applicable laws, regulations, and guidelines in analyzing potential impacts of this action, including those established by NEPA. In making an informed decision of potential environmental impacts, APHIS used the best available scientific information, data and expert advice.

One comment stated that an environmental impact assessment is needed.

APHIS response: APHIS has determined that the analysis in its EA showed no significant impact on the quality of the human environment if APHIS was to grant the petitioner's request to deregulate Pioneer 305423 soybean and that APHIS did not have to prepare an environmental impact statement (EIS). The EA took a hard look at the need for action, the issues, alternatives, and environmental consequences. APHIS also reviewed the assessment of plant pest risk for Pioneer 305423 soybean and carefully considered all comments submitted by respondents to the public involvement efforts. As a result of this analysis, APHIS prepared a final EA, from which came the NEPA decision document and a finding of no signification impact (FONSI) that discussed, under each of the ten Council for Environmental Quality (CEQ) points of significance, why each point was not significant, and why an EIS was not required. The agency followed CEQ NEPA regulations and Agency NEPA implementing procedures.

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

OECD Unique Identifier:
DP-3Ø5423-1

Final Environmental Assessment

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Agency Contact
Cynthia Eck
Document Control Officer
Biotechnology Regulatory Services
USDA, APHIS
Riverdale, MD 20737

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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. 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. Products are regulated according to their intended use and some products are regulated by more than one agency.

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 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 uses 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 when APHIS determines that it is unlikely to pose a plant pest risk.

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) 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[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

[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)

and nutritional assessment to FDA for Pioneer 305423 soybean in 2007. Pioneer 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). The text of the FDA's scientific and regulatory assessment response for Pioneer 305423 is available at the FDA website (FDA, 2009). Because Pioneer 305423 soybean 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 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, were made available for public comment for 120 days, from September 2, 2009 until December 28, 2009. Comments that were received within the comment period were fully analyzed and used by APHIS to determine if the petition to deregulate the Pioneer 305423 soybean should be granted.

Decision to Be Made

APHIS will also use the information from this EA, and the comments received, to inform and to assist APHIS' decisionmaker to determine whether to grant nonregulated status, or to continue to regulate Pioneer 305423 soybean under the regulations at 7 CFR part 340, or that an Environmental Impact Statement for Pioneer 305423 soybean is necessary prior to the decision of whether to grant nonregulated status to this soybean variety.

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. Pioneer identifies users of the modified oil as both food manufacturers and industrial product manufacturers, especially those who are presently using soybean oil products.

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 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) would 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

To determine the areas of soybean production, APHIS used data from the National Agricultural Statistics Service (NASS) 2002/2007 Census of Agriculture to determine where soybean is produced in the United States (USDA-NASS 2009). Only 17 states in 2008 produced more than 1 million acres among the 31 that produced soybean according to the 2002 Census of Agriculture. These states include IA, IL, MN, IN, MO, NE, OH, SD, AR, ND, KS, MI, MS, WI, NC, KY, TN (USDA-NASS, 2008). In the U.S., soybeans were harvested on 72.1 million acres in 2008 (USDA-NASS, 2008). This

harvested soybean acreage was only one percent below the record high acreage in 2006 (USDA-NASS, 2008a).

Pioneer states that this variety will "offer growers another value-added specialty soybean option" and do not expect that soybean market dynamics will change with the availability of this product (Pavely, 2007). Properties of soybean oil from this variety will be preferred over those commodity soybeans with less stability, and over the inferior flavor properties that currently characterize hydrogenated commodity oils (Pavely, 2007). Thus, products derived from Pioneer 305423 soybean will likely only replace commodity soybean products, and not create new demand. APHIS concludes that it is not likely that "new," previously uncultivated land will be brought into soybean production beyond that currently or historically used for such production.

Processed soybeans are the largest source of protein feed and the second largest source of vegetable oil in the world. Soybeans represent 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 has been a major development in agriculture over the past 20 years. Weed control had been one of the biggest challenges for soybean growers. Infestation with weeds during an entire growing season have resulted in soybean yield losses ranging from 12 to 80% (Barrentine, 1989). By the early 1990's, Gianessi et al. (2002) reported over 70 individual herbicides or combination products registered for weed control in soybean. 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 accompanying 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 0.13% of U.S. soybean production in 2008) was devoted to the production of organic soybean (USDA-NASS, 2010).

Weed control is one of the biggest challenges for all soybean farmers. United States soybean farmers began switching from the use of tillage for weedcontrol to herbicides in the late1950s. 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). As a result, soybean is a 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 0.13% of U.S. soybean production (USDA-NASS, 2010). In 2008, 98,199 acres of certified or exempt organic soybean were harvested in United States. 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 transfer of pollen from GE soybean into their organic production fields (Organic Farming Research Foundation (2002). Growers may choose to plant only organic seed; 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; and 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. Additionally, organic growers must 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 (FAO, 2009; Lusas, 2004). After the oil is extracted the remaining solid materials in the form of flakes are toasted and ground to produce soybean meal.

Oil

For total world vegetable oil production, soybean is ranked number one among the major oilseed crops, and in the US supplies 71% of all fats and oils consumed (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 product shelf life and have a desirable taste and texture (The Pennsylvania State University, 2006). Although use of soybean oil in food is a large part of the market for soybean products, soy oil is also used for industrial products as Pioneer notes, and modified oils may be used for biodegradable products such as certain types of lubricants.

Meal

Soybean meal, which contains about 50% protein by dry weight, remains the primary product from soybean, and of this more than 95% of domestic soybean meal is consumed by livestock (ABG, 2007). 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 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 (Manach et al., 2004).

Antinutrients

Soybean grain contains several key antinutrients, such as oligosaccharides, lectins, phytic acid and protease inhibitors (OECD, 2001). The trypsin and chymotrypsin inhibitors can inhibit digestion of proteins (OECD, 2001). Others such as lectins are sugar-binding proteins, preventing digestion of proteins resulting in decreased animal growth (OECD,

2001). The activity of these inhibitors is destroyed during the heat treatment processing of the soybean products. 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 and can chelate various minerals as well (OECD, 2001). Livestock producers commonly add phytic acid degrading enzymes to animal feed formulas (Vats and Banerjee, 2004).

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. 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.”

APHIS has assessed the potential for environmental impacts for each alternative in the “Environmental Consequences” sections below.

A. No Action: Continuation as a regulated article

Under the "no action" alternative, APHIS would deny the petition. 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. *Grant nonregulated status to Pioneer 305423 soybean, "in whole"*- 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).

Alternatives Considered but Rejected from Further Consideration

Geographic restrictions
APHIS considered geographic restrictions based upon geographic variation in plant pest risk. As presented in APHIS plant pest risk assessment for Pioneer 305423 soybean, there is no geographic differences in the plant pest risks for Pioneer 305423 soybean

(USDA-APHIS 2009). This alternative was rejected and not analyzed in detail because Pioneer 305423 soybean is unlikely to pose a plant pest risk and therefore, APHIS will have no regulatory authority over Pioneer 305423 soybean and will be unable to impose regulatory restrictions on this GE soybean variety.

V. 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. The analysis of potential environmental consequences in the following sections address the potential impacts to the human environment from the alternatives analyzed in this EA, namely taking no action and granting nonregulated status to Pioneer 305423 soybean, "in whole".

SCOPE OF THE ENVIRONMENTAL ANALYSIS

Although the preferred alternative would allow for plantings of Pioneer 305423 soybean to occur anywhere in the U.S., APHIS limited the environmental analysis to those areas that currently support soybean production. To determine areas of soybean production, APHIS used data from the National Agricultural Statistics Service (NASS) 2002 Census of Agriculture to determine where soybean is produced in the United States (USDA-NASS, 2009). Only 17 states in 2008 produced more than 1 million acres among the 31 that produced soybean according to the 2002 Census of Agriculture. These states include IA, IL, MN, IN, MO, NE, OH, SD, AR, ND, KS, MI, MS, WI, NC, KY, TN (USDA-NASS, 2008). Because the oil derived from this Pioneer 305423 soybean will likely replace that from existing soybean varieties, it is not likely that new land will be planted to soybean by growers. The most recently harvested area of soybean production (72.1 million acres) is already only one percent below the record high acreage of 2006 (USDA-NASS, 2008a).

A. No Action

Under the "no action" alternative, Pioneer 305423 soybean would continue to be a regulated article. APHIS' assessment of environmental consequences under the no action alternative is described below.

A. Soybean

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

The food/feed nutritional and safety assessment for Pioneer 305423 soybean has been reviewed by 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 accompanying scientific literature (Reference section), APHIS has concluded that Pioneer 305423 soybean would have no significant impacts on human or animal health

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). In 2008, the production of organic soybeans represented about 0.13% of U.S. soybean production (USDA-NASS, 2010). Conventional production practices that use GE varieties (such as herbicide tolerant varieties and new GE traits planned by four companies) will likely still dominate in terms of acreage, or perhaps increase in acreage (Bonny, 2009), even without granting nonregulated status to Pioneer 305423 soybean under the "no action" alternative. Available seed for both GE and conventional varieties will remain the same under the "no action" alternative. Pioneer 305423 soybean variety will remain unavailable for commercial production. Soybean is currently produced in more than 30 states according to the 2008 State Soy Crop Statistics (USDA-NASS, 2008), and under the "no action" alternative, this area for production will likely 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). 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, when used properly, are a safe and effective method to control certain weeds in soybeans.

Under the "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. APHIS has no authority under the Plant Protection Act to regulate herbicide use. The use of herbicides is regulated by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) restrictions administered by the EPA, which mandate registration and use of all pesticides. EPA includes instructions and restrictions

on how herbicides can be applied, and has determined that there is no unreasonable environmental risk if the user adheres to the directions. Directions include application restrictions that minimize impacts on nearby environments. Violators of the regulations are liable for all negative consequences of their actions; therefore, farmers who use herbicides are very likely to follow its label restrictions, and thereby limiting any potential adverse impacts.

The large scale commercial cultivation of GE 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 (Cerdeira and Duke, 2006). Under the 'no action' alternative, APHIS expects no change to the availability or use of GE glyphosate tolerant soybean varieties. These GE varieties will remain available for commercial use. Thus, it is likely that under the 'no action' alternative, greater than 90% of the soybean acres produced in the U.S. will continue to be GE varieties and production of organic varieties will remain unchanged (Bonny, 2009).

If APHIS chooses 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.

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 commercially available 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. Additionally, other seed high in oleic oil (e.g. GE sunflower oil) will also remain in commerce.

A variety of soybean oil choices are presented to food manufacturers, particularly in response to the requirement by FDA that those oils with trans-fats be labeled. These choices may be altered linolenic acid varieties or interesterified soybean oils (Eckel et al., 2007). In response to any soybean oils with altered fatty acid ratios that are used in food products, substantial efforts in reformulating the entire product may be needed (Eckel et al., 2007). Although these specialty oils are marketed exclusively as identity preserved

(see Summary and Section I.B, Taveley, 2007) and are not likely to be found in commodity soybean oils, food manufacturers need to be especially attentive to altered fatty acid ratios that may be present in commodity oils.
Pioneer proposes to use current industry standard mechanisms to maintain product identity, from seed production to delivery to customers after oil refining (Pioneer Public Comment APHIS Docket 2007-0156-0045; Regulations.gov (2009). These methods include sales of seed for planting to growers under contracts to deliver produced specialty oilseed at defined locations, and also to meet standards for oil quality. Other agreements by oilseed crushers and refiners could specify precisely the acceptable range of fatty acids that would meet established standards for the commodity oil. The Biotechnology Industry Organization in cooperation with its member companies is also developing stewardship standards for any specialty oilseed products that may differ substantially from commodity oils (Jeff Barach, Grocery Manufacturers Association, personal communication, 2009).

High oleic oils are found to be useful for industrial products, such as biodegradable lubricants (National Ag-Based Lubricants Center, 2008; Machinery Lubrication (2010). Such products are used in food processing applications or farm tractor transmissions. High oleic oils are used in the industrial sector for base oils that are produced for hydraulic or gear oils (National Ag-Based Lubricants Center, 2008). Because properties of oils are dependent upon properties of constituent fatty acids, the specific content deriving from differing soybean varieties is of interest to these manufacturers. Again, specialty soy oils (See Section I.B., Taveley, 2007) are marketed as identity preserved products, and are not likely to be found in commodity soybean oils.

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 is the potential for gene flow to sexually compatible plant species. Based on the plant pest risk assessment (USDA-APHIS, 2009), APHIS has determined that Pioneer 305423 soybean is not a plant pest and that gene flow between this product and weedy and wild relatives will not occur in the United States. Weedy and wild relative species do not exist naturally in the United States (USDA-NRCS, 2008). Therefore, soybean lacks sexually compatible wild relatives in the United States and its territories. APHIS does note that gene flow can take place between a field planted with Pioneer 305423 soybean and a neighboring soybean crop. 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). Although the biology of the soybean crop limits the amount of gene flow that may occur between two soybean fields, soybean farmers may implement certain management practices to minimize gene flow between production fields (e.g., plant at different dates than neighboring soybean, to encourage differing flowering times, establish isolation distances to avoid mechanical mixing and short-distance pollen flow).

The food/feed nutritional and safety assessment for Pioneer 305423 soybean has been reviewed by 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 accompanying scientific literature (Reference section), APHIS has concluded that Pioneer 305423 soybean would have no significant impacts on human or animal health with overall impacts similar to the no action alternative.

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 agricultural fields that have been in crop production for multiple years.

Pioneer 305423 soybean has been field tested under permits issued or notifications acknowledged by APHIS in the United States since 2002. Field test sites included the major soybean growing areas of the Midwest and the winter nursery areas in Hawaii. Agronomic and phenotypic data were collected by the applicant 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, APHIS agronomists evaluated the potential for weediness as compared to conventional soybean. The evaluation concluded that there were no biologically significant differences in weediness potential between Pioneer 305423 soybean and other soybean varieties (see Section VIII-A-D and X-C in Pavely, 2007). No differences were found in dormancy or germination potential, in responses to diseases or insects, seedling vigor, plant maturity,

plant height, and the lowered linolenic acid content would be correlated with reduced cold tolerance. These results show that the agronomic and phenotypic characteristics of Pioneer 305423 soybean were either not altered when compared to nontransgenic (conventional) soybean or were characteristic of less fitness (cold tolerance potential). Therefore, granting nonregulated status to Pioneer 305423 soybean under the "preferred" alternative is not expected to substantially alter the range of soybean cultivation as the new GE trait (high oleic acid) does not change the production needs or growth habits of GE soybean varieties compared to conventional varieties (USDA-APHIS, 2009).

Pioneer 305423 soybean oil will be marketed as "TREUS^{TM2}" high oleic soybean oil. This soybean variety will likely be introduced to areas where soybean is currently grown for oil production as an alternative to other choices (such as conventional and GE varieties producing altered fatty acids) already available in the market (see Summary, Pavely (2007)). Therefore, the current distribution of soybean production acreage will likely remain unchanged as a result of APHIS granting nonregulated status to Pioneer 305423 soybean.

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 expected change in herbicide use with Pioneer 305423 soybean under the preferred alternative. Therefore, potential impacts from the use of EPA registered herbicides are the same as those from the no action alternative.

Production of mid and high oleic soybean

In 2003, Dupont Protein Technologies provided grower contracts for production of G-94-1, G-94-19 or G-168 GE high oleic soybean. Production over several thousand acres through 2005 revealed no environmental or agronomic issues. Beginning in 2007, growers in Iowa planted mid-oleic, low linolenic producing varieties (Stockhausen, 2008). These varieties, available through the Iowa State University Research Foundation, such as IA3036 and IA3039 have oleic acid content of between 49 and 53% (Iowa State University, 2010). These lines were grown under contracts with commercial oilseed crushers (J. J.G. Minot, personal communication, IA State Univ. Res. Fndtn). No agronomic issues were identified, nor impacts on agricultural production noted.

Organic and Conventional 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

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

the production operation to achieve and document compliance with the National Organic Standards, including the prohibition on the use of excluded methods. Excluded methods include a variety of methods used to genetically modify organisms or influence their growth and development by means that are not possible under natural conditions or processes.

Organic certification involves oversight by an accredited certifying agent of the materials and practices used to produce or handle an organic agricultural product. This oversight includes an annual review of the certified operation's organic system plan and on-site inspections of the certified operation and its records. Although the National Organic Standards prohibit the use of excluded methods, they do not require testing of inputs or products for the presence of excluded methods. The presence of a detectable residue of a product of excluded methods alone does not necessarily constitute a violation of the National Organic Standards (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 Pioneer 305423 soybean. 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 (Vendors, 2009).

If Pioneer receives regulatory approval from all appropriate agencies, Pioneer 305423 soybean will likely be widely available to growers and breeders. Other farmers who choose not to plant or sell Pioneer 305423 soybean or other transgenic soybeans will not likely be significantly impacted by the expected commercial use of this product because:

(a) non-transgenic soybeans will likely still be sold and will be readily available to those who wish to plant these varieties;

(b) soybean is a highly self-pollinated plant and therefore buffer requirements to isolate GE and organic varieties would be minimal;

(c) 92% of the 2008 soybean acreage in the United States is already planted to transgenic herbicide tolerant varieties with organic soybean currently coexisting in the largely biotech agricultural environment; 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 Pioneer 305423 soybean is granted nonregulated status, similar to the no action alternative there 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).

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.

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., 2007).

Similar to the no action alternative, a variety of soybean oil choices are presented to food manufacturers and for use in industral products. These specialty oils are marketed exclusively as identity preserved (see Summary and Section I.B, Taveley, 2007) and will

not likely be found in commodity soybean oils. Pioneer 305423 soybaen would be an additional soybean variety available to this specility food oil market.

Oil Composition

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 a GE 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 have received regulatory approval for food use in the Australia, Canada, Japan, New Zealand, and the United States (AgBios, 2010). 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 (AgBios, 2010).

Data that support the safety of *GmFad2-1* gene was reviewed by FDA resulting in their issuing a FDA Biotech Consultation BNF #000039 (FDA, 1996b). 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 completed the voluntary consultation of the Pioneer 305423 soybean for "Food and Feed Safety and Nutritional Assessment" and had no further questions concerning Pioneer 305423 soybean based on the information provided by Pioneer (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 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

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

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), less in linoleic acid (from an average of 52% down to 3.6%), less in linolenic (9.4% to 5.4%) and less in palmitic acid (from an average of 10.3% down to 6.28%) than conventional soybeans. A mid-oleic acid soybean variety is offered by Iowa State Research Foundation producing more than 50% oleic acid (Stockhausen, 2008). High oleic acid levels are found in other commonly consumed premium edible oils (e.g., olive oil 55-83% oleic, Vossen (2007) and high oleic sunflower (at least 75% oleic, CODEX, 2009) and high oleic canola oil (70% oleic, Canolainfo.org, 2010). The consumption of high levels of oleic acid is not considered to pose any safety concerns (Lunn, 2007; FDA, 2009). 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 22 and 30 percent of most animal fats and is also an important constituent of most vegetable fats (2-17 percent, but 35-45 percent of palm oil) (Table 1, Nishina et al., 1993; Beare-Rogers et al., 2001).

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, Appendix 7, 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 (non-GE) 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, pp 62-63). 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) of Pioneer 305423 soybean 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 (non-GE) 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 (non-GE) soybean lines and concludes there were no statistically significant differences were seen between Pioneer 305423 soybean and control (non-GE) 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 (non-GE) 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.

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 on agricultural 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. There 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. 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 as a result of granting nonregulated status to Pioneer 305423.

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 this high oleic soybean variety. This 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.

Toxcity and Allergencity
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 (NIH-NCBI, 2010) 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 taken into account (by the dosage range) 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.

Nutrition

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.

Pest and Disease

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, pp. 158-160) 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 significantly different (lower; adjusted P-value < 0.05) in Pioneer 305423 soybean as compared to the control line although remaining within the statistical tolerance interval and the "combined literature range." 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, pp. 158-160).

Soil Communities

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.

Threatened and Endangered Species

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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VII. PREPARERS AND REVIEWERS

Biotechnology Regulatory Services
Michael Gregoire, Deputy Administrator
Beverly Simmons, Associate Deputy Administrator for Emerging and International Programs
Sidney Abel, Assistant Deputy Administrator

BRS, Environmental Risk Analysis Division
Michael Watson, Ph.D., Division Director
Margaret Jones, Ph.D., Senior Biotechnologist (Reviewer)
Karen Green, Biotechnologist (Preparer of Environmental Assessment)
Luc Hebou, Biotechnologist (Reviewer)

BRS, Policy and Coordination Division

John Turner, Ph.D., Director
Andrea Huberty, Ph. D., Branch Chief (Reviewer)
Richard Coker, Regulatory Analyst
Michael Blanchette, Environmental Protection Specialist (Reviewer)

AGENCY CONTACT

Ms. Cynthia Eck, Document Control Officer
USDA, APHIS, BRS
4700 River Road, Unit 147
Riverdale, MD 20737-1237
Phone: (301) 734-0667
Fax: (301) 734-8669
Cynthia.A.Eck@aphis.usda.gov

VIII. Appendix 1 - Biotechnology Consultation Note to the File BNF No. 000110

Date: January 14, 2009

Subject: High Oleic 305423 Soybean

Keywords: soybean, *Glycine max*, high oleic 305423 soybean, TREUS™, OECD unique identifier DP-3Ø5423-1, omega-6 desaturase, *FAD2-1*, *gm-fad2-1*, seed-specific silencing, *gm-hra*, soybean acetolactate synthase

1. Introduction

In a submission dated December 27, 2006, Pioneer Hi-Bred International, Inc. (Pioneer), a DuPont Company, submitted to the Food and Drug Administration (FDA) a safety and nutritional assessment of the bioengineered high oleic 305423 soybean line containing the transformation event DP-3Ø5423-1. Pioneer provided additional information on August 31, 2007, January 30, February 19, March 18, November 21, December 12, and December 19, 2008. Pioneer concluded that food and feed derived from the 305423 soybean are as safe and nutritious as food and feed derived from conventional soybean varieties currently being marketed.

2. Intended Effect

The intended effect of the modification in 305423 soybean is to produce soybean seeds with increased levels of monounsaturated fatty acid (oleic) and decreased levels of polyunsaturated fatty acids (linoleic and linolenic). To accomplish this objective, Pioneer inserted a fragment of the soybean microsomal omega-6 desaturase gene (*FAD2-1*) into

soybean. The fragment of the *FAD2-1* gene does not code for a protein. Transcription of the gene fragment under the control of a seed-preferred KTi3 promoter acts to silence the expression of the endogenous soybean omega-6 desaturase, which results in an increased level of oleic acid and decreased levels of linoleic and linolenic acids in the soybean seed. A gene (*gm-hra*) encoding a modified version of the soybean acetolactate synthase (*als* gene) that confers tolerance to a sulfonylurea herbicide was used as a selectable marker for the transformation.

3. Genetic Modifications and Characterization

3.1. Parental Variety

The publicly available soybean variety "Jack" was used as the recipient in the DNA transformation to create 305423 soybean.

3.2. Transformation DNA and Method

Pioneer used microprojectile bombardment to co-transform secondary plant cell embryos with two purified linear DNA fragments: a 2924 base pair fragment containing the *gm-fad2-1* cassette, and the 4512 base pair fragment containing the *gm-hra* cassette. The *gm-fad2-1* cassette includes the promoter region from the soybean Kunitz trypsin inhibitor gene (KTi3), a fragment of the *FAD2-1* gene that corresponds to approximately 40% of the middle portion of the *FAD2-1* gene, and the 3' untranslated region of the KTi3 gene (KTi3 terminator).

The *gm-hra* cassette includes a promoter and an intron from the 5'regulatory region of the S-adenosyl-L-methionine synthetase (SAMS) gene from soybean, the *gm-hra* gene that encodes the GM-HRA protein, and the terminator from the endogenous *als* gene.[1]

3.3. Characterization, Stability, and Inheritance of the Introduced DNA

In order to characterize the introduced DNA, Pioneer conducted Southern blot and sequencing analyses of the DNA inserted into the 305423 soybean. Pioneer reports that the 305423 soybean contains four genetically linked insertions. The 305423 soybean has multiple intact and partial copies of the *gm-fad2-1* cassette that contain, in total, eight copies of the KTi3 promoter, seven copies of the *gm-fad2-1* gene fragment, and five copies of the KTi3 terminator. Pioneer states that it appears that multiple copies of the *gm-fad2-1* gene fragment are necessary for effective co-suppression of the endogenous gene. One copy of the KTi3 promoter is associated with a small non-functional fragment of plasmid backbone DNA. Pioneer determined that a single intact *gm-hra* cassette is inserted into the genome of 305423 soybean. Pioneer noted that further analysis of 305423 soybean for plasmid backbone sequence using Southern hybridization showed that 305423 soybean contains neither the hygromycin gene nor the bacterial plasmid origin of replication present on the plasmids from which the *gm-fad2-1* cassette and the *gm-hra* cassette are derived. Pioneer sequenced the inserted DNA and reported that the sequence confirmed the results of the Southern analysis.

Each of the four insertions in 305423 soybean was screened for the presence of open reading frames (ORFs) containing both a start and stop codon that spanned any novel junctions. Pioneer identified two such ORFs. Pioneer reports that neither ORF contains the necessary regulatory elements for transcription. Northern blot analysis detected no transcripts of the ORFs in developing seeds from either 305423 soybean or the control soybean. Pioneer concluded that it is very unlikely that a protein is expressed from either ORF. Pioneer also reports that screening of the ORFs against a database containing known protein toxins[2] and to a database of known allergens[3] showed no biologically significant identities to known protein toxins or allergens. Based upon their analyses, Pioneer concluded that there are no safety concerns resulting from these ORFs.

Pioneer reports that Southern blot analyses across three generations showed that the inserted DNA in 305423 soybean is stably integrated into the genome. The same event-specific hybridization pattern was observed for all but one plant that apparently lost the *gm-hra* cassette due to a recombination event. Pioneer investigated the frequency of recombination in 305423 soybean by examination of 1000 additional segregating 305423 soybean plants by PCR-based assays and found no other recombinants.

Pioneer conducted chi square analysis of trait inheritance data. They report that the expected segregation ratios were observed in crosses showing the Mendelian inheritance and stability of the introduced trait. Pioneer reports that when both traits were analyzed in the same plants, data confirmed co-segregation of the *gm-fad2-1* gene fragment and the *gm-hra* gene.

4. Introduced Protein – GM-HRA

4.1. Identity, Function, and Characterization

Pioneer describes the GM-HRA protein as a modified version of the endogenous soybean acetolactate synthase (ALS) enzyme and that this modified ALS enzyme confers tolerance to ALS-inhibiting herbicides. ALS enzymes are widely distributed in nature and have been isolated from bacteria, fungi, algae, and plants. ALS-inhibiting herbicides inhibit plant growth by blocking the action of the ALS enzyme thereby inhibiting branched-chain amino acid biosynthesis. The mature GM-HRA protein differs from the endogenous ALS enzyme at two specific amino acids and is responsible for GM-HRA insensitivity to ALS-inhibiting herbicides. The *gm-hra* gene was used only as a selectable marker for the transformation.

Pioneer characterized 305423 soybean-produced GM-HRA protein using various methodologies[4] and demonstrated its equivalence with *Escherichia coli*-produced GM-HRA protein. The *E. coli*-derived GM-HRA protein was used for *in vitro* and *in vivo* biochemical and toxicological studies.

The GM-HRA protein levels in 305423 soybean were measured in replicated samples of leaf, root, forage and grain tissues using a quantitative enzyme-linked immunosorbent assay (ELISA). Pioneer reports that the mean GM-HRA protein concentrations in 305423 soybean leaf, root, forage, and grain were 4.0, 0.18, 5.7, and 2.5 nanograms per milligram

(ng/mg) tissue dry weight respectively. Pioneer concluded that the above results confirm that the expression of the GM-HRA protein in 305423 soybean is constitutive.

4.2. Assessment of Potential Toxicity and Allergenicity

Pioneer reports the results of a global sequence similarity search of the GM-HRA amino acid sequence against the National Center for Biotechnology Information (NCBI) Protein dataset. The search was conducted using the BLASTP algorithm.[5] GM-HRA showed sequence similarity to other ALS proteins. None of the proteins returned by the search was identified as a toxin. Pioneer concluded that the GM-HRA protein did not share relevant sequence similarities with known protein toxins.

Pioneer conducted an acute oral toxicity study in mice. A single dose of 582 mg per kilogram of body weight (mg/kg bw) of GM-HRA protein was administered by oral gavage to five male and five female mice. No clinical symptoms of toxicity, body weight loss, gross organ lesions or mortality were observed. Pioneer concluded that the result of this study shows that the GM-HRA protein does not cause acute toxicity.

Pioneer reports that while soybean is one of the major food allergens, none of the identified allergens is a member of the ALS family and the ALS protein from soybean has not been characterized as a soy allergen. Pioneer compared the amino acid sequence of the GM-HRA protein to the amino acid sequences of known allergens in the Food Allergy Research and Resource Program (FARRP, version 6.0) database using the FASTA34 sequence alignment program.[6] None of the identified alignments met or exceeded the threshold of greater than or equal to 35% identity over 80 amino acids and no contiguous stretches of 8 or greater amino acids were shared between the GM-HRA protein and the proteins in the allergen database. Additionally, the GM-HRA protein is not glycosylated.

Pioneer also reports that the GM-HRA protein is rapidly (< 30 seconds) hydrolyzed in simulated gastric fluid (SGF) to fragments of < 3 kDa; and when subjected to simulated intestinal fluid (SIF), the GM-HRA protein, including the low weight molecular fragments seen in SGF, is completely and rapidly (< 2 minutes) hydrolyzed.

Pioneer concluded that the GM-HRA protein is unlikely to be a toxin and is not a potential allergen.

5. Evidence for Silencing of FAD2-1 *Gene Expression*

Pioneer determined that a 597 base pair *gm-fad2-1* gene fragment, which is identical to a portion of the coding sequence of the endogenous soybean microsomal omega-6 desaturase gene (*FAD2-1*), resulted in the down regulation of expression of the endogenous *FAD2-1* gene. Pioneer examined the expression of the endogenous *FAD2-1* gene in the leaf and developing seed of ten 305423 soybean plants and five control Jack soybean plants. Northern blots indicated that transcripts of the *FAD2-1* gene in developing seeds of 305423 soybeans were greatly diminished when compared to the

control soybean. This greatly decreased level of transcription confirms that the endogenous *FAD2-1* gene is effectively silenced.

6. Food and Feed Uses of Soybean

Pioneer describes historical and current uses of soybean varieties in food and animal feed. The primary use of commodity soybeans is for soybean meal that is consumed by animals. Raw soybeans contain several antinutritional factors (trypsin inhibitors, urease, and hemagglutinins). Heat treatment is the most common processing method used to minimize the activity of such factors. Soybean oil is the major soybean fraction consumed by humans. Soybean oil accounts for 80% of total United States consumption of oils and fats.

Pioneer states that the 305423 soybean variety is intended to be used for the production of high oleic soybean oil. The oil is intended to be a highly stable vegetable oil suitable for frying applications without the need for hydrogenation which produces *trans* fatty acids and "hydrogenated flavor." Pioneer states that it is aware of no food or feed uses of current soybean varieties for which the 305423 soybean variety would not be equally suitable.

7. Overview of Compositional Analysis

Pioneer assessed the composition of grain and forage from the 305423 soybean and a null segregant (non-transgenic isoline) control. Pioneer states that the null segregant plants are an appropriate control because they are almost genetically identical to the corresponding 305423 soybean plants with the exception that they do not carry the transgenic DNA. Both the transgenic and control soybeans were grown at six field locations in soybean-growing areas of North America using a randomized complete block design with three replicates at each location. Pioneer also analyzed grain and forage from four different commercial soybean varieties.

Pioneer measured 52 components in grain and 5 in forage. Pioneer used the composition data obtained from the commercial varieties to calculate 99% tolerance intervals with 95% confidence for all measured components. To interpret the composition results for 305423 soybeans, Pioneer used the confidence intervals and established a combined literature range using data from published literature and databases on soybean composition.

Pioneer performed statistical analyses of composition data obtained for 305423 soybean and control soybean using mean values calculated from data aggregated from all tests sites. Pioneer used a linear mixed model analysis of variance (ANOVA). In order to hold the rate of false positive results to 5% or less, Pioneer employed the false discovery rate (FDR) approach (Benjamini and Hochberg (1995) and Westfall et al. (1999)). Pioneer reported the composition data by providing mean values, ranges, FDR-adjusted P-values, unadjusted P-values, tolerance intervals, and literature ranges, as available. Pioneer discussed analytical results in the context of FDR-adjusted P-values. Unless so indicated,

statistical analyses using the unadjusted and adjusted P-values are in agreement. Pioneer used a P-value of 0.05 to denote a statistically significant difference between the control and the 305423 soybean.

Pioneer analyzed grain samples for proximates (protein, fat, and ash), acid detergent fiber (ADF), neutral detergent fiber (NDF), fatty acids, amino acids, isoflavones, and antinutrients. Compositional analysis of forage samples included proximates, ADF, and NDF. Table 1 contains the complete list of all measured components.

Table 1. Components measured in grain and forage

Proximates* & Fiber*	Fatty Acids**	Amino Acids	Isoflavones+	Antinutrients
ash fat protein acid detergent fiber (ADF) neutral detergent fiber (NDF)	myristic (14:0) palmitic (16:0) palmitoleic (16:1) heptadecanoic (17:0) heptadecenoic (17:1) stearic (18:0) oleic (18:1) linoleic (18:2) linoleic (18:2) isomer (9,15) linolenic (18:3) arachidic (20:0) eicosenoic (20:1) behenic (22:0) lignoceric (24:0)	methionine cystine lysine tryptophan threonine isoleucine histidine valine leucine arginine phenylalanine glycine alanine aspartic acid glutamic acid proline serine tyrosine	genistin genistein malonylgenistin daidzin daidzein malonyldaidzin glycitin glycitein malonylglycitin	stachyose raffinose lectins phytic acid trypsin inhibitor

* Proximates and fiber were measured in both soybean grain and forage. All other components were measured in grain only.
** The levels of eleven additional fatty acids were near or below the lower limit of quantitation.
+ The levels of acetylgenistin, acetyldaidzin, and acetylglycitin were below the limit of quantitation.

7.1 Compositional Analysis of Soybean Grain

Intended Compositional Change – Fatty Acids

Pioneer analyzed the fatty acid composition of the oil extracted from the grain of the 305423 and control soybeans. Pioneer provides the levels of 14 fatty acids (see Table 1) calculated as percentages of the total fatty acids. Pioneer states that the fatty acid analysis confirmed the expected high oleic acid phenotype as shown by a substantial increase in the mean level of oleic acid to 76.5% in 305423 soybean as compared to a mean level of 21% in the control soybean. As expected, the increase in the level of oleic acid was accompanied by a decrease in the level of linoleic acid. The mean level of linoleic acid in the control soybean was 52.5% and that in the 305423 soybean was 3.6%. The level of

linolenic acid also decreased since linolenic acid is formed in soybeans directly from linoleic acid. Pioneer also notes that the levels of two minor fatty acids, heptadecanoic and heptadecenoic, increased in the 305423 soybean to 0.8% and 1.2% of the total fatty acids, respectively. Pioneer explains that the increase in the levels of heptadecanoic and heptadecenoic fatty acids is not unexpected because the GM-HRA enzyme most likely results in the increased concentration of 2-ketobutyrate, which is the substrate in the biosynthesis of hepta fatty acids in soybeans. Pioneer also reported changes in the levels of all remaining fatty acids, which are not biologically significant.

Proximates and Amino Acids

Pioneer reports that the mean levels of protein and fiber in grain from the 305423 and control soybean are not statistically significantly different. While the mean level of fat was statistically significantly lower in the 305423 soybean than in the control using the unadjusted P-value, no statistical difference was detected using the FDR-adjusted P-value. The mean level of ash was statistically significantly lower in the 305423 soybean as compared to the control soybean. Mean levels of the proximates and fiber in grain from 305423 and control soybean lines were within the 99% tolerance intervals for the reference varieties and within the combined literature range.

Pioneer reports that there are no statistically significant differences observed in the mean levels of amino acids between 305423 and control soybean grain with the exception of threonine and glutamic acid. The mean levels of these two amino acids were statistically significantly increased in 305423 soybean when the unadjusted P-values were used, but not when the FDR-adjusted P-values were used. All these levels were within the 99% tolerance intervals.

Isoflavones

Pioneer analyzed grain from the 305423 and control soybeans for twelve isoflavones, of which nine were quantified (see Table 1). The mean levels for daidzin, malonylgenistin, and malonyldaidzin were statistically significantly increased in the 305423 versus the control soybean. Mean genestin levels were only shown to be statistically significantly increased when the unadjusted P-value was used. Mean values for all the measured isoflavones were within the 99% tolerance intervals.

In both the control and 305423 soybean the mean levels of genistin, glycitin, malonylgenistin, malonylglycitin, and malonyldaidzin were above the combined literature range. Daidzin levels were only above the combined literature range in the 305423 soybean. No literature data were available for the level of glycitein. The values for the other two isoflavones (genistein and daidzein) were within the combined literature range.

Antinutrients

Pioneer measured several antinutrients in soybean grain including non-digestible oligosaccharides stachyose and raffinose, lectins, phytic acid, and trypsin inhibitor (see Table 1). No statistically significant differences were observed between the 305423 and control soybean in mean values for raffinose, lectins, and phytic acid. The mean value for trypsin inhibitor was statistically significantly lower for 305423 soybean as compared to the control soybean. The reduction in the mean value of trypsin inhibitor in 305423 soybean was expected as Pioneer reported that the promoter used to drive expression of the *FAD2-1* gene, the Kunitz trypsin inhibitor promoter, has been shown to silence the KTi3 gene which encodes the Kunitz trypsin inhibitor. The mean value for stachyose was statistically significantly increased when the unadjusted P-value was used, but not when the FDR-adjusted P-value was used. Mean values for all the measured antinutrients were within the 99% tolerance intervals and within the combined literature ranges.

7.2 Compositional Analysis of Soybean Forage

Pioneer analyzed forage for protein, fat, ash, ADF, and NDF. Pioneer reports that no statistically significant differences were observed between the mean levels of these components in forage from the 305423 and control soybeans with the exception of the level of fat. The mean level of fat was statistically significantly decreased in 305423 using the unadjusted P-value, but not when using the FDR-adjusted P-value. All mean levels were within the 99% tolerance intervals and combined literature ranges, with the exception of NDF. The mean level of NDF in both the control and 305423 soybean was above the combined literature range.

7.3 Endogenous Allergens

Pioneer conducted a study to assess whether the transformation process may have increased the overall allergenicity of 305423 soybean compared to conventional soybean. Using sera from clinically reactive soy allergic patients, Pioneer conducted IgE immunoblot and ELISA studies using protein extracts from 305423 soybean and conventional soybean. Pioneer reports that the SDS-PAGE Coomassie blue-stained protein profiles for 305423 and control soybean extracts appeared to be the same; they are similar in their IgE binding profile, and showed the same IgE binding capacity for 305423 and control soy extracts. Pioneer concluded that the levels of endogenous allergens in and the allergic potential of 305423 soybean are comparable to those in nontransgenic control soybean.

8. Fatty Acids Intake

8.1 Human Diet

Pioneer generated estimates of dietary exposure to various fatty acids from the consumption of soy oil derived from conventional soybeans, as well as 305423 soybeans. Pioneer concluded that the intake of oleic acid would increase, while the intakes of

linoleic acid and *trans* fatty acid would decrease. Pioneer states that based on very conservative intake estimates calculated on the assumption that high oleic soybean oil would replace all soybean oil in commercial applications, the dietary intake of linoleic acid would still fall within the current intake levels. Pioneer also notes that a variety of other oils used by the food industry would provide significant amounts of linoleic acid in the diet. Other noted changes in dietary intakes of fatty acids would result in small increases in the consumption of minor fatty acids, heptadacanoic acid (C17:0), heptadecenoic acid (C17:1), and the (9, 15) isomer of linoleic acid (cis-9, cis-15-octadecadienoic acid).

Pioneer states that the 17-carbon fatty acids, heptadecanoic and heptadecenoic acids, occur at low levels in commonly consumed foods. For example, heptadecanoic acid is commonly found in meat (lamb, beef, pork), butter, and tofu, while heptadecenoic acid is found in foods such as tofu, beef, cheese, and olive oil. Pioneer stated that odd-chain fatty acids such as 17-carbon fatty acids are readily metabolized.

8.2 Animal Diets

When oil is removed from the soybean a defatted meal is generated that is used as a primary protein supplement for animal feed. Pioneer provided examples of swine and poultry diets to demonstrate that the reduced intake of linoleic acid, an essential fatty acid, would not lead to a nutritional deficit for animals consuming feeds containing meal derived from 305423 soybeans. Corn, the major ingredient of animal diets, is the primary source of linoleic acid in such diets. Even though soybean meal derived from 305423 soybeans would have reduced amounts of linoleic acid, the quantity of linoleic acid provided by corn is several-fold above the animals' requirement.

9. Common or Usual Name of the Oil Product

Pioneer concluded that based on the intended increase in the level of oleic acid and decrease in the levels of linoleic and linolenic acids, a new common or usual name is appropriate for the oil from 305423 soybeans to distinguish this oil from the conventional soybean oil as defined in the Food Chemicals Codex (FCC). Pioneer proposed the name "high oleic soybean oil" for the oil that will be produced from 305423 soybeans.

10. Conclusion

Pioneer has 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. At this time, based on Pioneer's data and information, the agency considers Pioneer's consultation on the 305423 soybean to be complete.

Mary D. Ditto, Ph.D.

[1]The *gm-hra* cassette also contains three Flp recombinase target sequences; however, these sequences were not used in the development of the 305423 soybean. The presence of these sites does not cause recombination. Recombination requires the presence of the specific Flp recombinase enzyme that is not present in plants.

[2]National Center for Biotechnology Information (NCBI) Protein Dataset release 156.0. The NCBI dataset incorporates all non-redundant entries from all GenBank nucleotide translations along with protein sequences from SWISS-PROT, PIR, PRF, and PDB databases.

[3]Allergen database derived from the Food Allergy Research and Resource Program (FARRP, version 6.0).

[4]Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), Western blot analysis, glycoprotein staining, mass determination of the tryptic peptides by matrix assisted laser desorption ionization mass spectrometry (MALDI-MS), and N-terminal amino acid sequence analysis.

[5]The E-score was set at 1.0 to ensure that proteins even with limited similarity would not be overlooked.

[6]University of Nebraska Allergen Database, version 6, January 2006; www.allergenonline.com.

From FDA website page:
http://www.fda.gov/Food/Biotechnology/Submissions/ucm155595.htm

Determination of nonregulated status for Pioneer DP-305423-1 soybean

In response to petition 06-354-01p from Pioneer Hi-Bred, APHIS has determined that Pioneer 305423 soybean and progeny derived from it are unlikely to pose a plant pest risk and are no longer to be considered regulated articles under APHIS's biotechnology regulations at 7 CFR part 340, as APHIS has no authority to continue to regulate a GE organism once APHIS has determined that the GE organism is not likely to pose a plant pest risk. Permits or acknowledged notifications that were previously required for environmental release, interstate movement, or importation under those regulations are no longer a requirement for Pioneer High Oleic Event 305423 soybean and its progeny. Importation of seeds and other propagative material will still be subject to APHIS foreign quarantine notices at 7 CFR part 319 and Federal Seed Act regulations at 7 CFR part 201.

This determination is based on APHIS' analysis of field, greenhouse, and laboratory data, references provided in the petition, and other relevant information as described in the Plant Pest Risk Assessment for Event 305423 soybean, the Environmental Assessment and in APHIS's response to public comments that indicate that Event 305423 soybean is unlikely to pose a plant pest risk.

The Plant Pest Risk Assessment (http://www.aphis.usda.gov/brs/not_reg.html) concluded that Pioneer High Oleic 305423 soybean is not likely to pose a plant pest risk and should be granted nonregulated status for the following reasons: (1) agronomic performance, disease and insect susceptibility, and compositional profiles (except for the intended changes in the fatty acid profile) of Event 305423 soybean are similar to those of its non-genetically engineered soybean counterparts and/or other soybean cultivars grown in the U.S., and are unlikely to have direct or indirect plant pest effects on raw or processed plant commodities; (2) the new gene products cause no greater changes to plant metabolism or seed composition (except for the intended changes in the fatty acid profile) than conventional soybean; (3) gene introgression from Event 305423 soybean into wild relatives in the United States and its territories is extremely unlikely and is not likely to increase the weediness potential of any resulting progeny nor adversely affect the genetic diversity of related plant any more than would cultivation of traditional or other specialty soybean varieties; (4) it exhibits no characteristics that would cause it to be weedier or more difficult to control as a weed than the non-genetically engineered parent soybean line or any other cultivated soybean; and (5) horizontal gene transfer is unlikely to occur between Event 305423 soybean and soil bacteria.

In addition, APHIS has completed an Environmental Assessment (EA) for this action and has determined that granting nonregulated status to Event 305423 soybean and its progeny would have no significant impact, individually or cumulatively, on the quality of the human environment and will have no effect on federally listed threatened or endangered species, species proposed for listing, or their designated or proposed critical habitat (http://www.aphis.usda.gov/brs/not_reg.html). APHIS also concludes that new varieties bred from Event 305423 soybean are unlikely to exhibit new plant pest properties, i.e., properties substantially different from any observed for Event 305423, or

those observed for other soybean varieties not considered regulated articles under 7 CFR part 340.

Sidney W. Abel for

Michael C. Gregoire

JUN 7 2010

Date

Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture