Finding of No Significant Impact

Animal and Plant Health Inspection Service

Petition for Nonregulated Status for Pioneer Soybean DP-356043-5

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture (USDA), has prepared an environmental assessment (EA) prior to making its determination of whether to approve a petition (APHIS number 06-271-01p) for a determination of nonregulated status received from Pioneer Hi-Bred International (Pioneer), under APHIS regulations at 7 CFR part 340. The subject of this petition, soybean (*Glycine max*) line DP-356043-5 (hereafter noted as 356043), is genetically engineered to express modified glyphosate acetyltransferase (GAT4601) and acetolactate synthase (GM-HRA) proteins (enzymes) that allow the plant to tolerate applications of glyphosate and acetolactate synthase-inhibiting herbicides. On October 5, 2007, APHIS published a notice in the *Federal Register* (72 *FR* 56981-56983, Docket no. 2007-0019) announcing the availability of the draft EA for public review and comment for a 60-day comment period, ending December 4, 2007. APHIS received 110 comments regarding the EA and petition. APHIS' responses to the issues raised during the comment period are included as an attachment to this document.

In the draft EA, APHIS considered two alternatives: Alternative A – No Action: Continuation as a Regulated Article; Alternative B – Determination that 356043 soybean is No Longer a Regulated Article, in Whole. APHIS proposed Alternative B as its preferred alternative because of the lack of plant pest characteristics displayed by 356043 soybean. APHIS has not identified any greater plant pest risk characteristics in this transformed soybean than non-transformed or other non-regulated soybeans that would warrant denying the petition for 356043 soybeans. Based upon analysis described in the final EA and in APHIS' response to comments, APHIS has determined that the preferred alternative, to grant the petition in whole, will not have a significant impact on the quality of the human environment for the following reasons:

1. There should be no significant environmental impact as a result of gene introgression (e.g., cross-pollination) from this soybean line to other crop and non-crop plant species. In assessing the potential risks associated with gene introgression from 356043 soybean into its sexually

compatible relatives, APHIS considered two primary issues: a) the potential for gene flow and introgression; and b) the potential impact of introgression. The genus *Glycine* has approximately 9 species with *G. max* being placed in the subgenus *Soja* along with one other species, *G. soja* (previously *G.ussuriensis*). *G. max* is sexually compatible with only *G. soja*. *G. max* is the only *Glycine* species located in the USA other than a few *G. soja* plants in research plots. *G. max* has never been found in the wild. The likelihood of gene flow and introgression of 356043 soybeans into other species is essentially zero and consequently the potential impact of introgression into other crop or non-crop species is not reasonably foreseeable. Therefore, there should be no impact related to outcrossing from granting nonregulated status to 356043 soybean.

2. APHIS does not expect 356043 soybean to have any impacts on non-target organisms, including beneficial organisms and threatened or endangered species because all the studies conducted on 356043 soybean and specific proteins show no evidence of toxicity and GAT4601 and GM-HRA protein assessments showed low likelihood of allergenicity. Pioneer conducted extensive compositional analyses on 356043 soybean and noted minor compositional differences compared to non-transgenic control soybeans. Pioneer conducted three separate acute toxicity studies with individual soybean components (GAT4601, GM-HRA, and N-acetyl-L-aspartic acid) and noted no toxicities at the levels tested. Pioneer also conducted two whole food studies to assess wholesomeness/nutrition (broiler chicken study) and toxicity (90-day rat study) of soybean meal. The chicken study demonstrated the wholesomeness of 356043 soybean meal and the rat study demonstrated a lack of toxicity across a battery of microscopic and macroscopic assessments. Since publication of the draft EA, Pioneer has also completed a food safety consultation with FDA for 356043 soybean (BNF No. 108). Finally, considering all the noted analyses and studies related to the composition of 356043, there should be no significant impacts to the environment or to non-target organisms, including threatened and endangered species, from the introduction of 356043 soybean.

3. Soybean (*Glycine max)* is not considered to be a weed and it does not persist in unmanaged ecosystems. In the United States, soybean is not listed as a weed in the major weed references, it is not present on the lists of noxious weed species distributed by the Federal Government, and soybean has been grown throughout the world without any report that it is a serious weed.

APHIS has reviewed field performance data submitted by the petitioner. These data indicate that the engineered plant is not different in any fitness characteristics from its parent that might cause 356043 to become invasive. No data of which APHIS is aware indicates that the presence of the *gat4601* or *gm-hra* genes improve the ability of this soybean line to survive without human intervention, nor is there any foreseeable reason to conclude that these genes would affect this line's survival in the wild. Therefore, soybean is unlikely to become a weed through the introduction of the glyphosate and ALS-inhibitor resistance traits. For these reasons, granting nonregulated status to 356043 soybean should not increase the weediness or invasiveness potential relative to the release of any other soybean line.

4. APHIS evaluated the potential for significant impacts from cultivation of 356043 soybean and its progeny on non-target organisms, including those species federally listed as threatened or endangered species (TES), or species proposed for listing, and their proposed and designated critical habitat (http://endangered.fws.gov/wildlife.html#Species). APHIS reviewed the expected use of glyphosate and ALS-inhibitor herbicides on 356043. Glyphosate tolerant soybeans have been grown commercially and treated with glyphosate for over ten years. Similarly, ALS-inhibitor herbicides have been registered by EPA for use on soybeans for many years. EPA has not identified any significant issues related to label changes or metabolites from the use of glyphosate and registered ALS-inhibitors on 356043 soybeans. Therefore, there should be no significant impact to non-target organisms or TES, or their designated or proposed critical habitat from exposure to 356043 soybean or from exposure to label rates of glyphosate or ALS-inhibitors expected to be used in conjunction with 356043 soybean as a result of deregulating this line.

5. APHIS evaluated the potential for significant impacts to biodiversity from the cultivation of 356043 soybean. Analysis of available information demonstrates that 356043 does not exhibit any traits that should cause increased weediness, and that its unconfined cultivation should not lead to increased weediness of other sexually compatible relatives (of which there are none in the United States). 356043 has no effect on non-target organisms common to the agricultural ecosystem or federally listed TES or species proposed for listing. Glyphosate use and crop production practices are not expected to change significantly, therefore there should be no

indirect or cumulative impacts on biodiversity related to these practices. Use of ALS-inhibitor herbicides will likely increase to manage glyphosate tolerant weed populations, but the use of glyphosate and ALS-inhibitors on 356043 soybean according to product labels is not expected to cause significant impacts on biodiversity outside the agroecosystem based on the chemical and toxicological properties of these products. Based on these conclusions, there should be no significant impact to biodiversity by deregulating this line in whole.

6. If 356043 were to be grown commercially, the effects on agricultural practices (e.g., cultivation, spray programs, crop rotation practices, planting rates, etc.) from introducing 356043 into the environment should not be significantly different than for previously deregulated glyphosate tolerant or RR®/STS® soybean lines. APHIS has evaluated field trial data reports submitted on the 356043 event and progeny and no significant effects have been noted based on levels of herbicide tolerance, other agronomic characteristics and recommended application rates for glyphosate and ALS-inhibitor herbicides. Based on these conclusions, there should be no significant impact on commercial use by deregulating this line in whole.

7. APHIS does not expect 356043 to cause significant impact on the development of herbicide tolerant weeds or cumulative impacts in combination with other glyphosate tolerant or Roundup Ready®/STS® (sulfonylurea tolerant soybean) crops. Soybean production in the U.S is currently nearly saturated with glyphosate tolerant soybean varieties and no significant increase in soybean acreage is expected above the highest production year of 2006. In the prior five years, soybean production was relatively steady varying from 72 million acres to 75.5 million acres (http://www.nass.usda.gov/QuickStats/index2.jsp). As 356043 is expected to replace some of the current glyphosate tolerant and RR®/STS® products, the amount of glyphosate tolerant soybean planted in the U.S. is unlikely to increase based on whether or not 356043 is deregulated. Further, the introduction of alternative tools, such as 356043, permit the use of a wider range of options for farmers to minimize the development of glyphosate tolerance in weeds, essentially extending glyphosate's usefulness. Likewise, the amount of glyphosate applied to soybeans is also unlikely to increase because there have been no changes in label rates on a per application or yearly total basis. Therefore, APHIS concludes that 356043 should not have impacts on the rate of the development of herbicide tolerant weeds.

8. If 356043 were to be grown commercially, APHIS expects 356043 soybean will be used to breed soybean varieties suitable to a range of environments and maturity zones and replace some of the presently available glyphosate and ALS-inhibitor tolerant soybeans. The potential impact on organic farming should not change from the current situation and organic farmers or other farmers who choose not to plant or sell glyphosate tolerant soybean or other transgenic soybeans (a) will still be able to purchase and grow non-transgenic soybeans and (b) will be able to coexist with biotech soybean producers as they do now. This is because soybean is a highly self pollinated (pollination occurs within the flower, not often between flowers) plant with large, heavy seeds that are not easily dispersed; thus minimal buffer zones (enough to prevent physical or mechanical mixing by farm equipment and where separation can be accomplished with farm roads or equipment turning zones common to agriculture practices) is all that is needed to prevent cross-pollination to other soybeans or seed movement to adjacent agricultural land that may contain non-genetically engineered soybean. Based on these considerations, there is no apparent potential for a significant impact on conventional or organic farming by deregulating this line in whole.

9. APHIS' analysis of agronomic performance, disease and insect susceptibility, and compositional profiles of 356043 and its non-genetically engineered counterpart indicates no significant differences in composition between the two that would be expected to cause significant effects on raw or processed plant commodities from the deregulation of 356043. In addition to the presence of GAT4601 and GM-HRA proteins, the analysis showed minor differences in two fatty acids, minor differences in one isoflavone and one anti-nutrient and increased levels of two acetylated amino acids. Based on the analyses, there should be no direct or indirect plant pest effects on raw or processed plant commodities by deregulating this line in whole.

10. When considered in light of other past, present, and reasonably foreseeable future actions, and considering potential environmental effects associated with adoption of 356043 soybean, APHIS could not identify significant environmental impacts that would result from granting nonregulated status to 356043 soybean. Granting deregulation, in whole, should not have

significant human health or environmental effects, nor is it expected to establish a precedent for future actions with potentially significant effects. None of the effects on the human environment are highly controversial, highly uncertain, or involve unique or unknown risks. The effects are similar in kind to those already observed for currently commercially available and widely grown glyphosate tolerant or RR®/STS® soybean varieties and to those observed for the use of glyphosate and several other herbicides in agriculture production systems. None of the proposed alternatives are expected to threaten or violate Federal, State, or local law requirements.

Because APHIS has reached a finding of no significant impact, no Environmental Impact Statement will be prepared regarding this decision.

for Michael Gregoire
Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: JUL 15 2008

Attachment
Finding of no significant impact
Response to Comments
Petition 06-271-01p

On October 5, 2007, APHIS published a notice in the Federal Register (72 *FR* 56981-56983, Docket no. 2007-0019) announcing the availability of the Pioneer petition to deregulate 356043 soybean and of a draft environmental assessment (EA) for public review and comment for a 60-day comment period, ending December 4, 2007.

APHIS reviews a petition to determine if the genetically engineered (GE) organism should continue to be considered a regulated article under APHIS biotechnology regulations (7 CFR part 340). In order for a GE organism to be considered a regulated article, the organism must pose a plant pest risk and be modified by recombinant DNA techniques (genetic engineering as defined in the regulation). Prior to making a decision on a petition for APHIS to grant nonregulated status for a GE organism, APHIS usually prepares an EA to evaluate the significance of impacts on the environment arising from a decision to grant nonregulated status. APHIS prepares the EA as part of its obligation, like other Federal agencies, to meet the requirements of the National Environmental Policy Act (NEPA) of 1969. As part of the process, APHIS considers public comments on the proposed deregulation as well as the EA.

APHIS' response to comments below has also been reflected in some revisions and clarifications of the draft EA, so that the revised, final EA takes these issues into account.

APHIS received 110 comments during the comment period. There were 18 comments from groups or individuals that supported deregulation and 92 that submitted comments opposing deregulation.

Commenters writing in support of granting nonregulated status included weed scientists, growers, seed supply retailers, state and national soybean associations, and other individuals. Two large agribusiness companies (including the petitioner) also wrote in support and to provide clarification for some submitted comments. Supporters of deregulation cite a number of benefits associated with having this product available for use: increased options for weed control in soybean fields, the ability to combat an increasing number of glyphosate tolerant weed populations, longer-lasting broad spectrum weed control, increased marketplace choice of seeds, increased market competition, benefits to the environment from decreased tillage and use of fewer environmentally hazardous pesticides, reduced fuel usage, improved productivity, improving the availability of a safe, affordable food supply, and preserving the utility of herbicide tolerance traits. Several comments also focused on Pioneer/Dupont's history of effective product stewardship programs.

Many comments were received that oppose deregulation based on general opposition to development and use of genetically engineered plants without citing specific issues in the EA or petition. Lengthier comments were received from individuals and organizations that are generally opposed to development of genetically engineered organisms. Responses to relevant comments received are addressed below.

1. **Comment:** One comment indicated that Pioneer had not provided data (such as pollen viability and morphology) on reproductive biology of 356043 soybean.

Response: As noted in Section VII-B (p. 67) of the petition, plant breeders and others skilled in agricultural sciences observed the flowers of 356043 soybean plants throughout the development process. Because soybeans are a self-pollinating crop, changes in pollen viability and morphology would be expected to impact seed set, and therefore yield, and other agronomic characteristics. Data submitted in the petition gave no indication of altered weediness potential, flower morphology, yield, dormancy or germination in 356043 soybean. In addition, neither the GAT4601 nor the GM-HRA proteins have properties of toxins or toxic modes of action. Therefore, these proteins would not be expected to affect pollen viability and morphology. Additional specific data on pollen viability and morphology were considered of limited value and not needed to assess the potential impact of 356043 soybean on weediness potential or potential pollinator species. The document cited by the commenter (Appendix II of the U.S. and Canada Bilateral on Agricultural Biotechnology) is a guidance document, and as such, does not describe data required by APHIS to make a determination of nonregulated status.

2. **Comment:** Several comments indicated that damage to symbiotic organisms along with the associated effects of reduced nitrogen fixation, reduced yields, and related effects had not been adequately analyzed.

Response: The results of King *et al*. (2001) demonstrated a negative impact of glyphosate on *Bradyrhizobium japonicum*, an important nitrogen-fixing symbiont that colonizes soybean roots. As nitrogen-fixation is critical for soybean yield, if this effect were significant, soybean yields would be expected to show a decline with glyphosate use. An investigation into the impact of using glyphosate on previously deregulated lines of glyphosate tolerant-soybeans concluded that there is no significant reduction in yield or nitrogen assimilation when glyphosate is used at label rates (Zablotowicz and Reddy, 2004; Zablotowicz and Reddy, 2007). National Agricultural Statistics Service (NASS) statistics indicate that the average nationwide yield of soybeans continues to increase or remain relatively steady with the adoption of the first generation of Roundup Ready® soybeans (http://www.nass.usda.gov/QuickStats/PullData_US.jsp). With respect to 356043 soybeans, Pioneer multi-location yield studies conducted in 2006 demonstrated no yield reduction compared to a near-isoline control when 356043 soybeans were sprayed with glyphosate and chlorimuron. Based on yield and other data supplied by Pioneer and analyzed by APHIS, APHIS reasonably concludes that deregulation of 356043 and use of glyphosate on developed soybean lines should have no significant impact on symbiont organisms.

References

King, C.A., L.C. Purcell, and E.D. Vories. 2001. Plant growth and nitrogenase activity of glyphosate-tolerant soybean in response to foliar glyphosate applications. Agron J. 93:179-186.

Zablotowicz, R. M. and K. N. Reddy. 2004. Impact of glyphosate on the *Bradyrhizobium japonicum* symbiosis with glyphosate-resistant transgenic soybean: A minireview. Journal of Environmental Quality. 33: 825-831.

Zablotowicz, R.M. and K.N. Reddy. 2007. Nitrogenase activity, nitrogen content, and yield responses to glyphosate in glyphosate-resistant soybean. Crop Protection 26: 370-376.

3. **Comment:** Several comments stated that APHIS had not considered the impacts of spraying two herbicides on agricultural practices, non-target and beneficial organisms, and threatened and endangered species.

Response: The use of multiple herbicides with different modes-of-action on crops (whether tank-mixed or applied sequentially) is already a common agricultural practice. Growers already spray both glyphosate and ALS-inhibiting herbicides on their soybeans, so there will be no significant change to current agricultural practice and therefore no expected cumulative impact. While the use of ALS-inhibiting herbicides is expected to increase with the availability of 356043 soybean, the amounts are not predicted to reach the historic high levels seen in the 1990's (Pioneer Letter to USDA, 1/18/08). As noted in the EA, tank mixes or sequential applications of glyphosate and ALS inhibitor herbicides are already likely used on RR®/STS® varieties (EA p. 18). It can reasonably be expected that herbicides used on 356043 soybeans will be labeled for this use and used at (or below) approved label rates. APHIS has consulted with EPA regarding herbicide label changes and use of glyphosate and ALS-inhibitors on 356043 soybean. They have not identified any significant issues related to either herbicide label changes or metabolites from the use of glyphosate or registered ALS-inhibitors on 356043 soybean (EPA, personal communication).

4. **Comment:** One comment suggested Tier 1 studies of the GAT4601 protein and the metabolites NAA and NAG against several key indicator species including beneficial organisms in order for USDA-APHIS to address the impact of 356043 soybeans on threatened and endangered species.

Response: Pioneer provided data indicating that the GAT4601 protein does not have significant amino acid sequence homology to known toxins. Additionally, Pioneer provided a summary of data of a mouse acute toxicity study using GAT4601 protein indicating no evidence of acute toxicity in mice (Petition, p. 63). Pioneer also conducted a 13-week rat feeding study (a common study used to assess toxicity) using 356043 soybean (Appenzeller et al., 2008). That study demonstrated a lack of toxicity of 356043 soybean when used as a significant portion of the rat diet. FDA received and reviewed similar data that ultimately led to a completed consultation (BNF No. 108) on this product (http://www.cfsan.fda.gov/~lrd/biocon.html). Tier 1 studies on non-target (NTO) and beneficial organisms are most appropriate for molecules like BT proteins (*Bacillus thuringiensis* toxin proteins used to provide protection against insect pests) which are known to be toxic to certain species. In addition, it is unlikely that changes which improve upon desired enzyme function will result in the creation of a toxic protein (Pariza and Johnston, 2001). Using information provided by Pioneer from mouse acute toxicity studies and rat feeding studies, APHIS addressed issues of potential toxicity of 356043 in the EA (pp.14-17). Similarly, NAA and NAG metabolites in 356043 soybean are not known or expected to cause any adverse effects to non-target or beneficial organisms. Therefore NTO testing is not warranted. The comment referencing possible adverse effects on honeybees cited an editorial article and interview that mentions fungicides and honeybees but does not make reference to substantiated data on effects of glyphosate or genetically engineered soybeans that would warrant further testing. Therefore, deregulation of 356043 soybean does not raise any new issues with respect to non-target or beneficial organisms.

References

Appenzeller, L.M., S.M. Munley, D. Hoban, G.P. Sykes, G.P., Malley, L.A., and Delaney, B. 2008. Subchronic Feeding Study of Herbicide-Tolerant Soybean DP-356Ø43-5 in Sprague-Dawley Rats. Food and Chemical Toxicology, doi: 10.1016/j.fct.2008.02.017

Pariza, MW and Johnston, EA. (2001) Evaluating the Safety of Microbial Enzyme Preparations Used in Food Processing: Update for a New Century. Regulatory Toxicology and Pharmacology 33: 173-186.

5. **Comment:** One comment suggested the need for a rigorous toxicological assessment for 356043 soybean.

Response: Neither the GAT4601 and GM-HRA proteins expressed in 356043 soybean nor the metabolites that are elevated (NAA, NAG, 17:0 and 17:1) are known toxins. The composition of 356043 soybean is comparable to that of control and commercial soybean varieties. The commenter notes that the broiler chicken feeding study referenced in the petition (p. 96) is not a relevant toxicological assessment and APHIS agrees. Toxicity studies have been conducted with N-acetyl-L-aspartic acid (NAA) and acute toxicity was not noted up to doses of 2000 mg/kg bodyweight (http://www.sciencedirect.com/science/journal/02786915). A repeated dose toxicity study was also conducted based on OECD guidelines for 28-day studies. Significant adverse biological effects were not noted up to the maximum dose of 1000 mg/kg/day (http://www.sciencedirect.com/science/journal/02786915). Additionally, Pioneer completed a rat subchronic (90-day) feeding study to assess the food safety of 356043 soybean. (Appenzeller, et al., 2008). No biologically relevant adverse effects were noted in rats fed both sprayed and unsprayed 356043 soybean. Additionally, Pioneer has completed its food safety and nutrition Consultation with FDA on this product (BNF No. 108). While APHIS does not rely entirely on FDA's completed Consultation in its assessment, this information, along with relevant compositional data, literature reviews and published papers on these various proteins and metabolites leads APHIS to conclude that further toxicological assessment is unwarranted.

6. **Comment:** Two comments asked whether the compositional analysis was conducted on soybeans sprayed with herbicides, stating that if no herbicides were applied, the compositional assessment may not be representative. Differences in composition of meal from sprayed and

non-sprayed 356043 soybeans in a published broiler study (McNaughton *et al*., 2007) were also pointed out.

Response: In order to isolate the potential impact of the transgenes on the nutritional composition of soybeans, the compositional analysis (as presented in 06-271-01p) was done on 356043 and control soybeans that were not sprayed with herbicides. For testing purposes, control soybeans obviously cannot be sprayed with glyphosate.

For regulatory authorities outside the U.S., Pioneer did submit information from a compositional comparison between 356043 soybean treated with herbicides (glyphosate, chlorimuron, and thifensulfuron) and control soybeans not treated with herbicide. With the exception of fatty acids 17:0 and 17:1 and the acetylated amino acids NAA and NAG, the only analytes that showed statistical differences were palmitic acid, oleic acid, linoleic acid, and malonylglycitin. However, all mean values for these analytes were within the statistical tolerance interval and the literature range for soybean grain.

Analysis of poultry diets is conducted to ensure balanced diets are fed to the animals. As one commenter suggests, the crude compositional assessment in the published broiler study is suggestive, but not statistically powerful enough, to identify compositional differences. The commenter makes broad statements about apparent compositional differences between sprayed and unsprayed 356043 soybeans without also considering the small sample sizes assessed (2) and data from the other reference soybeans analyzed for the study. A multi-site, multi-replication study, as was done for the compositional study summarized the Petition, is best suited for understanding potential compositional changes.

Reference

McNaughton, J., M. Roberts, B. Smith, D. Rice, M. Hinds, J. Schmidt, M. Locke, K. Brink, A. Bryant, T. Rood, R. Layton, I. Lamb, and B. Delaney. 2007. Comparison of broiler performance when fed diets containing event DP-356Ø43–5 (Optimum GAT), nontransgenic near-isoline control, or commercial reference soybean meal, hulls, and oil. Poultry Science 86: 2569-2581.

7. **Comment:** One comment indicated that the reference varieties used for compositional comparison were not specified, and that the locations where the reference varieties were grown were mostly different than where 356043 and control soybeans were grown.

Response: The soybean varieties used as reference lines were commercial varieties sold by Pioneer: 92M10, 92B12, 92B63, 92M72.

Conducting the nutrient composition study on conventional soybean varieties separately from the transgenic study is appropriate because the tolerance intervals are not used for direct comparison between the transgenic soybean and the non-transgenic isoline. Tolerance intervals are helpful in understanding the amount of natural variation that can be observed in contemporary commercial soybean varieties. As long as the soybean studies are conducted in commercial soybean growing areas with varieties appropriate for those areas, it is not necessary that the conventional soybean varieties be grown in the exact same locations as the transgenic soybean.

8. **Comment:** One comment indicated that consumption of tofu made from 356043 soybeans could greatly increase the exposure to these unusual 17:0 and 17:1 fatty acids. The commenter noted that "While this may not be problematic, it may have long-term health implications, and it is troubling that neither DuPont-Pioneer nor APHIS assessed this matter." The commenter also notes purported differences in four isoflavones from data provided in the Petition.

Response: Soybeans used for tofu and other traditional soy foods (such as edamame, natto, miso, yuba, soy sauce, tempeh, *etc*.) generally come from special soybean varieties, commonly called "food-grade" soybeans, distinguished from commodity soybeans by having a larger seed and special taste/texture characteristics (http://web.aces.uiuc.edu/value/factsheets/soy/fact-tofu-soy.htm). Soybean varieties grown in the U.S. for the Asian food market, including tofu, are grown under contract and are generally (>90%) non-GE (Pioneer Marketing communication). Therefore, the levels of 17:0 and 17:1 fatty acids in tofu are not expected to increase significantly above their current levels. The commenter provided no data or references suggesting that consumption of 17:0 or 17:1 fatty acids constitute any sort of health risks. Given these facts, along with the fact that many foods are known to contain both of these types of fatty acids (http://www.nutritiondata.com/topics/fatty-acids), APHIS did not identify significant issues associated with consumption of these soybeans.

For FDA (BNF No.108), a dietary assessment for 17:0 and 17:1 was conducted assuming 100% of the soybeans consumed were 356043 soybean, and that all traditional soy foods were made with 356043 soybeans. 356043 soybeans completed the FDA consultation process on September 21, 2007. FDA's summary of the exposure data for fatty acids in 356043 soybean can be found in their Note to File (http://www.cfsan.fda.gov/~rdb/bnfm108.html).

Regarding the noted "differences" in isoflavones noted by the commenter, APHIS also noted levels of these isoflavones, as well as other isoflavones and antinutrients in 356043 soybean. Three of the levels of isoflavones fell within the statistical norms based on adjusted P-values (noted response to this statistical analysis in #27 of this document) provided by Pioneer and are, therefore, not statistically different than control soybeans used for comparison. The average (mean) of one isoflavone concentration was noted as being statistically different in 356043 soybean compared with the control soybean (malonyldaidzin). While the average concentration of this isoflavone were different (~12% as noted by the commenter), the ranges of concentrations between 356043 and the control overlapped a great deal, as noted in the table. While Pioneer provided some information regarding the literature range normally found for this isoflavone in soybeans, it apparently was not complete. APHIS did identify a reference (Lee, et al., 2002) which noted wide variations in a number of isoflavones and higher malonyldaidzin (the isoflavone noted as different in 356043 soybean) concentrations than those found even in 356043 soybean. Given all this information, there appear to be wide variations in concentrations of these compounds in soybeans (likely due to differences in cultivar, environment, analytical methods, sampling time, etc.). The commenter provided no data or references indicating that this difference was biologically meaningful and APHIS could identify no other references that might result in any significant environmental impacts from this difference in increased malonyldaidzin.

Lee, L.J., W. Yan, J.K. Ahn, I.M. Chung. 2003. Effects of year, site, genotype and their interactions on various soybean isoflavones. Field Crops Research 81: 181-192.

9. **Comment:** One comment stated that "Pioneer's own composition study demonstrates that the concentrations of two fatty acids are increased to levels that exceed the specified range for soybean oil in the Codex Alimentarius standard. As a result, soybean oil derived from 356043 soybeans cannot be marketed as commodity soybean oil."

Response: While this is an interesting point, the difference in levels of two fatty acids, 17:0 and 17:1, between 356043 soybean and control soybean does not constitute a safety issue. Codex Alimentarius standards are referred to in the comment, but generally, U.S. companies trade in oils using quality standards (such as color, viscosity, etc.) and not through the Codex standards. Additionally, because soybean oil is a highly blended commodity, it is unlikely that oil from 356043 soybean would be exported in a percentage high enough to affect levels of 17:0 and 17:1 in the commodity soybean oil, and therefore cause it to not meet Codex standards. Prior to the time when 356043 soybeans reach a market share level where they may influence commodity soybean oil characteristics, DuPont and Pioneer can reasonably be expected to work with organizations such as Codex Alimentarius as well as soybean processors, exporters, commodity associations, etc. to identify any implications for international trade of soybeans.

Pioneer's data on control soybeans show that levels of 17:0 fatty acids specified by Codex apparently do not reflect modern soybean varieties, as noted below.

	Codex Alimentarious	Commodity beans (petition, Table 21, p. 83)	ILSI database*
Level of 17:0	ND – 0.1%	Average = 0.110% Range = 0.0896 – 0.130%	0.085 – 0.146 (n=97)

* The ILSI database (http://www.cropcomposition.org/) represents a compilation of crop analyses from a number of companies engaged in agricultural life sciences. Through ILSI, the participants have standardized and pooled their crop data in order to make the data available to scientists from academia, government agencies, and industry, and to the general public.

Ultimately, Pioneer and growers of 356043 soybeans will need to deal with marketing issues associated with sale of these soybeans and associated products.

10. **Comment:** One commenter suggested that a dietary exposure assessment for 17:0 and 17:1 be conducted, including the highest exposed sub-populations, namely non-nursing infants and children.

Response: This dietary exposure assessment was conducted and submitted to FDA as part of BNF No.108, and included the population subgroup with the highest predicted exposure (children 1-2 years). 356043 soybeans completed the FDA consultation process on September 21, 2007. FDA's summary of the exposure data for fatty acids in 356043 soybean can be found in their Note to File (http://www.cfsan.fda.gov/~rdb/bnfm108.html).

11. **Comment:** One comment suggests that APHIS cannot address the impact of the potential increase in 2-ketobutyrate, which may have toxic effects on NTO's such as microbes.

Response: 2-ketobutyrate is a common cell metabolic component found in an amino acid biosynthesis pathway in cells. It can act as a substrate for the GM-HRA protein (enzyme) and is also a substrate in the 17-carbon fatty acid biosynthetic pathway. As Pioneer notes in its Petition (Appendix 6, p. 141), the alteration in the GM-HRA enzyme may increase the 2-ketobutyrate found in the plant. The commenter claims that there is ample literature showing that 2-ketobutyrate is toxic to bacteria. Two journal articles are cited in support of this statement (LaRossa et al., 1987 and Danchin et al., 1984). However, the literature is not conclusive on the topic of 2-ketobutyrate toxicity. More recent work concludes that there is not a definitive correlation between the accumulation of 2-ketobutyrate and toxicity in microorganisms (Epelbaum et al., 1996 and Landstein et al., 1995). In addition, results published by Shaner and Singh (1993) demonstrate that growth inhibition of corn treated with imazaquin is not due to the accumulation of 2-ketobutyrate and that the growth rate of the plants is unchanged in the presence of high levels of 2-ketobutyrate. 2-ketobutyrate does not result in growth inhibition of microbes or plants and is therefore not likely to be toxic to non-target organisms. Given a weight of this evidence, including considerations of toxicity testing studies, feeding studies, compositional analysis and numerous field evaluations, further assessment related to 2-ketobutyrate is considered unwarranted.

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Shaner, D., Singh, B. 1993. Phytotoxicity of Acetohydroxyacid Synthase Inhibitors Is Not Due to Accumulation of 2-Ketobutyate and/or 2-Aminobutyrate. Plant Physiology. 103:1221-1226).

12. **Comment:** Two comments noted issues associated with increasing use of herbicides and the likelihood of increased development of herbicide tolerant weeds. One commenter spends several pages talking about herbicide tolerant weeds and use of herbicides and comments further on APHIS' treatment of this issue in its draft Programmatic Environmental Impact Statement (http://www.aphis.usda.gov/biotechnology/EIS_index.shtml). The commenter cites comments by some weed scientists relating to an increased reliance on glyphosate alone as a means of weed control and how this has been identified as problematic. The commenter also talks about increased use of glyphosate and decreased use of ALS inhibitors and notes that some weeds having developed tolerances to both herbicides as well as the acreages of land which may contain ALS tolerant weed populations (up to 9.9 million acres). One commenter also believes that Pioneer is developing new products with higher tolerance to glyphosate (based on a Pioneer patent) and this will lead to cumulative impacts that APHIS should address in its NEPA documents. The commenter also suggests that APHIS has the option of imposing conditions on this deregulation decision (based on a section in APHIS' 2007 draft Environmental Impact Statement) and should impose such conditions.

Response: APHIS acknowledges and addresses the occurrence of herbicide tolerant weeds and includes discussion of management strategies to deal with herbicide tolerant weeds in the 356043 soybean EA (pp. 11-12). The development of herbicide tolerant weeds is reasonably well understood by weed scientists (Heap, 1997; Jasieniuk, et al., 1996). Although weed scientists cannot predict which weeds will become tolerant to a particular herbicide, several noted weed scientists (including one who was cited by a commenter) provided comments on management methods to mitigate the development of herbicide tolerant weeds. APHIS considers the potential cumulative impacts of other glyphosate tolerant crops, increased use of glyphosate, glyphosate tolerant weeds, and reasonably foreseeable future actions in the 356043 soybean EA (pp. 25-28). APHIS does not agree that more extensive analysis is warranted.

APHIS notes some documentation of the appearance of glyphosate tolerant weeds (http://www.weedscience.org), particularly over the past 6-8 years in the U.S. Estimates of acres that may have some glyphosate tolerant weeds on them range from approximately 230,000 to 2.49 million acres (acreages are reported in ranges only and therefore lack some accuracy). In terms of the total U.S. farmland dedicated to growing major crops in the U.S. (over 225 million acres in 2007 when considering wheat, corn, soybean, and cotton alone), the total acreage of land that may have some glyphosate tolerant weeds on it is relatively small (somewhere between approximately 0.1 and 1%). As noted by the commenter, the acreage with weeds tolerant to ALS inhibitors may be several times this. It is reasonable to expect that growers who find it necessary to address glyphosate tolerant weeds in their fields may choose to adopt 356043 soybean or one of the currently available RR® /STS® varieties. If either of these options is not sufficient, it can be expected that a grower will utilize another means of weed control (e.g., tillage, use of another herbicide, etc.). It can further be expected that growers will rotate between methods of weed control over time as they see fit based on the presence or absence of herbicide tolerant weeds in their fields. What this means is that usage of various herbicides will fluctuate over time (as has happened in the past and is documented in the EA, p. 10). APHIS is not aware of significant environmental impacts resulting from these fluctuations in herbicide use in the past and would not expect significant impacts to occur in the future.

APHIS also notes some documentation of the appearance of ALS inhibitor tolerant weeds (http://www.weedscience.org) dating back as far as 1987. Subject to the same limitations in the accuracy of data noted above, estimates of affected acreage ranged from approximately 100,000 to 300,000 acres. One also needs to consider that there are numerous ALS inhibitor herbicides (http://www.weedscience.org/summary/ChemFamilySum.asp?lstActive=&lstHRAC=3&btnSub2=Go) and noted tolerant weeds may not be tolerant to all such herbicides (i.e., other ALS inhibitors may still be effective for significant weed control in crop plantings). A further consideration is still the relatively limited acreage (up to 9.9 million acres out of over 225 million acres total cropland) affected by ALS tolerant weeds. Further, ALS inhibitors are still being used widely and effectively on many crops including those developed specifically for use of ALS inhibitors (e.g., Clearfield® varieties of corn, wheat, rice, canola, soybean, and sunflower from BASF: http://www.agproducts.basf.com/ag-products.asp). Pioneer documented current ALS inhibitors labeled for use on soybeans in additional information submitted to APHIS on 1/18/2008 and that list alone included 9 different ALS inhibitors. There are currently approximately fifty ALS inhibitor herbicides (http://weedscience.org).

No increased soybean acreage is expected because approximately 90% of the present soybean acreage is already devoted to glyphosate tolerant (GT) varieties. Acres planted to soybean in 2007 were at their lowest level since 1996, reflecting a 16% decrease in acreage compared to 2006. In the prior five years, soybean production was relatively steady varying from 72 million acres to 75.5 million acres (http://www.nass.usda.gov/QuickStats/index2.jsp). Furthermore, the percentage of soybean acreage in the United States planted to glyphosate-tolerant soybean varieties is not expected to dramatically increase beyond current levels for the following reasons: 1) a certain small percentage (<1%) of soybean growers choose to grow organic soybeans, and genetically engineered soybean varieties cannot be grown and certified as organic, 2) some growers will choose to grow non-genetically engineered soybeans for other marketing reasons (e.g., food-grade soybeans), 3) a certain percentage of soybean growers each year may choose to rotate out of glyphosate tolerant soybeans and use herbicides with alternate modes of action and/or tillage as recommended to avoid weed shifts or the selection of glyphosate tolerant weeds, and 4) growers may simply choose another variety without the glyphosate tolerance trait that is better suited to their specific growing conditions and their needs. Based on this analysis, there should not be any significant cumulative impact because there should not be any incremental increase in acreage of GT soybeans if APHIS were to grant the petition for non-regulated status. Also, no change in the label rate of glyphosate use is anticipated for the 356043 soybean.

If APHIS chooses the no action alternative, there would also be no significant cumulative impact due to increased acreage of GT soybeans since most of the present area of soybean production in the United States is already planted to glyphosate tolerant varieties. Further, if APHIS were to choose the no action alternative or delay its decision, soybean growers would be forced to use varieties with single tolerance to glyphosate or RR®/STS® varieties. It is possible that such a course of action would serve to exacerbate the problem of weeds developing tolerance to glyphosate as growers would not have the option of using 356043 soybean for several years. In a reference cited in one comment (Yancy, 2005), weed scientists "York, Culpepper and Wilcut believe the effective life of glyphosate can be extended another 15 years by judicious use of other active ingredients in the battle against weeds." Dr. York wrote in to support APHIS' decision to deregulate this product in whole and does note Pioneer's history of good product stewardship (comment APHIS-2007-0019-0088 in the docket). A number of other weed scientists submitted comments in support of Pioneer's requested deregulation and specifically note that introduction of this product would help to address the development of glyphosate tolerant weeds by allowing growers to use another herbicide chemistry.

Regarding development of new plant varieties that may have increased tolerance to glyphosate, APHIS will make the appropriate assessments of such a product if and when such a development is reasonably foreseeable.

Regarding a "conditional deregulation," an issue discussed in APHIS' 2007 draft Environmental Impact Statement; that was an option mentioned in proposed regulations and APHIS has not promulgated new regulations that would allow such a "conditional deregulation." As such, current regulations in 7 CFR 340 do not allow APHIS to make such a determination.

Reference

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Yancy, C.H. (2005) Weed Scientists develop plan to combat glyphosate resistance. Southeast Farm Press, June 1, 2005
(http://southeastfarmpress.com/mag/farming_weed_scientists_develop/)

13. **Comment:** There will be increased soil erosion from mechanical tillage to control resistant weeds.

Response: It is reasonably foreseeable that growers will utilize the most effective and economical means of weed control at their disposal. While mechanical tillage is certainly one recommended practice for controlling herbicide tolerant weeds and an important part of Integrated Weed Management (IWM) practices, growers will have the option to simply switch to a different herbicide rather than till. This is because of the cost involved in mechanical tillage, including fuel and labor, as well as the risk of soil compaction and erosion.

14. **Comment:** Glyphosate use is linked to plant disease, mineral deficiencies, and reduced yield.

Response: APHIS has previously reviewed the literature (Gordon, 2005, Ebelhar et al. 2005) and agrees that preliminary information indicates that glyphosate application to glyphosate tolerant soybeans (referring specifically to previously deregulated Roundup Ready® soybean line 40-3-2, not 356043 soybean) may affect manganese uptake or metabolism especially in soils with low levels of manganese, that are on bottomlands, are sandy, and/or that have high pH levels (pH 6.5 or more). Whether these apparent micronutrient deficiencies affect all glyphosate tolerant varieties or just a few varieties still remains to be determined. These deficiency effects may cause lower yields, but research has shown that these deficiencies can be corrected by the addition of manganese (5 to 7.5 lb/acre banded pre-plant), and result in a typical yield increase of 3 to 5 bushels/acres, but can be up to an increase of 10 bushels/acre (Gordon, 2005, and personal communication with Dr. Susan Koehler 6/1/07). Very few acres are affected by this problem (Gordon, 2005, and personal communication with Dr. Susan Koehler) and it is not considered a significant problem. The problem is manifested on soils that are typically high yielding, that are sandy with high pH, or on soils with extremely low pH to which lime is applied to adjust the pH, and glyphosate application tends to enhance the deficiency problem. The cost of applying supplemental manganese (about $2.5/acre) would be recovered about 7 fold with even a minimal yield boost of 3 bushels/acre bringing in a typical price of about $6.00- $6.50/bushel.

APHIS also previously reviewed information (Kremer et al. 2005) reporting the apparent stimulation of the growth of selected rhizosphere fungi (*Fusarium* spp.) on the roots of hydroponically-grown glyphosate tolerant soybean (referring to Roundup Ready® soybean line 40-3-2, not 356043 soybean) plants treated with glyphosate. In this case, no noticeable effect was observed on yield of soybeans grown in the field (Njiti et al. 2003). As noted in a review (Cerdeira and Duke, 2006), glyphosate is toxic to many microorganisms, including some plant pathogens, and its influence on plant diseases in glyphosate tolerant crops is variable, sometimes reducing and other times increasing disease. In soybean, glyphosate application was reported to reduce the incidence of infection with the Asian soybean rust pathogen, *Phakopsora pachyrhizi,* in glyphosate tolerant soybeans in preliminary greenhouse experiments (Feng et al., 2005). The reported increased susceptibility of glyphosate tolerant soybeans in Michigan to white mold disease, caused by *Sclerotinia sclerotiorum,* was demonstrated not to be linked to the resistance transgene, to glyphosate, or its formulation components (Lee et al. 2000 and 2003). Additionally, glyphosate tolerant soybeans have not consistently shown more susceptibility to root rot and damping off diseases caused by *Rhizoctonia solani* Kuehn (Harikrishnan and Yang, 2002) or to *Fusarium solani*-caused sudden death syndrome (Sanogo et al., 2000, 2001, and Njiiti et al. 2003). Glyphosate tolerant and glyphosate-sensitive cultivars had similar responses when treated with glyphosate and exposed to root rot, damping-off, and sudden death syndrome.

As is the case with all plant species, various susceptibilities to pests and diseases have developed and continue to evolve over time, or greater susceptibility to an abiotic stress is discovered in isolated or unique environments for a particular variety or similar set of varieties. There are numerous soybean varieties available to farmers, and soybean breeders are continuing to develop improved varieties. Over the course of time, breeders have developed and will continue to develop varieties suited to a number of growing environments to address issues related to disease and pest resistance and tolerance to various abiotic stresses. Agricultural extension agents and seed companies make recommendations on varieties that are best suited to local growing conditions and pressures. That soybean yields continue to improve with the widespread adoption of glyphosate tolerant crops indicates that the effects of glyphosate on micronutrient uptake and potentially increased susceptibility to disease are insignificant relative to the benefits of flexible and effective weed control. It is reasonably foreseeable that these biological evolutionary processes will continue to occur and growers and developers of new agricultural varieties will continue to adapt new plant varieties and methods to address them.

References

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Harikrishnan, R., and X.B. Yang. 2002. Effects of herbicides on root rot and damping-off caused by Rhizoctonia solani in glyphosate tolerant soybean. Plant Dis. 86:1369–1373.

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15. **Comment:** Surfactants in glyphosate formulations are toxic to tadpoles and juvenile stages of certain frogs and APHIS' assessments of impacts on non-target and TES were limited to trait, not herbicide use. The commenter states that under NEPA and ESA obligations, APHIS must exercise independent judgment in evaluating TES of both trait and both herbicides.

Response: The commenters raise the point that a surfactant in Roundup® is toxic to tadpoles and juvenile frogs. APHIS recognizes this fact and notes that EPA registration does not permit growers to use glyphosate formulations with surfactant in or near fresh water (where tadpoles and frogs reside). When used according to the label, glyphosate does not have unreasonable adverse effects, according to EPA's glyphosate Re-registration Eligibility Decision (http:www.epa.gov/oppsrrd1/RED/old_reds/glyphosate/pdf). To make such determinations, EPA requires more than 100 different scientific studies and tests from applicants (http://www.epa.gov/pesticides/regulating/). Many plant and wildlife species can be found near or in cities, agricultural fields, and recreational areas. Before allowing a pesticide product to be sold on the market, EPA ensures that the pesticide will not pose any unreasonable risks to wildlife and the environment. EPA does this by evaluating data submitted in support of registration regarding the potential hazard that a pesticide may pose to non-target fish and wildlife species. In considering whether to register a pesticide, EPA conducts ecological risk assessments to determine what risks are posed by a pesticide and whether changes to the use or proposed use are necessary to protect the environment. A pesticide cannot be legally used if it has not been registered with EPA's Office of Pesticide Programs. Commercialization of 356043 soybean should not lead to increased use of glyphosate because nearly all soybean produced in the U.S. is already sprayed with glyphosate, EPA registration does not change the maximum glyphosate application rate for 356043 soybean, and 356043 soybean is expected to be a replacement for existing GT products. Furthermore, 356043 soybean is not modified in a way that would allow it to be grown in wetter environments; therefore, it should not increase the exposure of amphibians to glyphosate herbicides containing harmful surfactants. For these reasons, APHIS reasonably concludes that the deregulation of 356043 soybean should not have a significant impact on wildlife and should have no effect on species federally listed as threatened or endangered, or species proposed for listing, or proposed or designated critical habitat.

16. **Comment:** Two comments suggested that additional acetylated amino acids be measured in 356043 soybean and that Pioneer provided misleading data in its Petition.

Response: APHIS disagrees with the suggestions to require further quantification of other acetylated amino acids. The suggestions to require data that assesses additional acetylated amino acids in 356043 soybean is apparently based, in part, on the fact that the native enzymes from *Bacillus licheniformis* have the ability to use additional amino acids as substrates under specific *in vitro* conditions. As noted in the Petition, however, the GAT4601 enzyme has different kinetic and specificity properties than the native enzymes. The definitive source of information about the ability of amino acids to serve as substrates for the GAT4601 protein in 356043 soybean under appropriate physiological conditions (see comment APHIS-2007-019-109 to this docket) is found in Appendix 7 of the Petition. The study conducted with GAT4601 demonstrated that kinetic parameters could only be established for aspartate and glutamate. Pioneer provided further clarification of this issue in its public comment submission to the docket (APHIS-2007-0019-0109.1). APHIS reasonably concludes, therefore, that other potential acetylated amino acids do not need to be measured.

17. **Comment:** One comment mentioned the YopJ protein from *Yersinia pestis*, citing a publication on the bacterial use of acetyltransferases as virulence factors (Worby and Dixon, 2006).

Response: The reference cited in this comment is a brief review with editorial perspective about a publication that was published in the same issue of this journal (Mukherjee et al., 2006). A recent Science article reports that a particular protein from *Yersinia pestis* (the causative bacteria from plague or "black death" in the Middle Ages) called YopJ can circumvent normal cell signaling machinery to promote its own survival. In the study, a cell-free system was used to demonstrate that the YopJ protein mediates enzymatic O-acetylation of the hydroxyl side-chain located on specific serine and threonine residues on particular intracellular signal transduction proteins.

The reference cited provides interesting insight into the pathology associated with *Y. pestis* and other pathogenic *Yersinia* species, but it is not relevant to the N-acetylation activity of the GAT4601 protein. An extensive bioinformatic (genetic) analysis with the GAT4601 protein revealed no similarity between the GAT4601 protein and the YopJ protein. Further, the active site of GAT proteins is restricted to small molecules such as glyphosate and certain amino acids (Siehl et al., 2007). Therefore, they are not capable of acetylation of proteins such as signal transduction proteins. USDA-APHIS concludes, therefore, that the activity of the YopJ protein in *Yersinia pestis* is not relevant to the safety assessment of the GAT4601 protein.

References

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Siehl, D., Castle, L., Gorton, R. and Keenan, R. 2007. The molecular basis of glyphosate resistance by an optimized microbial acetyltransferase. Journal of Biological Chemistry 282(15): 1146-1155.

Worby CA and Dixon JE. 2006. Bacteria seize control by acetylating host proteins. Science 312:1150-1151.

18. **Comment:** One comment criticized FDA's consultation process.

Response: While this comment is specific to FDA, APHIS consults frequently with FDA and EPA in the course of our review of petitions for nonregulated status. APHIS has first-hand knowledge of the scientific rigor that each of the agencies of the Coordinated Framework bring to bear when conducting reviews. This knowledge provides APHIS with additional confidence in its own conclusion that a determination of nonregulated status for 356043 soybean should pose no significant impact on the environment.

19. **Comment:** Two comments focus on statements in the Petition and the EA related to a history of safe use of the GAT 4601 protein and *Bacillus licheniformis*.

Response: The draft EA contains a statement indicating that "The GAT protein sequence, which is derived from the bacterium *Bacillus licheniformis*, has been considered safe for food and feed in the U.S., Canada, and Europe (EU Commission, 2000; FDA, 2001)." (EA, Section V.D., p. 18). APHIS recognizes this misstatement and has corrected the language in the final EA. The sentence should read "The bacterium *Bacillus licheniformis* has been considered safe for specific food and feed uses in the U.S., Canada and Europe (EU Commission, 2000; FDA, 2001; http://canadagazette.gc.ca/partI/2006/20060805/html/notice-e.html)." *Bacillus licheniformis* is, in fact, used extensively in the detergent, starch and food industries to produce large quantities of industrial enzymes (Schallmey, et al., 2004). A review by FDA scientists further notes a history of safe use of *B. licheniformis* for a variety of food enzyme uses (Olempska-Beer, et al., 2006). Pioneer provided further clarification and documentation of the safety of *B. licheniformis* in a comment that they provided to the docket (APHIS-2007-0019-0109.1).

Regarding the GAT 4601 protein, although GAT4601 is a synthetic protein, it is 84% identical and 94-95% similar at the amino acid level to the translated protein sequences of each of the three original *gat* alleles from *B. licheniformis* from which *gat4601* was derived. The GAT4601 protein retains the characteristics found in other N-acetyltransferases that are ubiquitous in plants and microorganisms (Neuwald and Landsman, 1997). GAT4601 contains the definitive motif for the GNAT family of N-acetyltransferases (Marchler-Bauer *et al.,* 2005). This superfamily of enzymes is present in all organisms, including plants, mammals, fungi, algae, and bacteria (Dyda *et al*., 2000). Therefore, due to the similarity of GAT4601 to the GNAT family of proteins, GAT4601 can be viewed as having a history of safe use in food.

Families/superfamilies of enzymes obtained from microorganisms found in diverse habitats retain their general structure and enzymatic properties, but they may differ in certain functional characteristics such as stability and substrate specificity. There are no known instances where natural variation within an enzyme family has resulted in the generation of a toxin active via the oral route, and it is unlikely that changes which improve upon desired enzyme function will result in the creation of a toxic protein (Pariza and Johnson, 2001).

FDA has completed its evaluation of 356043 soybean containing the GAT 4601 protein (BNF No. 108), and has posted an agency response letter and note to the file. FDA has indicated that it has no further questions on the safety of 356043 soybean (http://www.cfsan.fda.gov/~rdb/bnfl108.html, http://www.cfsan.fda.gov/~rdb/bnfm108.html).

References

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Olempska-Beer, ZS, Merker, RI, Ditto, MD, and DiNovi, MJ. 2006. Food-processing enzymes from recombinant microorganisms—a review. Regulatory Toxicology and Pharmacology. 45: 144-158.

Pariza, MW and Johnston, EA. (2001) Evaluating the Safety of Microbial Enzyme Preparations Used in Food Processing: Update for a New Century. Regulatory Toxicology and Pharmacology 33: 173-186.

Schallmey, M., Singh, A., and Ward, OP. 2004. Developments in the use of *Bacillus* species for industrial production. Can. J. Microbiol. 50: 1-17.

20. **Comment:** GM-HRA has two amino acid mutations, as opposed to the ALS protein in both STS® soybean and imidazolinone-tolerant crops which have one. Thus, any claims of comparable environmental safety or impact of 356043 soybeans to products currently on the market are not justified.

Response: USDA-APHIS does not agree that the one additional amino acid substitution in the GM-HRA protein renders it substantially different from ALS proteins currently in the environment. ALS proteins are present in many species, including bacteria, fungi, algae and higher plants (Friden *et al.,* 1985; Falco *et al.,* 1985; Mazur *et al*., 1987; Reith and Munholland, 1993) and this family of proteins show variability between species (http:www.freepatentsonline.com/6444875.html).

According to FDA, proteins that function as enzymes do not raise concern. Exceptions include enzymes that produce substances that are not ordinarily digested and metabolized by vertebrates, or that produce toxic substances (FDA, 1992). Families/superfamilies of enzymes obtained from microorganisms found in diverse habitats retain their general structure and enzymatic properties, but they may differ in certain functional characteristics such as stability and substrate specificity. As noted previously, there are no known instances where natural variation within an enzyme family has resulted in the generation of a toxin active via the oral route (Pariza and Johnson, 2001).

References

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21. **Comment:** One comment disagreed with Pioneer's statement that acetylated amino acids have a history of safe use in food. The commenter further claims that there is no history of safe use of these "unusual amino acid variants" for human consumption.

Response: Pioneer's assertion that "acetylation of proteins is commonly employed in the food industry" (Petition, Addendum 2, p. 3) is based on information contained in El-Adawy, 2000. Chemical modification is one method proposed to improve the functional properties of the proteins for food processing (Li-Chan *et al*., 1979; Matheis & Whitaker, 1984). Chemical modification, particularly acylation with acetic and succinic anhydrides, has been used to improve functional properties of many plant proteins including wheat (Grant, 1973), soybean (Franzen and Kinsella, 1976) and chickpea (Liu & Hung, 1998). Acylated proteins have been applied in preparation of some products such as coffee whiteners (Melychyn & Stapley, 1973), flavoring agents for roasted meat (Mosher, 1974), carbonated beverages (Creamer *et al*., 1971), mayonnaise and salad dressings (Evans & Irons, 1971) margarine and ice cream (Evans, 1970) and cheese-like gels (Chen *et al*., 1975).

N-acetyl-L-methionine has, in fact, been approved by FDA as a food additive (21 CFR 172.372; http://www.cfsan.fda.gov/~acrobat/opa-appa.pdf). In addition, an online search reveals that N-acetylcysteine and N-acetyltyrosine are readily available as dietary supplements from a wide variety of retailers. Most importantly, NAA and NAG have a history of safe consumption, based on their presence in commonly consumed foods such as soybean, eggs, chicken, turkey, beef, mushrooms, fruits, vegetables, milk, sardines, walnuts, chocolate, tea, roasted coffee beans, and grains including corn, wheat, rice and barley (as documented by Pioneer in its Petition and its comment submitted to the docket APHIS-2007-0019-0109).

References

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Creamer, L. K., Roeper, J., & Lohrey, E. H. (1971). Preparation and evaluation of some acid soluble casein derivatives. New Zealand Journal Dairy Science and Technology, 6, 107-1 14.

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Evans, M. (1970). Unilever method for preparing emulsion of edible oils. Netherlands Patent Appl. 6,919,619; German Patent 1,964,954.

Evans, M., & Irons, L. (1971). N-Succinylated egg yolk proteins. German Patent 1,95 1,247.

Franzen, K. L., & Kinsella, J. E. (1976). Functional properties of succinylated and acetylated soy protein. Journal of Agricultural and Food Chemistry, 24, 788-795.

Grant, D. R. (1973). The modification of wheat flour proteins with succinic anhydride. Cereal Chemistry, 50,417-428.

Li-Chan, E., Helbig, N., Holbek, E., Chan, S., & Nakai, S. (1979). Covalent attachment of lysine to wheat gluten for nutritional improvement. Journal of Agricultural and Food Chemistry, 27, 822-877.

Liu, L. H., & Hung, T. V. (1998). Functional properties of acetylated chickpea proteins. Journal of Food Science, 63,331-337.

Matheis, G., & Whitaker, J. R. (1984). Chemical phosphorylation of food protein: an overview and a prospectus. Journal of Agricultural and Food Chemistry, 32,699-705.

Melychyn, P. & Stapley, R. B. (1973). Acylated soybean protein for coffee whiteners. US Patent 3,764,711.

Mosher, A. J. (1974). Flavouring agent and process for preparing same. US Patent 3,840,674.

22. **Comment:** One comment disagreed with Pioneer's statement that acetylated amino acids have a history of safe use in feed.

Response: Pioneer's assertion that "one well-characterized industrial application of acetylated amino acids is the use in livestock feed in cases when it is unsuitable to use free amino acids" (Petition, Addendum 2, p. 3) is based on information contained in Boggs et al., 1975 and Amos et al., 1975. These studies demonstrated acetylated methionine may be substituted for methionine in rat diets. Amos speculated that such substitution would be useful in ruminant diets. In conversation with David Baker at the University of Illinois (Professor Emeritus of Animal Sciences and member of the National Academy of Sciences; phone: 217-333-0243), Pioneer has since learned that the cost of substitution of methionine with acetylated methionine has made it cost prohibitive to implement at the commercial level.

However, NAA and NAG have a history of safe consumption in feed based on their presence in soybean, corn and, to a lesser extent, wheat, rice and barley (comment to the docket APHIS-

2007-0019-0109). Therefore, livestock animals have historically consumed acetylated amino acids, including NAA and NAG, from these sources.

References

Amos, H.E. Schelling, G.T., Digenis, G.A., Swintosky, J.V., Little. C.O., and Mitchell, G.E. 1975. Methionine Replacement Value of *N*-Acetylmethionine and Homocysteinethiolactone Hydrochloride for Growing Rats. Am Soc Nutrition. 105: 577-580.

Boggs, R.W., Rotruck, J.T., and Damico, R.A. 1975. Acetylmethionine as a Source of Methionine for the Rat. Am Soc Nutrition. 105: 326-330.

23. **Comment:** One comment discussed the language in the 4th paragraph of the Petition, Addendum 2, p. 3 with respect to glutamate and glutamine. The comment also mentioned the lack of literature specific to the deacetylation of NAA and NAG by mammals or humans. Another comment said that the literature cited for N-acetylated proteins or acetylated amino acids other than NAA and NAG is not relevant for NAA and NAG.

Response: APHIS contacted Pioneer about this issue and determined that this paragraph contained a typographical error. The sentence should have read, "Such results have been reported for glutamine (Magnusson et al., 1989; Neuhauser and Bassler, 1986; Arnaud et al., 2004), cystine (Du Vigneaud et al., 1934) and threonine and methionine (Boggs, 1978)". As stated in this paragraph, specific information about nutritional and metabolic studies with aspartate and glutamate are not available. However, data and information on other acetylated amino acids are applicable to NAA and NAG, and it is reasonable and appropriate to rely on information about other amino acids when assessing the safety of these substances.

There are at least four different classes of acylases (also known as deacetylases) that have been identified in mammals, and they are widely distributed throughout the human body. For example the Type II acylase (also known as aspartoacylase), which is known to deacetylate NAA, is present in multiple tissues, including liver and kidney (Surendran et al, 2003). Acetylated amino acid nutrient supplements such as N-acetyl-L-methionine are known to substitute directly for their dietary constituent amino acid (i.e., L-methionine) by taking advantage of one or more of the acylases to release free amino acids following consumption. Similar observations have been reported for other N-acetylated amino acids including glutamine, phenylalanine, tryptophan and threonine. Although specific data on the bioavailability of NAA and NAG have not been reported in the literature, data on the bioavailability of other N-acetylated amino acids, as well as the wide distribution of acylases in the human body, lead Pioneer to conclude that NAA and NAG will also be deacetylated as a routine part of digestion or metabolism.

Reference

Surendran, S., Michals-Matalon, K., Quast, M.J., Tyring, S.K., Wei, J., Ezell, E.L., and Matalon, R. 2003. Canavan disease: a monogenic trait with complex genomic interaction. Mol Genetics and Metabolism. 80: 74-80.

24. **Comment:** One comment pointed out that Pioneer did not report their methodology for measuring concentrations of NAA and NAG in food.

Response: While Pioneer did not submit specific methodologies for measurement of concentrations of NAA and NAG in soybean or other tested foods, APHIS does not typically require specific methodologies to be submitted as part of a Petition requesting deregulation. Petitioners may submit methodologies (and Pioneer did submit methods for some of its tests) but these are not required for APHIS analysis.

Compositional methodology for acetylated amino acids in 356043 soybean were shared with FDA as part of the BNF No. 108 consultation process, which concluded favorably on September 21, 2007. The method was validated prior to use. Seed was extracted with chloroform/methanol/water and the resulting aqueous phase contained acetylated amino acids. N-acetyl-L-aspartate and N-acetyl-L-glutamate were measured by HPLC/mass spec using MS/MS with selective reaction monitoring.

25. **Comment:** One comment suggests that a complete assessment of the potential neurological and reproductive effects of NAA must be included.

Response: NAA synthesized by the brain is an essential substance that provides acetate for proper brain development in healthy individuals. NAA is present at relatively high levels in the brain. Although there could be modest increases in dietary exposure to NAA resulting from commercialization of 356043 soybeans, this increase is negligible (< 2000X) compared to the amount of NAA being produced in the brain (see Petition, Addendum 1, pp. 2-3). In addition, acylases that can metabolize acetylated amino acids are widely distributed in the human body. Because NAA is already being produced in our bodies at much higher amounts than that which could be consumed from 356043 soybean, there is no need to conduct an assessment on the neurological and reproductive effects of NAA.

26. **Comment:** One commenter asserts that the acetylation process is meant to increase the bioavailability of amino acids. Another comment speculates on the potential of the GAT enzyme to acetylate other proteins that might have potentially hazardous unintended effects.

Response: Acetylation of amino acids is designed to increase the stability of amino acids (see, for example, Showden et al., 2002). An extensive review of the literature did not find any evidence that acetylated amino acids are more bioavailable than their constituent counterparts. Numerous studies have been conducted to determine whether the N-acetylated amino acid would substitute 1:1 with the non-acetylated form. The references noted (Petition, Addendum 1, p. 3) were cited because they demonstrate comparable substitution of the acetylated form of some amino acids either in feeding trials or with pharmacokinetics.

As noted, Pioneer conducted numerous analyses on soybean composition and field performance and collected data on a large number of different characteristics of 356043 soybean. Other than the relatively minor differences noted in a small number of these characteristics, no other effects were noted that affected plant performance in the field. Pioneer also addresses the potential for acetylated amino acid incorporation into protein in its comments to the docket APHIS-2007-0019-0109.

Reference

Snowden, M. K., Baxter, J. H., Mamula Bergana, M., Beyzer, I. and Pound, V. 2002. Stability of N-acetylglutamine and glutamine in aqueous solution and in a liquid nutritional product by an improved HPLC method. Journal of Food Science 67: 384-389.

27. **Comment:** One comment notes use of a particular statistical method (adjusted p-values) used by Pioneer and believes that some of the resulting data should be disregarded. No rationale or citation is provided to support this assertion. The commenter also questions the value of other information related to the range of variability found experimentally and in the literature.

Response: APHIS disagrees with the commenter and finds the use of adjusted p-values, tolerance intervals and literature range information both useful and reasonable for analyses provided in the petition. The use of adjusted p-values has been recommended in the literature and widely employed across a number of disciplines to deal with data such as that generated by Pioneer to improve statistical analyses and control the "false discovery rate" in experiments when numerous comparisons are conducted in a single study (Benjamini and Hochberg, 1995 and 2000). The false discovery rate adjustment of p-values conservatively addresses the issues associated with multiple comparative testing while retaining the power for detecting real differences. The value of using known literature ranges in compositional analyses is also useful when assessing experimentally significant differences which may be of no biological significance (e.g., differences in composition associated with growing in Georgia soils versus Iowa soils, which can be expected).

References

Benjamini, Y. and Hochberg, Y. 1995. Controlling the False Discovery Rate: a Practical and Powerful Approach to Multiple Testing. J. Roy. Statist. Soc. B. pp. 289-300.

Benjamini, Y. and Hochberg, Y. 2000. On the Adaptive Control of the False Discovery Rate in Multiple Testing With Independent Statistics. J Educational and Behavioral Statistics. 25: 60-83.

28. **Comment:** One commenter claims that the EA fails to analyze potential harm to wildlife, endangered species and overall biodiversity. The commenter cited news articles claiming harm to rats and mice fed glyphosate tolerant soybean.

Response: APHIS is aware of the news article cited by the commenter and APHIS did consider potential effects of 356043 soybean on wildlife, threatened and endangered species and biodiversity (Section V., D., E., F). Data associated with the cited news article has never been published in a peer-reviewed journal and has been discounted (http://www.food.gov.uk/multimedia/pdfs/acnfpgmsoya.pdf, Marshall, 2007). FDA has completed reviews of glyphosate tolerant soybean and has found no safety issues (http://www.cfsan.fda.gov/~lrd/biocon.html, BNF No. 104 and No. 01). Several studies have been published in peer-reviewed journals that contradict the claimed adverse effects on rats (Brake and Evenson, 2004, Teshima, et al., 2000, Zhu, et al., 2004) and have noted no food safety issues associated with glyphosate tolerant soybean fed to mice. Numerous other studies have assessed the food safety aspects of genetically engineered foods and the overwhelming majority has not identified significant issues (http://www.agbioworld.org/biotech-info/articles/biotech-art/peer-reviewed-pubs.html).
APHIS stands by its safety assessment in this EA.

References

Brake, DG and Evenson DP. 2004. A generational study of glyphosate-tolerant soybeans on mouse fetal, postnatal, pubertal and adult testicular development. Food and Chemical Toxicology 42: 29-36.

Marshall, A. 2007. GM soybeans and health safety--- a controversy reexamined. Nature Biotechnology 25: 981-987.

Teshima R, Akiyama, H., Okunuki, H., Sakushima, J., Goda, Y., Onodera, H., Sawada, J., and Toyoda, M., 2000. Effect of GM and Non-GM Soybeans on the Immune System of BN Rats and B10A Mice. J Food Hyg Soc of Japan. 41: 188-193.

Zhu, Y., Li, D., Wang, F., Yin, J., and Jin, H. 2004. Nutritional assessment and fate of dna of soybean meal from roundup ready or conventional soybeans using rats. Arch. Anim. Nutr. 58: 295-310.

29. **Comment:** One commenter claims issues associated with “gene stacking” and believes that APHIS should do further analysis. The commenter speculates about various “unintended” effects and creation of “super weeds” and potential creation of new allergens or toxins.

Response: The comment provided no particular compelling references or data that would support further assessment. Genes are “stacked” as a matter of course when plant breeders make hybrid plants, a process that has been used to develop new and better plant varieties for centuries. Pioneer provided, and APHIS reviewed, extensive genotypic, phenotypic and compositional data in its petition and subsequent submissions and outside of those differences noted, identified no other issues associated with “unintended effects” or toxins or allergens.

30. **Comment:** One commenter claims that "gene shuffling" (the process used to develop the *gat4601* gene) is inherently dangerous and is likely to "produce variants with unexpected and unknown toxicity or immunogenicity."

Response: The commenter provided no compelling references or data that would support this claim. APHIS considered data submitted by Pioneer from several acute toxicity studies (GM-HRA, GAT4601, NAA, whole food 13-week rat study) and a chicken broiler feeding study. Pioneer also conducted bioinformatic analyses to assess potential allergenicity on the GAT4601 and GM-HRA proteins which showed a low likelihood of allergenicity of these proteins. APHIS also considered extensive field data submitted by Pioneer. Nothing in any of the studies indicated the generation of "unintended" effects or production of toxic components in 356043 soybean. Therefore, APHIS concludes that no further assessment is warranted.

31. **Comment:** One comment claims that the analysis of alternatives is inadequate in that it only considers the "no action" alternative and approval in whole and does not consider an "in part" determination alternative.

Response: USDA/APHIS has been considering petitions requesting nonregulated status since 1992 and has considered a wide variety of species including tomato, corn, cotton, soybean, canola, squash, papaya, and plum. While APHIS regulations at 7 CFR § 340.6 (d)(3)(i) allow for an "in part" determination, such a determination has never been made and an "in part" consideration as an alternative is not required. Many previous environmental assessments have also not considered an "in part" determination. In a preliminary assessment of 356043 soybean, an "in part" consideration was deemed unnecessary given that other herbicide tolerant soybean lines have been granted approval in whole (93-258-01p, 96-068-01p, 98-014-01p, and 06-178-01p) and APHIS could not identify any new issues that would warrant such a consideration.

32. **Comment:** One commenter claims that APHIS improperly uses an Environmental Impact Quotient pesticide assessment program in its analysis of potential effects. The commenter claims that the program has weaknesses and that APHIS should conduct a detailed ecological assessment for all ALS-inhibitor herbicides that will be allowed on 356043 soybean.

Response: APHIS disagrees that a full ecological assessment for all potential ALS-inhibitors that might be used on 356043 soybean is warranted. The noted pesticide environmental impact program (developed and managed at Cornell University) is useful in that it does consider and summarize potential adverse pesticide (including herbicide) effects on applicator, farm worker, consumer, fish, birds, bees, beneficial and terrestrial organisms. Pioneer did not submit, and APHIS does not require submission of, information on all ALS-inhibitors that may ultimately be registered for use on 356043 soybean. The potential list of such herbicides is extensive (http://www.weedscience.org/summary/ChemFamilySum.asp?lstActive=&lstHRAC=3&btnSub2=Go) and it cannot readily be ascertained which ALS-inhibitors will be used on 356043. ALS-inhibitors have been used on soybeans in the past (EA, table p. 10). It can be expected that all such ALS-inhibitors will be appropriately assessed and registered for use by EPA.

33. One commenter claims that since the exact physiological role of the GAT enzyme is not known, APHIS should not have allowed 356043 soybean to be field tested and references 7 CFR 340.3(b)(3).

APHIS does not agree that the function of the introduced genetic material was not known. The function of the *gat4601* gene in soybean results in production of GAT protein, in acetylation of glyphosate, and in tolerance of soybean to applications of glyphosate. The reference to APHIS regulations at 7 CFR 340 refers to the section applicable to notifications. Regardless of whether 356043 soybean had qualified under this section of the regulations or not, 356043 soybean could still have been field tested under 7 CFR 340.4 which describes permitting procedures for field releases.

34. One commenter notes that Pioneer provided no data regarding levels of acetylated aminomethylphosphonic acid (AMPA) or an assessment of its potential toxicity.

Pioneer provided information to APHIS (12/20/2007) of its assessment of N-acetyl AMPA, as well as N-acetylglyphosate, safety testing. Their assessment included an assessment of structure-activity relationships and comparisons with toxicology data from structurally similar compounds. They also referenced a subchronic rat toxicity study using N-acetylglyphosate (which gets metabolized to N-acetyl AMPA in the body). They noted no adverse effects in the study. Several factors are considered that lead APHIS to conclude that N-acetyl AMPA has low likelihood for significant environmental impacts: expected low gastrointestinal absorption (based on the chemical structure and physiological pHs) therefore low potential exposure, structural activity relationship comparisons with known toxins and the absence of significant adverse effects on rats when assessed as a metabolite of N-acetylglyphosate.

35. One commenter claims that approximately 99% of commercially grown herbicide tolerant plants are Monsanto's Roundup Ready® crops and believes that APHIS needs to analyze the current and future states of herbicide tolerant crop systems. The commenter also believes that APHIS has failed to act regarding issues associated with the development of herbicide tolerant weeds in herbicide tolerant GE plants. The commenter also spends significant time commenting on APHIS' draft programmatic EIS (APHIS docket 2006-0112) which published in July 2007 and was available for comment at that time.

APHIS is aware of the wide adoption rates of a number of genetically engineered herbicide tolerant (HT) crops and has been in contact for a number of years with the EPA on issues surrounding increased glyphosate use, weed shifts, and increased development of glyphosate tolerant weeds. While the adoption rate of GE Roundup Ready® crops is high, APHIS noted that, in addition to GE plants with herbicide tolerance, there are also non-GE crops that have been developed to be tolerant to herbicides (APHIS docket 2006-0112, draft EIS, pp. 119-120) for which APHIS has no regulatory oversight. One of the major categories of these plants are those developed to be tolerant of some of the ALS inhibitors (e.g., Clearfield® products including corn, wheat, rice, canola, sunflower and STS® soybean products) and these have been grown on millions of acres, starting as early as 1993. These non-GE HT crops were grown on over 6 million acres in 2007 (Doane Market Research and Biotech Traits Commercialized: Outlook 2010 data). Other HT crops were developed as early as 1983, long before the development of GE plants (APHIS docket 2006-0112, p. 120).

See the detailed discussion regarding herbicide tolerant weeds in our response to comment 12. Moreover, given that 356043 soybean is most likely going to replace acreages of glyphosate tolerant soybeans currently on the market (and not increase acreages of HT soybeans), APHIS identified no new issues about glyphosate use or glyphosate tolerant weeds that would require specific consultation with EPA on this particular product. As noted previously, a number of weed scientists, including Dr. York (who is cited by the commenter), provided comments supporting approval of 346043 soybean (APHIS-2007-0019-0088). The commenter's issues related to APHIS' draft programmatic EIS (APHIS docket 2006-0112) are not relevant to this determination of nonregulated status for the 356043 soybean. Those issues are expected to be addressed in the final programmatic EIS when it is published.

36. One commenter believes that APHIS must consider economic costs associated with increased production costs from HT weed populations, increased herbicide expenditures, other increased production costs, and the potential for losses from decreased yields. The commenter cites a number of press articles and editorials that speculate on a variety of potential costs, most referring to costs in Roundup Ready® cotton.

As Pioneer has indicated in the Petition and APHIS has noted in the EA (p. 4), 356043 soybean was developed specifically to provide herbicide tolerance to both glyphosate and ALS inhibitors. Weed scientists who provided comments on this Petition and EA, showed support for this approach as part of an integrated weed management program (which Pioneer has recommended to growers). It is not clear, therefore, that use of 356043 soybean will necessarily result in increased costs to growers from increasing populations of herbicide tolerant weeds. It is also not clear that adopters of 356043 soybeans will incur other increased production costs from using 356043 as production costs vary widely based on crop rotation, weather, seed and technology fees assessment, chemical costs, fuel costs, pest infestations and other factors.

USDA/APHIS Environmental Assessment

In response to Pioneer Hi-Bred International Petition 06-271-01p seeking a Determination of Nonregulated Status for Herbicide Tolerant 356043 Soybean

OECD Unique Identifier DP-356Ø43-5

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

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TABLE OF CONTENTS Page

I. Introduction

A. Background

Soybean *(Glycine max* L.) is a member of the Phaseoleae tribe of the Leguminosae family with its center of origin in eastern Asia. Soybean is grown as a commercial crop in over 35 countries. In the United States it is grown on greater than 70 million acres in at least 31 states with over a million acres grown in each of the following states: IA, IL, MN, IN, MO, NE, OH, SD, AR, ND, KS, MI, MS, WI, NC, KY, TN (USDA-NASS, 2006).

Soybeans do not persist as they are not frost tolerant and do not survive freezing winter conditions. Soybean plants are not weedy and are not found outside of cultivated areas, nor do they compete well with other cultivated plants. They have never been found in the wild in the U.S. (Hymowitz & Singh, 1987). Volunteer plants that might grow under certain environmental conditions can be easily controlled mechanically or with herbicides. Additional information on the biology of soybean can be found within an OECD (Organization for Economic Co-Operation and Development) consensus document (OECD, 2000).

Soybean is a highly self pollinated species. The soybean stigma[1] is receptive to pollen approximately 24 hours before anthesis[2] and remains receptive for approximately 48 hours. The anthers[3] mature in the flower bud and directly pollinate the stigma of the same flower, resulting in a cross pollination rate typically less than one percent between adjacent plants under normal field planting conditions (Caviness, 1966, OECD, 2000). Cross-pollination greater than 4.6 m from a pollen source has been rarely observed although it has been observed that insects can sometimes transfer the pollen (Caviness, 1966). A recent study in Arkansas has shown that different soybean cultivars vary in the amount of cross pollination that can occur under optimal growing conditions and in the presence of honeybee populations. Some cultivars averaged anywhere from 0.09-2.5% cross-pollination in rows 6 m long and 102 cm apart (Ahrent and Caviness, 1994).

In 2006, glyphosate tolerant varieties of soybean were planted on approximately 89 percent of soybean acreage in the United States. Only 11 percent of the total soybean acreage was planted with conventional soybean varieties and of that roughly 122,000 acres (0.17% of soybean acres) was devoted to organic soybean production (USDA-ERS, 2006). Ultimately, soybean growers make their choices of soybean varieties based on several factors: yield, weed and disease pressures, cost of seed, pesticides and other inputs, technology fees, human safety, potential for crop injury, and ease and flexibility of the production system (Gianessi, 2005).

USDA data from the 2007 growing year showed that soybean plantings were at their lowest levels since 1996, reflecting an 11% decrease in acreage compared to 2006 (http://www.nass.usda.gov/Newsroom/2007/03_30_2007.asp). USDA's March 2008

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1 Receptive part of a flower, on which pollen is deposited, leading to fertilization and seed production.
2 The period during which a flower is open and functional
3 The part of the flower on which pollen is produced.

Prospective Planting Report (http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1136) suggests that planting will increase about 11% over 2007 plantings, but remain below 2006 acreages.

Pioneer's herbicide tolerant 356043 soybean has been genetically engineered to express modified glyphosate acetyltransferase (GAT4601[4]) and acetolactate synthase (ALS) proteins. These genes impart tolerance to both glyphosate and ALS-inhibiting herbicides (e.g., sulfonylureas and imidazolinones). The *gat4601*[5] gene is derived from *gat* genes from *Bacillus licheniformis*, a common soil bacterium. Expression of the *gat4601* gene is driven by a synthetic promoter[6] (SCP1). The gene that confers tolerance to ALS-inhibiting herbicides is *gm-hra* and is a modified soybean *als* gene. Expression of the *gm-hra* gene is controlled by a soybean promoter. A single copy of these genes and other DNA regulatory sequences were introduced into soybean somatic[7] embryos using microprojectile bombardment[8]. Genetic segregation data collected over five generations demonstrated that *gm-hra* and *als* genes were stably inherited.

Pioneer's dual herbicide tolerant soybeans have been developed to allow growers to choose herbicides with different modes of action to best address their individual weed populations. Glyphosate is a broad spectrum herbicide that is effective on many weeds when applied post-emergence. ALS-inhibiting herbicides can effectively control many weeds when applied either post-emergence or pre-emergence. Growers may choose to grow 356043 soybeans if they have identified or are concerned about development of glyphosate tolerant weed populations in their fields. Growers will have an option to apply ALS-inhibitors in fields to address such weeds but are also likely to consider the increased herbicide and application costs as well as potential persistence of some ALS-inhibitors in their fields that may affect crops used in rotation with 356043 (i.e., some ALS-inhibitors persist in soils and may adversely affect a different crop grown the following season, unlike glyphosate which does not persist long in soils). Growers may also choose to grow these soybeans if they perceive some other benefit they can derive from their use (e.g., cost savings or other economic gain).

Herbicide tolerant (HT) soybeans have been the subject of several determinations of nonregulated status by USDA APHIS since 1993. APHIS has made determinations on five separate petitions requesting nonregulated status for HT soybeans: 93-258-01p, 96-068-01p, 98-014-01p, 98-238-01p, and 06-178-01p.

In 1994, APHIS deregulated the first HT soybean; Monsanto's glyphosate tolerant soybean, 40-3-2, (OECD Unique Identifier MON-Ø4Ø32-6) (Petition 93-258-01p, 1993 and EA, 1994). This event was the result of incorporating the *cp4 epsps* gene derived from

4 By convention, GAT4601 refers to the specific protein
5 By convention, *gat4601* refers to the specific gene
6 A promoter is a region of DNA that controls expression of a gene.
7 Refers to a non-sex cell
8 A method of introducing DNA into cells by physically shooting small particles of gold or tungsten to which the gene(s) of interest has been attached.

Agrobacterium sp. strain CP4, a common soil bacterium. Since the 1994 deregulation, glyphosate tolerant/Roundup Ready® soybeans have gained in market share such that in 2005 Roundup Ready® soybeans were planted on approximately 89% of the U.S. soybean acreage (USDA-NASS, 2006), and 60% of the area planted to genetically engineered[9] (GE) crops worldwide (James, 2005). Since the adoption of Roundup Ready® soybean, cost to control weeds has decreased and farmers have saved in excess of $200 million per year in weed control costs (Gianessi, 2005). In addition to significant convenience in weed control, use of glyphosate tolerant soybean has encouraged the use of conservation-tillage. Because chemical weed control is so effective, soil tillage can be avoided thereby reducing soil erosion and fuel consumption used for the tillage (Service, 2007).

Additional herbicide tolerant lines of soybean that APHIS has deregulated are from petition submissions by AgrEvo (merged into Aventis in 2000 and Bayer CropScience in 2002) in 1996 (96-068-01p) and 1998 (98-014-01p and 98-238-01p) requesting deregulation of glufosinate tolerant soybean lines (seven in total). None of the glufosinate tolerant lines have been commercialized. Most recently, in July 2007, APHIS granted nonregulated status to glyphosate tolerant MON 89788 (petition 06-178-01p), a soybean very similar to the first deregulated soybean.

Pioneer has field tested 356043 soybean since 2003. Agronomic performance, disease, and phenotypic assessments were conducted in trials at over 35 different locations in 15 states across the United States. Pioneer assessed 356043 soybean for possible allergens and toxins and submitted this data, as well as extensive compositional analysis data, to USDA as part of the petition process. Pioneer analyzed GAT4601 and GM-HRA (the modified ALS protein) proteins to look for similarities to known allergens and toxins and none were identified. Pioneer noted no other biologically meaningful differences (with respect to allergens and toxins) between 356043 soybean and the comparator control soybean[10] lines (Petition, pp. 63-64). Pioneer also conducted toxicity studies using purified GAT4601, GM-HRA proteins and N-acetylaspartic acid (NAA). None showed evidence of toxicity at the levels tested.

Field tests of 356043 soybean plants in the United States have been authorized by APHIS under several permits (Appendix 5, page 140 of the petition). Pioneer evaluated 356043 soybean plants extensively to confirm that they exhibit the desired agronomic characteristics, are tolerant to glyphosate and ALS-inhibitors, are genetically stable under field conditions, and that they do not present a plant pest risk. They assessed seed germination, seed dormancy, response to abiotic stresses, plant growth, yield, days to maturity, disease incidence, insect damage and a number of other agronomic parameters (Petition pp. 65-76). The field tests have been conducted in agricultural settings under physical and reproductive confinement conditions.

B. USDA Regulatory Authority

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9 Genetic engineering refers to methods used to modify organisms by recombinant DNA techniques
10 Non-GE soybean

APHIS regulations at 7 CFR part 340, which were promulgated pursuant to authority granted by the Plant Protection Act (7 U.S.C. 7701-7772), regulate the introduction (importation, interstate movement, or release into the environment) of 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 and is also a plant pest, or if there is reason to believe that it is a plant pest. These soybean plants have been considered regulated articles because they contain non-coding DNA regulatory sequences derived from plant pathogens (cauliflower mosaic and tobacco mosaic viruses). An organism is no longer subject to the regulatory requirements of 7 CFR part 340 when it is demonstrated not to present a plant pest risk. Section 340.6 of the regulations, titled "Petition for Determination of Nonregulated Status," provides that a person may petition APHIS to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk, and therefore should no longer be regulated. If APHIS determines that the regulated article is unlikely to present a greater plant pest risk than the unmodified organism, then APHIS can grant the petition in whole or in part. In such a case, APHIS authorizations (i.e., permits or notifications) would no longer be required for environmental release, importation, or interstate movement of the non-regulated article or its progeny.

As a Federal agency subject to compliance with the National Environmental Policy Act (NEPA),[11] APHIS has prepared this environmental assessment (EA) to consider the environmental effects of this proposed action (deregulation) and the alternative to that action (no action) consistent with NEPA regulations.[12] This EA has been prepared in order to specifically address whether the unconfined cultivation and use in agriculture of Pioneer's 356043 soybeans would significantly affect the quality of the human environment[13].

C. U.S. Environmental Protection Agency and Food and Drug Administration Regulatory Authorities

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: the U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS), the Environmental Protection Agency (EPA), and the U.S. Department of Health and Human Services' Food and Drug Administration (FDA). Products are regulated according to their intended use, and some products are regulated by more than one agency. Together, these agencies ensure that the products of modern biotechnology are safe to grow, safe to eat, and safe for the

11 42 United States Code (U.S.C.) 4321 *et seq.*

12 40 CFR parts 1500–1508, 7 CFR 1b, and 7 CFR part 372.

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

environment. USDA, EPA, and FDA apply regulations to biotechnology that are based on the specific nature of each GE organism.

Under the Coordinated Framework, the U.S. Environmental Protection Agency (EPA) is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended (7 U.S.C. 136 *et seq.*). FIFRA requires that all pesticides, including herbicides, be registered prior to distribution or sale, unless exempt by EPA regulation. In order to be registered as a pesticide under FIFRA, it must be demonstrated that when used with common practices, a pesticide will not cause unreasonable adverse effects in the environment. Under the Federal Food, Drug, and Cosmetic Act (FFDCA), as amended (21 U.S.C. 301 *et seq.*), pesticides added to (or contained in) raw agricultural commodities generally are considered to be unsafe unless a tolerance or exemption from tolerance has been established. Residue tolerances for pesticides are established by EPA under the FFDCA, and the U.S. Food and Drug Administration (FDA) enforce the tolerances set by EPA (http://www.epa.gov/opp00001/factsheets/stprf.htm). Pioneer submitted the appropriate regulatory package to EPA for registering the use of glyphosate herbicide on the HT 356043 soybean. Safe use of glyphosate and ALS inhibiting herbicides have been established by the EPA through the registration of glyphosate and ALS-inhibitors for use on soybeans and the setting of tolerances for these herbicides (EPA 1993, 1997a, 1997b, 2002, 2004a, 2004b, 2005, 2006, 2007).

The FDA policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the Federal Register on May 29, 1992, and appears at 57 FR 22984-23005. Under this policy, FDA uses what is termed a consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g., labeling) are resolved prior to commercialization and distribution of bioengineered food and feed. Pioneer submitted a food and feed safety and nutritional assessment summary to FDA for 356043 soybean in 2006. That review (BNF No. 108) was completed on September 21, 2007. FDA indicated that it had no further questions regarding the safety and nutritional assessment of 356043 for used in food and feed (http://www.cfsan.fda.gov/~lrd/biocon.html).

II. PURPOSE AND NEED

USDA-APHIS has prepared this Environmental Assessment (EA) in response to a petition (APHIS Number 06-271-01p) from Pioneer Hi-Bred International (referred to hereafter as Pioneer). Pioneer is requesting a determination of nonregulated status for genetically engineered (GE) herbicide tolerant (HT) 356043 soybean (*Glycine max*) derived from their transformation event, 356043 (referred to hereafter as 356043 soybean). Pioneer developed the GE 356043 soybean to tolerate glyphosate and acetolactate synthase (ALS) -inhibiting herbicides. 356043 soybean is currently a regulated article under USDA regulations at 7 CFR part 340, and as such, Pioneer has conducted interstate movements, importations, and environmental releases (including field testing) of 356043 soybean under notifications or permits issued by APHIS. Pioneer petitioned APHIS requesting a determination that

356043 soybean does not present a plant pest risk and therefore 356043 soybean and progeny derived from crosses with other nonregulated soybeans should no longer be regulated articles under these APHIS regulations. Under regulations in 7 CFR part 340, APHIS is required to make a determination on a petition for nonregulated status. This EA was prepared in compliance with the National Environmental Policy Act (NEPA) of 1969 as amended, (42 U.S.C. 4321 *et seq*.) and the pursuant implementing regulations (40 CFR 1500-1508; 7 CFR § 1b; 7 CFR part 372).

III. ALTERNATIVES

A. No Action: Continuation as a Regulated Article

Under the Federal "no action" alternative, APHIS would deny the petition and 356043 soybean would continue to be a regulated article under 7 CFR part 340. Permits issued or notifications acknowledged by APHIS would still be required for introductions of 356043 soybean. APHIS might choose this alternative if there were evidence that 356043 soybean was a plant pest risk or if there were insufficient evidence to demonstrate the lack of plant pest risk from the unconfined cultivation of glyphosate/ ALS-inhibitor tolerant soybeans.

Under this alternative, parties involved in commercial scale production, handling, processing or consumption of soybean will not have access to 356043 soybean. Other genetically engineered and conventional soybeans will still be available for purchase.

B. Proposed Action: Determination that Pioneer 356043 Soybeans are No Longer Regulated Articles, in Whole (Preferred Alternative)

Under this alternative, 356043 soybeans would no longer be a regulated article under 7 CFR part 340. Permits issued or notifications acknowledged by APHIS would no longer be required for introductions of 356043 soybean. APHIS might choose this alternative if there were sufficient evidence to demonstrate that 356043 soybeans are not a plant pest risk or to demonstrate the lack of plant pest and environmental risks from the unconfined cultivation of glyphosate/ ALS-inhibitor tolerant soybeans derived from this event.

Under this alternative, growers and other parties who choose not to plant transgenic soybean varieties or sell transgenic soybeans, should not be significantly impacted by the expected commercial use of this product. Non-transgenic soybeans will likely still be sold and will be available to those who wish to plant them for the reasons explained below. If Pioneer receives regulatory approval from all appropriate agencies, it will make 356043 soybean available to growers or breeders. Introduction of 356043 soybean is not expected to significantly alter the range of soybean cultivation. 356043 soybean will likely be introduced in areas where soybeans are currently grown.

APHIS has chosen the proposed action as the preferred alternative. This is based upon the determination of the lack of plant pest characteristics of Pioneer 356043 soybeans. The environmental assessment prepared by APHIS shows that the preferred alternative should not have a significant impact the human environment, as described in section V.

IV. Affected Environment

A. Soybean Production

As noted previously, soybean is grown as a commercial crop on over 70 million acres in at least 31 states in the U.S. (USDA-NASS 2006). Eighty-nine percent (89%) of the 2006 soybean acreage in the United States was planted with glyphosate tolerant varieties and, based on historical data, the introduction of 356043 soybeans is not likely to increase the number of glyphosate tolerant soybean acres planted or alter the range where soybeans are planted. Since this soybean is a new product, there is some uncertainty as to where 356043 will be readily adopted because is does not impart a selective cultivation advantage over soybean's production area, however, this soybean will likely be a replacement product for current commercially available herbicide tolerant soybeans based on the previous information. The highest potential for adoption may be in locations where glyphosate tolerant weed populations have been identified because of the dual action nature of 356043. As previously noted, however, soybean farmers make choices to grow soybean varieties based on several factors: yield, weed and disease pressures, cost of seed and other inputs, technology fees, human safety, potential for crop injury, and ease and flexibility of the production system (Gianessi 2005). Depending upon all these factors, growers will ultimately base their decisions regarding seed choice on their individual needs and desires.

APHIS notes that products similar to 356043 soybean have been available to growers since 2006 (http://www.deltaandpine.com/soybean_products_rrs.asp, accessed 5/28/2008). These products are tolerant to both glyphosate and ALS-inhibiting herbicides (i.e., sulfonylurea). They contain genes from Monsanto's Roundup Ready® technology and Delta and Pine Land's (D&PL) STS® (Sulfonylurea Tolerance System) technology. These varieties were developed through traditional breeding of Roundup Ready® soybean (genetically engineered) with D&PL STS® varieties (developed through mutation breeding). D&PL notes potential uses of their RR®/STS® soybean varieties in rotation with cotton, wheat and rice on its website (http://www.deltaandpine.com/soybean_agronomy_updates.asp, accessed 5/28/2008). None of the current D&PL RR®/STS® varieties are subject to oversight by USDA/APHIS since they were developed using traditional breeding of previously deregulated Roundup Ready® soybeans with STS® varieties that are not subject to USDA/APHIS oversight under 7 CFR part 340. It is foreseeable that 356043 soybean could compete directly with these D&PL varieties due to their trait similarities and may also compete with other glyphosate tolerant soybean varieties.

Finally, if 356043 soybean is deregulated, it is unlikely that the percentage or total soybean acreage in the United States planted with genetically engineered soybean varieties will dramatically increase beyond current levels for the following reasons: 1) a certain small percentage (<1%) of soybean growers choose to grow organic soybeans, and genetically engineered soybean varieties cannot be grown and certified as organic, 2) some growers will choose to grow non genetically-engineered soybeans for other marketing reasons, 3) a certain percentage of soybean growers each year may choose to rotate out of single glyphosate resistant soybeans and use seed tolerant to multiple herbicides (such as D&PL RR®-STS® soybean) with alternate modes of action and/or tillage to avoid weed shifts or the selection of glyphosate tolerant weeds, and 4) they may simply choose other varieties without herbicide tolerance traits that are better suited to their specific growing conditions. Soybean acreage is not expected to increase compared to 2006 (http://www.nass.usda.gov/Newsroom/2007/03_30_2007.asp), and in the prior five years, soybean production was relatively steady varying from 72 million acres to 75.5 million acres (http://www.nass.usda.gov/QuickStats/index2.jsp).

B. Weed Competition and Control

Uncontrolled weed populations can cause yield losses of up to 50-90 percent in soybean fields. Before the development of effective herbicides for the selective control of weeds in soybeans in the early 1960's, cultural practices including tillage, using weed free seed, row spacing, and crop rotation were the only ways to control weeds (Wax, 1973). By 1987, over 30 herbicides were being used on soybeans (Jordan et al, 1987). By the early 1990's, over 70 individual herbicides or combination products were registered for weed control in soybeans (Gianessi et al, 2002). Along with the increased number and use of herbicides on soybeans came plant species that developed resistance to numerous herbicide modes of action (Weed Science, 2006). With the 1996 commercial introduction of glyphosate tolerant soybeans, a major shift in herbicide usage occurred with an increasing use of glyphosate concurrent with the increased planting of glyphosate tolerant soybeans and a decrease in use of other soybean herbicides as noted in the following table (Gianessi, et al 2002).

Percent of U.S. Soybean Acres Treated with the Following Herbicides in 1995 vs. 2001 and 2006

	1995	2001	2006		1995	2001	2006
2,4-D	10	4	3	**Glyphosate**	20	76*	96
2,4-DB	1		<1	**Imazamox**		5	<1
Acifluorfen	12	3	<1	**Imazaquin**	15	2	1
Alachlor	4	<1	<1	**Imazethapyr**	44	9	3
Bentazon	12	1	<1	**Lactofen**	5	1	<1
Chlorimuron	16	5	4	**Linuron**	2		
Clethodim	5	4	3	**Metolachlor**	7		
Clomazone	4	<1		**Metribuzin**	11	2	2
Cloransulam		5	1	**Paraquat**	2		1
Dimethenamid	1			**Pendimethalin**	26	10	3
Ethalfluralin	1			**Quizalofop**	6	<1	<1
Fenoxaprop	6	3	<1	**S-Metolachlor**		<1	1
Fluazifop	10	3	1	**Sethoxydim**	7	1	<1
Flumetsulam	2	<1	<1	**Sulfentrazone**		5	1
Flumiclorac		<1	1	**Thifensulfuron**	12	2	1
Fomesafen	4	7	2	**Trifluralin**	20	7	2

* In 2001, 68 percent of U.S. soybeans were glyphosate tolerant (Pew, 2001) (USDA NASS, 2007)

The reasons for growers rapidly switching to glyphosate tolerant varieties are numerous (Gianessi, et al 2002). These varieties allow:

- Effective post emergence treatment,
- broad spectrum control of weeds with a single herbicide,
- flexibility in time of application,
- total lower costs of the glyphosate treatment vs. alternative programs,
- reduced tillage costs and
- reduced costs of fewer herbicide applications.

While glyphosate is the most used herbicide on soybean, there are currently at least eight different ALS inhibiting herbicides registered for use on soybean (chlorimuron, tribenuron, thifensulfuron, cloransulam, flumetsulam, imazethapyr, imazaquin and imazamox). Growers may use both glyphosate and ALS inhibiting herbicides as directed by the EPA labels, when needed.

As has occurred with weeds in other crops, some weeds in soybean acreage have developed tolerance to particular herbicide modes of action. Weeds commonly found in soybeans that have developed tolerance to glyphosate include horseweed (*Conyza canadensis)*, common waterhemp (*Amaranthus rudis*), common ragweed (*Ambrosia artemisiifolia*), giant ragweed (*Ambrosia trifida*), and Palmer amaranth (*Amaranthus palmeri)* (Weed Science, 2007). Additionally, two of these weeds, waterhemp and horseweed, have been noted to be tolerant to both ALS-inhibitors and glyphosate in localized areas (Weed Science, 2007). Weed scientists are developing management strategies to help ensure control of these weeds (Loux et al., 2004, Loux and Stachler, 2006), and companies and university scientists have developed and are developing alternative herbicide resistant crop strategies (Service, 2007). A primary use of 356043 soybean is that it can enable effective weed control using an ALS-inhibitor herbicide in areas where glyphosate tolerant weeds are present. As soybean varieties tolerant to both glyphosate and ALS-inhibitors are currently available, 356043 is likely to compete with these dual herbicide tolerant varieties in the event 356043 is deregulated as well as currently available glyphosate tolerant varieties.

A study assessing the ecological impact of glyphosate on weed resistance researched the fitness costs and benefits of herbicide tolerance of glyphosate tolerant *Ipomoea purpurea* (tall morning glory) (Baucom and Mauricio, 2004). In an agricultural field in Georgia thirty-two random *I. purpurea* plants, which had been sprayed with Roundup® for approximately 8 years were chosen for this evaluation. All seeds collected from each plant shared the maternal genetic contributions which were then used as the unit for the genetic analysis. Seeds from each of the 32 lines were self-pollinated for one generation; the seeds from the F2 generation were grouped according to each maternal line and planted in five spatial blocks to account for habitat heterogeneity. All plants were sprayed with enough Roundup® that had previously been shown to reduce biomass production by 90%. Results demonstrated that the tolerant line produced 35% fewer seeds in the absence of Roundup® than the most susceptible lines. These results suggest that in the absence of herbicide selection (e.g., spraying with Roundup®), herbicide tolerance would be lost in subsequent generations due to higher metabolic costs to resistant weeds. Therefore it is possible that weeds may lose their resistance trait if herbicide use is discontinued (Baucom and Mauricio, 2004).

To minimize the development of weed resistance, the petitioner recommends grower adoption of Integrated Weed Management (IWM) programs through communication, research, education, and participation in industry coalitions such as the Herbicide Resistance Action Committee (HRAC) (http://www.hracglobal.com/). The HRAC is an industry based group whose mission is to "Facilitate the effective management of herbicide resistance by fostering communication and co-operation between industry, government, and farmers." Specific recommended practices include:

- Scouting fields prior to the application of any herbicide to determine the species and the need for an herbicide application.
- Using alternative weed management practices, such as mechanical cultivation, delayed planting and weed-free crop seeds.
- Rotating crops with an accompanying rotation of herbicides to avoid using herbicides with the same site of action on the same field.
- Limiting the number of applications of a single herbicide or herbicides with the same site of action in a single growing season and in successive years.
- Using mixtures or sequential treatments of the herbicides having a different site of action.
- Scouting fields after application to detect weed escapes or shifts and applying alternative control methods to avoid seed deposition in the field.
- Cleaning equipment before leaving fields suspected to have resistant weeds to minimize the spread of weed seed.
- When using herbicides, using full label application rates and compatible tank mix partners.
- Where practical, using cover crops and other methods to reduce weed seeds in the soil.

Use of 356043 soybeans is amenable to the above integrated weed management program. By having herbicide resistance to two different modes of action, use of 356043 soybeans, in conjunction with a herbicide resistance management strategy, should facilitate practices that allow the application of more than one herbicide mode of action within a season and in successive years.

V. Potential Environmental Impacts.

Potential environmental impacts addressed in this EA are those that pertain to the use of Pioneer 356043 soybeans and its progeny in the absence of confinement. APHIS considered potential impacts related to gene introgression[14], soybean composition, weediness, non-target and threatened or endangered species, biodiversity, commercial use, agricultural

[14] Introgression is the successful, stable incorporation of a gene (or genes) from one organism into another as a result of repeated hybridizations.

practices, conventional or organic farmers, agricultural commodities, and possible cumulative impacts. If APHIS takes no action, commercial scale production of 356043 soybean and its progeny is effectively precluded, and the presently deregulated and commercially available herbicide tolerant soybean varieties would be the only available choice of herbicide tolerant varieties. Pioneer 356043 soybeans could still be grown under APHIS permit as they have been for the past several years. However, widespread, unconfined plantings of 356043 soybean would not be allowed as long as these soybean plants are considered regulated articles. If APHIS decides to choose the "No action" alternative there would be no notable environmental impacts associated with this action. As noted in the response to comments, however, if APHIS were to choose the "No action" alternative, an increase in the development of glyphosate tolerant weeds (along with increased use of other herbicides) may result.

A. Potential Impacts from Gene Introgression from Pioneer 356043 Soybeans into its Sexually Compatible Relatives.

In assessing the risk of gene introgression from 356043 soybeans into its sexually compatible relatives, APHIS considers two primary issues: 1) the potential for gene flow and introgression; and 2) the potential impact of introgression.

The genus *Glycine* has approximately 9 species with *G. max* being placed in the subgenus *Soja* along with one other species, *G. soja* (previously *G. ussuriensis*). *G. max* is sexually compatible with only *G. soja* and no other *Glycine* species. *G. max* is the only *Glycine* species located in the United States other than a few *G. soja* plants in research plots. *G. max* has never been found in the wild in the U.S. (Hymowitz and Singh, 1987). Therefore, it is not likely that gene flow and introgression of 356043 soybeans into other species of soybean will occur; thus, any potential environmental impact resulting from gene flow and introgression to other species is not anticipated under either the no action or proposed action alternative.

B. Potential Impacts on Soybean Composition

Data supplied in the petition and reviewed by APHIS (Chapter V, pp 24-50) support the conclusion that 356043 contains newly introduced genes *gat4601* (glyphosate acetyltransferase from *Bacillus licheniformis*, a common soil bacterium) and *gm-hra* (modified acetolactate synthase from soybean). Additionally, other DNA sequences were introduced that serve to control gene expression. Section IV (pp. 20-23) of the petition describes the genes and gene regulatory sequences introduced into 356043 soybean. Several of the additional gene sequences originate from soybean itself and others are from common plant viruses. The intended changes to 356043 result in the production of the proteins glyphosate acetyltransferase and a slightly modified acetolactate synthase that impart tolerance to glyphosate and ALS-inhibiting herbicides. Both the GAT4601 and GM-HRA (modified ALS) proteins were assessed by Pioneer for possible allergenicity and toxicity using internationally accepted guidance from the Codex Alimentarius Commission. Complete summaries of the food and feed safety assessments for the GAT4601 and GM-HRA proteins are found in sections VI-E and VI-F (pp. 63-64) of the petition. Pioneer's assessment of GM-HRA protein noted high similarity with ALS proteins found in bacteria, fungi, algae and other plants. Pioneer also analyzed protein sequence similarities with known and putative protein allergens and toxins and found no similarity that would indicate either allergenicity or toxicity of GM-HRA protein. ALS proteins have been the subject of previous FDA consultations in GE flax (http://www.cfsan.fda.gov/~lrd/biocon.html BNF 000050) and GE cotton (http://www.cfsan.fda.gov/~lrd/biocon.html BNF 000030), as well as 356043 soybean (http://www.cfsan.fda.gov/~rdb/bnfl108.html BNF 0000108). In all cases, FDA indicated that they had no further questions regarding safety and nutritional assessments submitted to the agency. A nearly identical ALS protein is found in a number of non-GE Clearfield® and STS® plant varieties which are grown widely across the globe (http://www.agro.basf.com/p02/AP-Internet/en_GB/portal/show-content/content/Products, http://www.deltaandpine.com/soybean_products_sts.asp). These products were developed in the 1980s and 1990s and have a history of safe use.

Additionally, Pioneer conducted extensive analyses to assess compositional differences between 356043, the comparator non-GE 'Jack' variety, and other standard non-GE soybean varieties. Compositional analyses were from 356043 and control soybeans that had not been sprayed with herbicides, in order to isolate the potential impact of the transgenes on the nutritional composition of soybeans. Proximate analyses for protein, fat, ash, and fiber were conducted on both soybean forage and grain. Soybeans were analyzed for 24 fatty acids (10 were undetectable), 18 amino acids, 8 isoflavones, 23 free amino acids, 5 anti-nutrients, and 2 acetylated amino acids (Section VIII., pp. 77-97 of the petition). The results of these analyses are discussed below.

Pioneer assessed 356043 for levels of 8 isoflavones (e.g., genistin, glycitin, etc.) and 5 antinutrients (e.g., stachyose, phytic acid, etc.). Eleven of the thirteen analyses showed no significant differences in concentrations of these compounds between 356043 and the non-transgenic Jack variety. Pioneer noted minor differences in mean levels of one isoflavone (malonyldaidzin, Table 26, page 93 of the petition) and one antinutrient (trypsin inhibitor, (Table 27, page 95). Although statistically different from control (Jack) lines, the levels of malonyldaidzin and trypsin inhibitor noted in 356043 can be found within the range of concentrations of these same compounds noted in the control. Because all these constituents are also found within the range of values for conventional soybeans, APHIS concludes that 356043 soybean poses no more of a plant pest risk or environmental risk from its composition than conventional soybean.

Pioneer noted increases in 2 minor fatty acids, heptadecanoic (C17:0) and heptadecenoic (C17:1) acids (Petition, pp. 81-83). These increases likely result from a change in the GM-HRA protein that shifts a metabolic pathway leading to production of the C17 fatty acids. Pioneer describes these changes in the Petition (Appendix 6, pp. 141-142). The major fatty acids in soybean consist of linoleic, linolenic, oleic, palmitic and stearic acids. In the control "Jack" variety, these major fatty acids make up over 98% of total fatty acids (Petition p. 83). Combined values of heptadecanoic and heptadecenoic fatty acids in the comparator "Jack" soybean are less than 0.25% of all the fatty acids. In 356043 soybean, these combined C17 fatty acids still make up less than 0.8% of the total fatty acids. Heptadecanoic and heptadecenoic acids are found in commonly consumed foods including tofu, beef, pork, lamb, bison, soy milk, salmon, frozen pizza and numerous other fast-food products (http://www.nutritiondata.com). Heptadecanoic acid is found in corn, soybean, sunflower, peanut and olive oils. Heptadecenoic acid is found in olive oil (Petition, p. 81). APHIS searched numerous scientific databases and could find no evidence that increased consumption of these fatty acids at these levels is associated with any adverse effects in humans. A dietary exposure assessment of the two fatty acids was conducted and submitted to FDA as part of BNF 108. FDA's summary of the exposure data for fatty acids in 356043 soybean can be found in the Note to File at FDA's website (http://www.cfsan.fda.gov/~rdb/bnfm108.html).

Pioneer also noted increased levels of 2 acetylated amino acids from their compositional analyses. The GAT protein preferentially targets glyphosate as substrate. However this enzyme also acetylates the amino acids aspartate and glutamate. Consequently, levels of N-acetylaspartate (NAA) and N-acetylglutamate (NAG), were elevated in 356043 to levels 230 and 8 fold, respectively, above the levels found in conventional soybean (petition Table 23 p. 87). Together these acetylated amino acids represent less than 0.15% of the total amino acids in 356043 soybean. It is unlikely that other amino acids could be acetylated, as kinetic parameters for GAT4601 (under conditions mimicking the soybean cytosol) could only be established for aspartate and glutamate (Petition, Appendix 7). No other amino acids, free or total, were found to differ significantly from levels found in conventional soybean.

N-acetylated amino acids are widely found throughout the plant and animal kingdom and so are present in many food sources (Table 1 and Table 2 p. 4 in addendum to Pioneer petition, and Pioneer comment to the docket APHIS-2007-0019-0109). As noted in an addendum to the petition (p. 3, Addendum 2), acetylation of proteins (which are made up of amino acids) is employed in the food industry to affect various properties of protein concentrates that may be added to food (El-Adawy, 2000, Ramos and Bora, 2004). Pioneer analyzed eggs, yeast, ground turkey, chicken and beef and found various levels of both NAA and NAG in these products (petition, p. 87 and pp. 3-4 of addendum 1, and Pioneer comment to the docket APHIS-2007-0019-0109). Because these amino acids are found in these common foods, APHIS concludes that they are normal components of the human diet.

NAA is an abundant amino acid in the central nervous system (CNS) (Demougeot, C., et al., 2004) but its biological function is not exactly clear (Chakraborty, G., et al., 2001). It is, however, essential for the formation and/or maintenance of myelin in the CNS (Chakraborty, G., et al., 2001). In mammals, NAG is found at high concentrations in the liver and small intestine but it is also found in the brain (Caldovic and Tuchman, 2003). Levels of NAG in the liver increase with increased protein consumption and are also affected by growth hormone levels (Caldovic and Tuchman, 2003).

Pioneer provided potential human dietary exposure estimates of NAA and NAG that would result from an assumption that 356043 would constitute 45% of consumed soybeans (in products such as tofu, soy milk, edamame, soybean oil, etc.). On average, addition of 356043 soybean to the diet of the U.S. population was estimated to increase consumption of NAA from 2.6 µg/kg body weight/day to 6.6 µg/kg body weight/day[15] (For a 120 lb person, this translates to an increased consumption from 143 µg/day to 363 µg/day. One µg is one-millionth of a gram). NAG consumption was estimated to increase from 1.9 µg/kg/day to 3.2 µg/kg/day. APHIS searched numerous scientific databases and found no indication that exposure to either NAA or NAG from food has led to adverse effects in humans. Humans and other mammals have enzymes (acylases) in various tissues that metabolize acetylated amino acids (Matalon, 2006; UniProtKB/SwisProt, 2008). The small increase in exposure to N-acetylated amino acids that might result in response to 356043 constituting 45% of consumed soybeans is not considered significant as the N-acetylated amino acids are readily metabolized to the free amino acid.

A rare human condition called Canavan's disease (CD) is caused by an inherited mutation in the aspartoacylase gene (aspartoacylase converts NAA into aspartate and acetate). This condition results in the inability to transform NAA to the free amino acid, aspartic acid, and leads to an accumulation of excess NAA in the brain. The resulting deficiency in metabolism of NAA leads to inadequate myelin formation in the brain and severe developmental abnormalities (Kirmani et al., 2002; Madhavarao et al., 2005; Mehta and Namboodiri, 1995). Pioneer submitted information on the potential impact of dietary exposure of NAA on individuals with CD. Analysis of the amounts of NAA excreted by healthy individuals compared to those with CD (Petition, Addendum 2) indicates that the vast majority of NAA within the body is produced endogenously (within the body) and does not result from dietary exposure (Petition, Addendum 2). Because the levels of NAA and NAG are negligible from dietary sources compared to the amounts produced endogenously by individuals with CD, individuals with CD are not expected to have adverse effects from consuming 356043.

As soybean meal is a major constituent of animal feed, particularly for chickens, cattle, and pigs, it is expected that animal exposure to NAA and NAG would also increase should APHIS deregulate 356043 soybeans. APHIS reviewed information submitted by Pioneer relating to the safety of the acetylated amino acids NAA and NAG (Petition, Addendum 1) and noted several points from their assessment:

- Acetylated amino acids are naturally occurring compounds that are found in many plants and animals.
- Up to 80% of cellular proteins in mammalian systems are estimated to be acetylated (Brown and Roberts, 1976; Driessen et al., 1985).
- Acetylation of proteins is used in the food industry (El-Adawy, 2000)
- Metabolism studies of other acetylated amino acids on rats, pigs and humans have not raised safety issues (Magnusson et al., 1989; Arnaud et al., 2004; Boggs, 1978; Boggs et al., 1975; Neuhauser and Bassler, 1986; Stegink et al., 1980 and 1982).
- NAA and NAG are components of human and animal diets and there is no indication that they are associated with adverse effects when consumed.
- The small increase in exposure to N-acetylated amino acids predicted to occur from consuming 356043 soybeans is not expected to have any adverse effects on animals as they have the enzymes to interconvert acetylated and deacetylated amino acids.
- Pioneer conducted a 42-day chicken feeding study where 356043 soybean was used as a large part of the chicken diet (Petition, Section VIII-D, p. 96). The study assessed mortality, weight gain, and feed efficiency parameters as well as various

carcass and organ data at the end of the period. There were no significant differences in the parameters analyzed and no noted adverse effects on the chickens.

Pioneer also submitted and APHIS reviewed a summary of data of a mouse acute toxicity study (Petition, p. 63) using GAT4601 protein. No evidence of acute toxicity was noted in that study. APHIS also reviewed a finalized manuscript submitted by Pioneer from a 13-week rat feeding study (Appenzeller, et al., 2008) after the comment period. This type of experiment is designed to assess toxicity of a food product. Diet analyses and animal testing were conducted using widely accepted Good Laboratory Practice (GLP) standards. Formulated diets used included both herbicide-sprayed and unsprayed 356043 soybeans. The study assessed rat mortality, overall weight gain, organ weights, gross and microscopic pathology, numerous blood and urinalyses, and neurobehavioral assessments. Compared with the non-transgenic controls or conventional reference diets, no biologically relevant adverse effects were noted in rats fed 356043 soybeans (sprayed or unsprayed). These results indicate that 356043 soybeans are as nutritious as conventional non-GE soybeans.

Considering all the information noted above on compositional similarities and differences, if APHIS chooses the proposed action to deregulate soybean 356043, significant environmental impacts are unlikely. Similarly, if APHIS chooses alternative A to continue to regulate 356043, there should also be no significant impact on the quality of the human environment.

APHIS has considered the effects of the alternatives on food and feed safety as one aspect of public health consistent with NEPA requirements. Under the Coordinated Framework, the safety of food and feed derived from soybean 356043 falls within the regulatory purview of the Food and Drug Administration under the Federal Food, Drug, and Cosmetic Act (FFDCA). Under 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 soybean 356043 must be in compliance with all applicable legal and regulatory requirements. FDA's final review for 356043 soybean was completed on September 21, 2007. As noted previously, FDA indicated that it had no further questions regarding the safety and nutritional assessment of 356043 for use in food and feed (http://www.cfsan.fda.gov/~lrd/biocon.html).

C. Potential Impacts Based on the Relative Weediness of Pioneer 356043 Soybean.

APHIS assessed whether 356043 soybean is any more likely to become a weed than the non-transgenic recipient soybean line or other soybean currently cultivated. The assessment encompasses a thorough consideration of the basic biology of the conventional soybean compared to evaluation of the unique characteristics of 356043 soybean.

In the United States, soybean is not listed as a weed in the major weed references (Crockett, 1977; Holm et al., 1979; Muenscher, 1980), nor is it present in the lists of noxious weed species distributed by the Federal Government (APHIS-USDA, 2006). Soybean has been grown throughout the world without any report that it is a serious weed. It is not generally persistent in undisturbed environments without human intervention. In the year following cultivation, soybean may grow as a volunteer under specific conditions and can be easily controlled by herbicides or mechanical means. It does not compete effectively with cultivated plants or primary colonizers (OECD, 2000). Soybean (*Glycine max)* has never been found in the wild in the U.S. (Hadley and Hymowitz, 1973). For these reasons, it is unlikely to become a weed.

In 2004 and 2005, Pioneer conducted field trials to evaluate phenotypic characteristics comparing 356043 to soybean variety "Jack," the recipient conventional parental line, at over 16 field trial locations in U.S. growing regions. Trials at numerous other locations were conducted in 2006 (Appendix 5, p. 140 of the petition). Data collected on agronomic performance characteristics are described in Chapter VII of the petition (pp. 65-76). Pioneer assessed aspects of performance, such as seed germination, seedling vigor, plant height, days to maturity, seed weight, yield, disease, and insect damage, along with qualitative phenotypic traits (flowers, pod color, etc.). Figure 26, on page 68 of the petition, notes many of the trial data collection locations. Table 12, on page 69, describes several of the field agronomic characteristics that Pioneer measured. Tables 13 through18, on pp 70-76, document summary data collected from those trials. There were no significant differences between 356043 and "Jack" for any of the assessed traits. Based on analysis of data on all these parameters, soybean 356043 is unlikely to pose any more of a plant pest risk from weediness than conventional soybean. If APHIS chooses the "no action" alternative, there should also be no change in the significance of impact on the environment as a result of potential increase in the weediness of soybean.

D. Potential Impact on Non-target and Beneficial Organisms

APHIS evaluated the potential for deleterious effects or significant impacts on non-target and beneficial organisms. First, APHIS notes that neither GAT nor GM-HRA proteins are known to have toxic properties. The bacterium *Bacillus licheniformis* has been considered safe for specific food and feed uses in the U.S., Canada and Europe (EU Commission, 2000; FDA, 2001; http://canadagazette.gc.ca/partI/2006/20060805/html/notice-e.html). GM-HRA protein is a modified form of the soybean ALS protein. Similar proteins (ALS) are present and have been commercialized in Clearfield® (BASF Corporation) and STS® products, which are grown on millions of acres in the U.S. every year. As such, ALS (the protein nearly identical to GM-HRA) is consumed regularly by anything that feeds on any Clearfield® or STS® products (e.g., corn, rice, sunflower, canola, wheat, and soybean). APHIS also notes that the metabolites that are elevated (NAA, NAG, 17:0 and 17:1) are found in many plants and animals and are not known to be toxic.

Pioneer collected extensive data on possible effects on non-target organisms in the field. They made observations on organisms such as beetles, grasshoppers, aphids, leaf miners, stinkbugs and whiteflies (Petition, Section VII-C, page 75). Data was compiled at all locations in 2003, 2004, and 2005. They also assessed insect damage in 2005 across 6 different locations (Petition, Table 15, p. 73). No significant differences were identified between 356043 and control soybeans in any instance.

Most soybean meal produced is used in animal feed products (Petition, Section VIII-D, p. 96). In order to address the wholesomeness and nutrition of 356043 compared with control soybeans, Pioneer conducted a 42-day chicken feeding study (a recognized model for assessing the wholesomeness of feeds) where 356043 soybean was used as a large part of the chicken diet (Petition, Section VIII-D, p. 96). The study assessed mortality, weight gain, and feed efficiency parameters over a 42-day period as well as various carcass and organ data at the end of the period. Pioneer did not observe any significant differences between 356043 soybeans and control soybeans in the parameters analyzed (Section VIII-D, page 96) and no adverse effects on the chickens were observed.

To address potential toxicity issues associated with 356043 soybean, Pioneer conducted acute toxicity studies using GAT 4601 protein, GM-HRA protein, NAA and a 13-week whole food rat feeding study. All were conducted using widely accepted methods for such studies (Petition, pp. 63-64 and Appenzeller, et al., 2008). None of these studies identified toxicity issues associated with 356043 soybean.

To assess unintended effects, APHIS analyzed data submitted by the developer to determine if there were changes to phenotype, germination, vegetative growth, reproductive parameters and response to biotic stressors (insect and disease stress) associated with 356043 soybeans in comparison to the various control lines (non-transgenic). These experiments are designed to document how the GE soybeans perform in the field environment compared to non-GE varieties (e.g., do the plants/seeds look, germinate, grow, flower, respond to insect and disease pressures similar to a non-GE variety). Data presented in Table 17- 18 (page 75-76 of the petition) indicates that the ecological interactions between 356043 soybeans and the control lines were similar. Pioneer noted no differences in field interactions with beetles, aphids, whiteflies, or other organisms that were different from control plants. Considering all the data noted from field observations, the broiler chicken feeding study and known safety information on GAT and ALS proteins, as well as changes in NAA and NAG concentrations, as well as minor fatty acid changes, APHIS concludes that 356043 soybeans are unlikely to pose a safety risk to non-target and beneficial organisms.

If APHIS chooses the no action alternative, there should also be no significant impact on non-target or beneficial organisms from not commercializing 356043 soybean.

E. Potential Impact on Threatened and Endangered Species

In addition to the analysis of potential impact to non-target organisms described above, APHIS also considered the potential impact on federally listed threatened or endangered species (TES) and species proposed for listing, as well as designated critical habitat and habitat proposed for designation, as required under Section 7 of the Endangered Species Act. In this analysis, APHIS considered the biology of 356043, as well as typical agricultural practices associated with cultivation of soybean. As noted previously, the 356043 soybean differs from non-transgenic soybean only in the expression of the *gat4601* and *gm-hra* genes which are responsible for herbicide tolerance to glyphosate and ALS-inhibiting herbicides, as well as increases in the amino acids N-acetylaspartate (NAA) and N-acetylglutamate (NAG), and increases in heptadecanoic and heptadecenoic acids. As discussed in Section V. B. of this EA, the proteins produced by the inserted genes and the increased amino and fatty acids do not raise safety issues. As noted above in Section D., consumption of GAT4601 protein, GM-HRA protein, and NAA have shown no toxicity in lab testing with mice and rats. The 356043 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 356043 soybeans indicate that these soybeans are not significantly different from non-transgenic soybeans and would not be expected to have any impact on TES that would be different from non-transgenic soybeans. The 356043 soybean is not sexually compatible with a federally listed TES or a species proposed for listing. The only TES animal listed that occupies habitat that is likely to include soybean fields and that might feed on soybeans is the federally Endangered Delmarva Peninsula Fox Squirrel. It is known to utilize certain agricultural lands readily, but its diet includes acorns, nuts/seeds of hickory, beech, walnut, and loblolly pine; buds and flowers of trees, fungi, insects, fruit, and an occasional bird egg (NatureServe 2007). Given all these factors and the lack of noted adverse effects on chickens (Petition, Section VIII-D) and rats (Appenzeller, et al., 2008) of 356043 soybean, consumption of 356043 soybean should have no effect on the Fox Squirrel.

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 356043 soybean is not expected to differ from typical soybean cultivation. The extent to which 356043 soybean will be grown is unknown, however, over 90% of the United States soybean acreage is planted with varieties tolerant to glyphosate, and it is expected that 356043 varieties will be used in areas with glyphosate tolerant weeds.

Although a shift to planting 356043 soybeans may result in an increase in the use of ALS-inhibiting herbicides, this increase is likely to occur anyway because of the availability of RR®/STS® soybean varieties. The ALS inhibitors currently registered for use on soybean can also be used on 356043 soybeans, with no change in label rates. In order to be registered as a pesticide by EPA under FIFRA, it must be demonstrated that when used with common practices, a pesticide will not cause unreasonable adverse effects in the environment, including effects on wildlife and TES (http://www.epa.gov/pesticides/factsheets/registration.htm). Thus, ALS-inhibitor herbicides currently registered for use on soybean varieties are not expected to pose any unreasonable risks to wildlife and the environment. Several of the ALS-inhibitor herbicides have comparable environmental impacts (as calculated by a Cornell University publication (http://nysipm.cornell.edu/publications/eiq/default.asp)) as glyphosate, which will likely continue to be used on large acreages across the U.S. Additionally, as noted in the table on page 10 of this EA, ALS inhibitors continue to be used and have been used effectively on soybeans in the past (e.g., chlorimuron, imazaquin, thifensulfuron, and others). As these herbicides have been used effectively and safely for many years on soybeans as well as other crops, there is no indication that their use on a higher percentage of acres would be associated with significant environmental impacts. Periodic registration review by EPA for these herbicides ensures that these products do not present unreasonable risks to humans, wildlife, fish, and plants (http://www.epa.gov/opp00001/factsheets/securty.htm). It is uncertain exactly which ALS-inhibiting herbicide(s) would be recommended for use on 356043 soybean but growers will have several options and be able to choose based on their needs.

After reviewing possible effects of deregulating 356043 soybean, APHIS has not identified any stressor 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. APHIS expects 356043 soybean to replace some of the presently available glyphosate tolerant and RR®/STS® soybean varieties, but APHIS does not expect that 356043 soybean will cause new soybean acres to be planted in areas that are not already devoted to agriculture. As noted previously, before allowing a pesticide product to be sold on the market, EPA ensures that the pesticide will not pose any unreasonable risks to wildlife and the environment. EPA does this by evaluating data submitted in support of registration regarding the potential hazard that a pesticide may pose to non-target fish and wildlife species. In considering whether to register a pesticide, EPA conducts ecological risk assessments to determine what risks a pesticide poses and whether changes to the use or proposed use are necessary to protect the environment. APHIS has considered the effect of 356043 soybean production on critical habitat (which is a subset of the environment and therefore also considered by EPA) and could identify no difference from affects that would occur from the production of other soybean varieties. Therefore, APHIS has determined that granting a petition of non-regulated status for 356043 soybean should 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.

F. Potential Impacts on Biodiversity

Analysis of available information indicates that 356043 exhibits no traits that would cause increased weediness, that its unconfined cultivation should not lead to increased weediness of other sexually compatible relatives (of which there are none in the United States), and it is likely to have no effect on non-target organisms common to agricultural ecosystems or threatened or endangered species recognized by the U.S. Fish and Wildlife Service. Based on this analysis, there is no apparent potential for significant impact to biodiversity. If APHIS chooses the no action alternative, there would also be no impact on biodiversity.

G. Potential Impacts on Commercial Use

Soybean is a globally traded commodity and the U.S. is the single largest exporter. The commercial use of soybeans in the U.S. would not be feasible without approvals from key trading partners. Pioneer informed APHIS that the company does not intend to commercially release 356043 soybean until all key soybean import markets with functioning regulatory systems have also granted approval of 356043 soybean. APHIS has evaluated field trial data reports submitted on this event and progeny and has noted no significant adverse effects on non-target organisms, no increase in fitness or weediness characteristics, and no effect on the health of other plants. Based on all these considerations, there is no apparent potential for significant impact on commercial use if

APHIS chooses either the "no action" alternative or grants nonregulated status to 356043 soybean.

H. Potential Impacts on Agricultural Practices

APHIS considered potential impacts associated with the cultivation of 356043 soybeans on current agricultural practices, in particular, those associated with weed control. Potential impacts include the development of herbicide resistant weeds through continued use of glyphosate and ALS-inhibitor herbicides and the stacking of herbicide resistance traits from previously deregulated as well as other non-GE herbicide tolerant soybean lines (i.e., Roundup Ready® and STS® varieties).

STS® (not GE, tolerant to ALS-inhibitor herbicides and not Roundup Ready®) varieties have been available for at least 10 years (http://web.aces.uiuc.edu/value/factsheets/soy/fact-sts-soy.htm) and an estimated 10 million acres of STS® varieties were grown in 2003 (http://web.aces.uiuc.edu/value/factsheets/soy/fact-sts-soy.htm). Pioneer notes STS® soybeans were grown on ~3.8 million acres in 2006 (Petition, p. 104). As noted previously in this EA, soybeans tolerant to both glyphosate and ALS-inhibiting herbicides (e.g., sulfonylureas) have been available to growers since 2006 (Section IV.A). Those varieties (marketed as Roundup Ready®/STS®) were developed by hybridizing GE Roundup Ready® varieties with STS® varieties developed through mutation breeding. Five (5) RR®/STS® varieties were available for the 2007 season (http://www.deltaandpine.com/soybean_products_rrs.asp). These varieties were developed to allow growers another option for weed control, which would be useful to growers in areas where glyphosate tolerant weeds exist or have developed. The significance of this is that soybeans tolerant to sulfonylurea herbicides (an ALS inhibitor) and Roundup® are already available to growers. Making 356043 soybean available to growers does not change this availability and impacts associated with glyphosate use and sulfonylurea herbicides would not be expected to change significantly.

APHIS does note two reports of weeds that have developed tolerance to both ALS-inhibitors as well as glyphosate in Missouri and Ohio (http://www.weedscience.org/Summary/UspeciesMOA.asp?lstMOAID=12&FmHRACGroup=Go). The species listed are horseweed (*Conyza canadensis)* and waterhemp (*Amaranthus rudis)*. Growers have adapted to the development of herbicide tolerant weed populations in the past and DuPont/Pioneer continues to recommend a variety of ways to manage such weeds through integrated weed management and product stewardship (Petition, pp. 105-107, pp. 148-151). As noted, weed scientists are developing management strategies to help ensure consistent control of these weeds (Loux et al. 2004, Loux and Stachler 2006), and companies and university scientists have developed and are developing alternative herbicide resistant crop strategies (e.g. Service, 2007). Pioneer has shown support for Integrated Weed Management programs in the past and it is likely that they will continue to do so in the future.

GE crop technology has improved growers' ability to control weeds, reducing the need to rely on soil cultivation and seed-bed preparation as a means to getting good weed control.

GE crop technology has also eliminated the potential damage caused by some soil-incorporated residual herbicides in crops used in rotation, including some ALS-inhibitors. Under traditional herbicide applications with conventional crops, a post-emergent herbicide application may result in ‘knock-back’ (crop damage from the residual herbicide application); this problem is less likely to occur in herbicide-tolerant crops because most of the herbicides (i.e., glyphosate, glufosinate) used on GE crops do not tend to have residual soil activity. The adaptation of no-till or reduced till systems results in time savings, reduced equipment usage, decreased soil erosions and run-off and decreased costs to growers. While no- or reduced-till systems are not new, the resultant weed control using engineered herbicide-tolerant crops allows the farmer to continue with the no-till/reduced-till systems long after conventional crops necessitate going back to full plowing due to excessive weeds.

Along with the typical no- or reduced-till systems, there is significant fuel savings associated with making fewer spray applications (relative to conventional crops) (Brookes and Barfoot, 2006). The authors also determined that the fuel savings has also resulted in reductions in carbon dioxide emissions. In 2005 this was estimated to be about 2.1 billion fewer pounds of CO_2 emitted (arising from reduced fuel use of 94 million gallons).

As noted in the petition (p.105), changes in crop rotation practices due to the availability of 356043 soybean are not expected. The type of rotation crop and the specific herbicide needs of the rotational crop will not be impacted, as growers will continue to choose rotational crops based on market needs and cultural practices. Recommendations for specific ALS inhibitor products for a grower planting 356043 soybean will take into account existing crop rotation practices (soybean/corn, wheat/soybean, soybean/cotton, soybean/rice, etc.), just as they do currently for conventional, glyphosate tolerant, or RR®/STS® soybean varieties. Glyphosate has no residual activity and therefore no re-cropping restrictions, so if growers choose to spray only glyphosate (and not ALS inhibitors or other herbicides labeled for soybean) on their 356043 soybean, there will be no re-cropping constraints.

No increases in glyphosate use are predicted from the decision to grant nonregulated status to 356043 because the adoption rate of glyphosate resistant soybeans is already extremely high and expansion of glyphosate resistant soybean plantings beyond the current amount are not reasonably foreseeable based on past trends and the economic benefits of planting other soybean varieties or corn. Soybeans resistant to ALS inhibitors were planted on approximately 5% of the total soybean acreage and therefore use of herbicides targeting ALS could conceivably increase especially if glyphosate resistant weeds become more widespread. However, there is some uncertainty as to the adoption rate of 356043 soybean given the fact that similar varieties with essentially identical herbicide use and weed management practices already exist in RR®/STS® varieties. Growers who choose to grow RR®/STS® varieties will be afforded another seed choice. Agricultural practices associated with either these varieties or 356043 are essentially the same. Growers who desire to use soybean varieties with tolerance to both glyphosate and ALS-inhibitors would purchase RR®/STS® varieties if 356043 varieties were not available. By this reasoning, impacts to existing agricultural practices should not be significant with either a decision to grant nonregulated status (proposed action) or the “no action” alternative. Similarly, the use of

ALS inhibitors for weed control is unlikely to be impacted by the decision to grant or not to grant nonregulated status to 356043 because soybeans can currently be sprayed with ALS inhibitors and because dual herbicide resistant varieties already have been commercialized and are widely used.

I. Potential Impacts on Conventional and Organic Farming

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 prohibited substances from adjoining land that is not under organic management. Organic production operations must also develop and maintain an organic production system plan approved by their accredited certifying agent. This plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition on the use of excluded methods. Excluded methods include a variety of methods used to genetically modify organisms or influence their growth and development by means that are not possible under natural conditions or processes. Organic certification involves oversight by an accredited certifying agent of the materials and practices used to produce or handle an organic agricultural product. This oversight includes an annual review of the certified operation's organic system plan and on-site inspections of the certified operation and its records. Although the National Organic Standards prohibit the use of excluded methods, they do not require testing of inputs or products for the presence of excluded methods. The presence of a detectable residue of a product of excluded methods alone does not necessarily constitute a violation of the National Organic Standards. The unintentional presence of the products of excluded methods will not affect the status of an organic product or operation when the operation has not used excluded methods and has taken reasonable steps to avoid contact with the products of excluded methods as detailed in their approved organic system plan. Organic certification of a production or handling operation is a process claim, not a product claim.

In 2003, of the total 73.4 million acres of soybeans in the United States (USDA-ERS 2006), 122,403 acres (0.17%) were certified organic soybeans (USDA-ERS 2005).

It is not likely that farmers, including organic and conventional farmers, who choose not to plant transgenic soybean varieties or sell transgenic soybeans, will be significantly impacted by the commercial use of this product. Non-transgenic soybean will likely still be sold and will be readily available to those who wish to plant it (e.g., STS® varieties). An internet search of "soybean seed company" identifies vendors that offer all types of soybean seeds for purchase including conventional and transgenic. A few of the many searchable sites available include www.lathamseeds.com and http://www.bo-jac.com. If Pioneer receives regulatory approval from all appropriate agencies, it will make 356043 soybean available to growers and breeders. It is not likely that other farmers who choose not to plant or sell 356043 soybean or other transgenic soybeans will be significantly impacted by the expected commercial use of this product as (a) non-transgenic soybeans will likely still be sold and will be readily available to those who wish to plant them; (b) soybean is a highly self-pollinated plant and buffer requirements would be minimal; (c) over 89% of the latest soybean acreage in the U.S. is already planted to transgenic RR® soybean varieties; and (d) APHIS expects that 356043 soybean may replace some of the presently available glyphosate tolerant soybean varieties without significantly affecting the overall total soybean acreage or glyphosate tolerant soybean acreage so organic farmers will be able to coexist with biotech soybean producers as they do now.

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 over 89% of the soybeans produced would likely be planted with the current herbicide tolerant biotech soybean varieties. If the 356043 line is granted nonregulated status, there also would be no direct impact on organic or other non-transgenic soybean farmers as the market share of transgenic soybean (which may include Monsanto's new MON89788 subject of a APHIS petition 06-178-01p) is unlikely to change by the introduction of 356043.

J. Potential Impacts on Raw or Processed Agricultural Commodities

Pioneer presented extensive data in their Petition relating to plant growth parameters, disease incidence, insect susceptibility, and forage and seed composition compared to the soybean variety “Jack” as well as other soybean varieties. APHIS analysis of this data indicate no differences between 356043 soybean and the non-transgenic Jack variety that would be expected to cause either a direct or indirect plant pest effect on any raw or processed plant commodity from deregulation of 356043 soybean. Compositional analysis of 356043 soybean detected increased levels of two minor fatty acids, heptadecanoic acid and heptadecenoic acid (discussed in Section V.B.). Together, these fatty acids make up less than 0.8% of soybean total fatty acids. Similarly, elevated levels of NAA and NAG still only represent less than 0.15% of the total soybean amino acids. Neither of these noted changes would be expected to alter how 356043 soybeans are handled, as soybean is a highly blended commodity and these changes are extremely small in terms of the overall composition of these soybeans. Consequently, no significant effects on raw or processed commodities are expected from these small differences in composition if APHIS were to grant nonregulated status to 356043 soybean. Similarly, no significant effects would be expected if APHIS chooses the “no action” alternative.

Given the reproductive biology of soybean (described in this EA, p. 3) and typical handling procedures for harvested soybeans, there is a low likelihood for economic impact to non-transgenic raw and processed soybean products should commingling or inadvertent outcrossing occur as a result of mechanical or physical interaction between 356043 fields and conventional or organic production fields in close proximity. In addition, as noted earlier, outcrossing may occur up to 2.5 % when rows of soybeans are planted with a separation distance of 102 cm. The impact of commingling may affect the marketability of conventional and organic soybean if detected during testing before or after sale. To date, APHIS is not aware of an incident where the value of a conventional or organic soybean crop was diminished by detection through testing or through the perception that commingling had occurred from existing glyphosate or ALS tolerant soybeans. However, it is important to note that separation distances as little as 10 meters result in only 0 – 0.01

percent outcrossing (http://www.funpecrp.com.br/gmr/year2007/vol2-6/gmr0322_full_text.html).

K. Potential Cumulative Impacts

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 undertakes such actions.

Past actions include previous determinations of nonregulated status for glyphosate tolerant and phosphinothricin tolerant varieties of soybean (http://www.aphis.usda.gov/brs/not_reg.html). Current glyphosate tolerant (GT) soybean acreage (~89% of all soybeans grown in the US) is almost entirely based on a USDA-APHIS determination made in 1994 and commercialized initially in 1996 (Monsanto Event 40-3-2). Along with that determination and gradual adoption of GT soybeans came increased use of glyphosate and concomitant decrease in use of other herbicides on soybean (outlined in Section IV.B table of this EA, p. 11). Additionally, APHIS has recently published a determination of nonregulated status for Petition 06-178-01p, Monsanto 89788 soybean that is tolerant to glyphosate. This new line was developed to replace the original 40-3-2 event. It is expected that the new MON 89788 varieties will replace the older 40-3-2 varieties over time.

Soybean production occurs on land that is dedicated to crop production. Most soybeans are planted in fields that have been in crop production for over 10 years. As with most agricultural production, continuous production of soybean would normally include the use of tillage and herbicides to limit the growth of weeds, limit the potential impact caused by insects, animals or disease, and to maximize production. Widespread use of 356043 soybean is expected to have an insignificant impact on overall soybean production, primarily because GT soybeans are currently planted on over 89% of soybean acreage and 356043 would most likely be grown in place of a portion of those transgenic soybean acres, not as additional acreage now occupied with non-transgenic soybeans.

APHIS is currently reviewing a petition from Pioneer requesting nonregulated status of a glyphosate and ALS-inhibitor tolerant corn (Petition 07-152-01p, 98140 corn). A future likely action is that 98140 corn will also obtain nonregulated status. It is possible that growers would choose to rotate 98140 corn with 356043 soybean. This rotation pattern is not likely to cause an increase in the number of herbicide tolerant weeds, as growers will be able to utilize both glyphosate and ALS-inhibitor herbicides. If a grower plants 356043 soybean and/or 98140 corn, it does not mean they will need to use both glyphosate and ALS-inhibitors; they may only need to use one, or they may use herbicides with other modes of action registered for the crop. Many herbicides are currently available to use on corn and soybeans (http://ipcm.wisc.edu/Publications/tabid/54/Default.aspx). Growers who apply recommended principles of integrated weed management (such as herbicide rotations using different chemistries) will best be able to delay the onset of resistant weeds.

Possible future actions from APHIS also include deregulation of glyphosate tolerant cotton (06-332-01p), alfalfa (04-110-01p) and creeping bentgrass (03-104-01p). Only cotton and alfalfa are sometimes rotated with soybean. If growers choose to rotate from either 356043 soybean or 98140 corn to a crop that does not have dual herbicide tolerance, there may be re-cropping issues to consider because of the residual re-plant restrictions for some of the ALS inhibitor herbicides. These issues are similar to re-cropping considerations that growers currently need to consider.

Although there is some uncertainty as to the adoption rate of 356043 soybean, there are a number of possible scenarios. For a grower, their decision to purchase 356043 is largely economic (i.e., all costs associated with growing and selling a crop are factored into which seed to purchase). Almost all buyers of 356043 soybeans will have previously grown GT soybean and 356043 will likely replace GT or RR®/STS® varieties. Growers will only likely buy 356043 if they can derive an economic benefit. Soybean 356043 will provide an extra weed control option since this product will allow use of ALS-inhibiting herbicides that could help a grower manage glyphosate tolerant weeds, if present. Using a variety of environmental toxicity parameters, researchers at Cornell University have developed a method to assess the environmental impacts of pesticides (http://nysipm.cornell.edu/publications/eiq/default.asp). This compilation indicates that both ALS-inhibitor and glyphosate-containing herbicides have comparable environmental impacts within agricultural production systems. Growers of currently available non-GE soybeans are currently able to apply both glyphosate and ALS-inhibitors to their soybeans. Since 356043 will most likely be used by growers that are presently using GT or RR®/STS® soybeans, and there are no significant environmental impacts from use of these two types of herbicides, cumulative impacts are likely to be similarly insignificant.

APHIS notes that the use of herbicides with different modes-of-action on crops is already a common agricultural practice. As part of its ongoing responsibilities for regulation of pesticides, EPA has assessed the impacts of application of glyphosate and ALS-inhibitors on soybeans, and other herbicide tolerant crops, and approved the appropriate pesticide label amendments and/or tolerances for those uses. In addition, EPA has reviewed aggregate dietary exposures of glyphosate and ALS-inhibitors in making its food safety determinations for these products under the Federal Food, Drug, and Cosmetic Act (e.g., EPA, 1993, 2004a). APHIS consulted with EPA regarding the use of glyphosate and ALS-inhibitors on 356043 soybean. They have not identified any significant issues related to herbicide label changes or metabolites from the use of either glyphosate or registered ALS-inhibitors on 356043 soybean (EPA, personal communication). While the use of ALS-inhibitors may increase with the availability of 356043 soybean, the amounts are not expected to reach the historic high levels seen in the 1990's. Thus, deregulation of 356043 soybean does not significantly change current agricultural practice, and no significant cumulative impacts would be expected.

Low use-rate herbicides such as ALS-inhibitors can cumulatively lessen impact on the environment by decreasing the amount of herbicide used, diminishing waste generation and energy use, while allowing easier handling, storage, and transport. Growers using low-use rate ALS-inhibitors would apply 95-99% less herbicide (active ingredient) to their crops, releasing much less into the environment. A significant reduction in energy input and waste generation is also expected during the chemical manufacturing of low-use rate herbicides. For example, the energy (gas, oil, electricity, etc.) needed to produce high use-rate herbicides (e.g., 2,4-D or dicamba) is 7 to 9-fold more than that required for a sulfonylurea (DuPont, personal communication). Waste streams of 10 – 100 kg of waste per kg final active ingredient are typical in agricultural chemical production (Brown, 1995). Waste streams for ALS-inhibitors are typically on the low end of this range. Coupled with the low-use rates needed, these result in lower energy use and waste generated per acre of treatment than for other herbicides.

Soybean acreage is not expected to increase significantly in the near future. Estimates of the 2007 soybean crop indicate that planting will be at the lowest level since 1996. This reflects an 11% decrease in acreage compared to 2006 (http://www.nass.usda.gov/Newsroom/2007/03_30_2007.asp) and in the prior five years, soybean production was relatively steady varying from 72 million acres to 75.5 million acres (http://www.nass.usda.gov/QuickStats/index2.jsp). Based on the above observations, Pioneer 356043 soybeans can be expected to replace some of the glyphosate tolerant soybeans on the market (which may include Monsanto's new MON89788, subject of APHIS petition 06-178-01p) and some of the RR®/STS® varieties available. Because the total amount of glyphosate tolerant and RR®/STS® soybean planted in the U.S. is unlikely to increase based on whether or not Pioneer 356043 is deregulated, and the development of glyphosate and ALS tolerant weeds is related to the amount of glyphosate and ALS tolerant soybean planted and glyphosate and ALS-inhibitors used, APHIS reasonably concludes that Pioneer 356043 should not have a significant cumulative impact on the development of herbicide tolerant weeds.

The release of 356043 soybean will not produce any other substance that is not normally produced by soybeans, nor is the composition of the seed produced by these soybeans significantly different from unmodified soybeans. Therefore, APHIS does not expect accumulation of a novel substance in soil, nor does APHIS expect significant impacts on organisms living in and around these agricultural fields because of exposure to 356043 soybean compared to soybeans currently planted.

Data supplied by the applicant, including results of 3 years of field tests in various environments, indicate that 356043 soybeans have not had observable or measurable impacts on ecosystems in which they have been grown (Petition, Section VII-C., pp. 75-76). Because there is not likely to be any incremental increase in the planting of transgenic soybean, 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 (soil, water, ecosystem quality, biodiversity, etc.) associated with the ecosystem in which 356043 soybean is planted. No resources should be significantly impacted due to cumulative impacts resulting from the proposed action. Similarly, no significant effects on resources would be expected if APHIS chooses the “no action” alternative.

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

Executive Order (EO) 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations," requires Federal agencies to conduct their programs, policies, and activities that substantially affect human health or the environment in a manner so as not to exclude persons and populations from participation in or benefiting from such programs. It also requires federal agencies to conduct their programs in a manner that will prevent minority and low-income communities from being subjected to disproportionately high and adverse human health or environmental effects.

EO 13045, "Protection of Children from Environmental Health Risks and Safety Risks," acknowledges that children may suffer disproportionately from environmental health and safety risks because of their developmental stage, greater metabolic activity levels, and behavior patterns, as compared to adults. The EO (to the extent permitted by law and consistent with the agency's mission) requires each Federal agency to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children. Each alternative was analyzed with respect to EO 12898 and 13045. None of the alternatives are expected to have a disproportionately adverse human health or environmental effect on minorities, low-income populations, or children.

EO 13112, "Invasive Species", requires 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-engineered and deregulated engineered glyphosate tolerant soybeans are widely grown in the United States. Based on historical experience with these soybeans and the data submitted by the applicant and reviewed by APHIS, these engineered 356043 plants are very similar in fitness characteristics to other soybean varieties currently grown. Due to the fact that soybeans have never been weedy or invasive species, they are not expected to have an increased invasive potential, the majority of soybeans planted in the U.S. are genetically engineered.

EO 12114, "Environmental Effects Abroad of Major Federal Actions" requires Federal officials to take into consideration any potential significant environmental effects outside the United States, its territories, and possessions that result from actions being taken. APHIS has given this due consideration and does not expect a significant environmental impact outside the United States should nonregulated status be determined for 356043 soybean or if the other alternative is chosen. It should be noted that all the considerable, existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new soybean cultivars internationally, apply equally to those covered by an APHIS determination of non-regulated status under 7 CFR Part 340. Any international traffic of Pioneer 356043 soybean subsequent to a determination of non-regulated status for 356043 soybean would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed under the International Plant Protection Convention (IPPC).

The purpose of the IPPC "is to secure a common and effective action to prevent the spread and introduction of pests of plants and plant products, and to promote appropriate measures for their control" (https://www.ippc.int/IPP/En/default.jsp). The protection it affords extends to natural flora and plant products and includes both direct and indirect damage by pests, including weeds. The IPPC set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (157 countries as of October 2006). 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 bioengineered organisms are consistent with the Plant Protection Act as well as with 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 138 countries are Parties to it as of January 5, 2007 (see http://www.biodiv.org/biosafety/default.aspx). Although the United States is not a party to the CBD, and thus not a party to the Cartagena Protocol on Biosafety, United States 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 United States Government has developed a website that provides the status of all regulatory reviews completed for different uses of bioengineered products (http://usbiotechreg.nbii.gov). These data will be available to the Biosafety Clearinghouse. APHIS continues to work toward harmonization of biosafety and biotechnology consensus documents, guidelines, and regulations, including within the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the United States, and 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* (see http://www.nappo.org/Standards/Std-e.html). APHIS also participates in the North American Biotechnology Initiative (NABI), a forum for information exchange and cooperation on agricultural biotechnology issues for the U.S., Mexico and Canada. In addition, bilateral discussions on biotechnology regulatory issues are held regularly with other countries including: Argentina, Brazil, Japan, China, and Korea.

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Determination of nonregulated status for Pioneer DP-356043-5 soybean

In response to petition 06-271-01p from Pioneer Hi-Bred, APHIS has determined that 356043 soybean and progeny derived from it are no longer regulated articles under APHIS regulations at 7 CFR part 340. Permits or acknowledged notifications that were previously required for environmental release, importation, or interstate movement under those regulations will no longer be required for 356043 soybean and its progeny. Importation of seeds and other propagative material would 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 this environmental assessment that indicate that 356043 poses no more of a plant pest risk than its non-genetically engineered counterpart. DP-356043-5 soybean will not pose a plant pest risk for the following reasons: (1) gene introgression from 356043 soybean into wild relatives in the United States and its territories is extremely unlikely; (2) based on its lack of toxicity and low likelihood of allergenicity, it does not pose a risk to non-target organisms, including beneficial organisms and federally listed threatened or endangered species, and species proposed for listing; (3) it exhibits no characteristics that would cause it to be weedier than the non-genetically engineered parent soybean line or any other cultivated soybean; (4) considering its cultivation in the agroecosystem, it does not pose a risk to non-target organisms, including threatened and endangered species, or designated critical habitat as a result of the use of EPA-registered glyphosate and ALS-inhibitor herbicides, as these have been used safely on soybeans for many years; (5) it does not pose a threat to biodiversity as it does not exhibit traits that increase its weediness, and its unconfined cultivation should not lead to increased weediness of other cultivated soybeans; (6) its commercial use should not have significant effects on agricultural practices; (7) it should not cause significant impacts on the development of herbicide tolerant weeds or cumulative impacts in combination with other glyphosate tolerant crops; (8) it commercial use should not have significant impacts on other growers, including organic growers; (9) agronomic performance, disease and insect susceptibility and compositional profiles of 356043 soybean are similar to those of its parent line and other soybean cultivars grown in the

United States, therefore no direct or indirect plant pest effects on raw or processed plant commodities are expected; (10) when considered in light of other actions, APHIS identified no significant environmental impacts that would result from granting nonregulated status to 356043 soybean.

In addition to our finding of no plant pest risk, there will be no effect on federally listed threatened or endangered species, species proposed for listing, or their designated or proposed critical habitat, resulting from a determination of nonregulated status for 356043 soybean and its progeny. APHIS also concludes that new varieties bred from 356043 are unlikely to exhibit new plant pest properties, i.e., properties substantially different from any observed for 356043, or those observed for other soybean varieties not considered regulated articles under 7 CFR part 340.

Sidney W Abel

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Deputy Administrator, Biotechnology Regulatory Services
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