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This section of the FEDERAL REGISTER contains documents other than rules or proposed rules that are applicable to the public. Notices of hearings and investigations, committee meetings, agency decisions and rulings, delegations of authority, filing of petitions and applications and agency statements of organization and functions are examples of documents appearing in this section.

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 96-098-2]

Dupont Agricultural Products; Availability of Determination of Nonregulated Status for Genetically Engineered Soybeans

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public of our determination that the Dupont Agricultural Products' soybeans designated as sublines G94-1, G94-19, and G168 derived from transformation event 260-05 which have been genetically engineered to produce high oleic acid oil, are no longer considered regulated articles under our regulations governing the introduction of certain genetically engineered organisms. Our determination is based on our evaluation of data submitted by Dupont Agricultural Products in its petition for a determination of nonregulated status and an analysis of other scientific data. This notice also announces the availability of our written determination document and its associated environmental assessment and finding of no significant impact.

EFFECTIVE DATE: May 7, 1997.

ADDRESSES: The determination, an environmental assessment and finding of no significant impact, and the petition may be inspected at USDA, room 1141, South Building, 14th Street and Independence Avenue SW., Washington, DC, between 8 a.m. and 4:30 p.m., Monday through Friday, except holidays. Persons wishing to inspect those documents are asked to call in advance of visiting at (202) 690-2817.

FOR FURTHER INFORMATION CONTACT: Dr. Ved Malik, BSS, PPQ, APHIS, 4700 River Road Unit 147, Riverdale, MD 20737-1236; (301) 734-8761. To obtain a copy of the determination or the environmental assessment and finding of no significant impact, contact Ms. Kay Peterson at (301) 734-4885; e-mail: mkpeterson@aphis.usda.gov.

SUPPLEMENTARY INFORMATION:

Background

On January 8, 1997, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 97-008-01p) from Dupont Agricultural Products (Dupont) of Wilmington, DE, seeking a determination that soybeans designated as sublines G94-1, G94-19, and G168 derived from transformation event 260-05 (sublines G94-1, G94-19, and G168) which have been genetically engineered to produce high oleic acid oil, do not present a plant pest risk and, therefore, are not regulated articles under APHIS' regulations in 7 CFR part 340.

On February 28, 1997, APHIS published a notice in the **Federal Register** (62 FR 9155-9156, Docket No. 96-098-1) announcing that the Dupont petition had been received and was available for public review. The notice also discussed the role of APHIS and the Food and Drug Administration in regulating the subject soybean sublines and food products derived from them. In the notice, APHIS solicited written comments from the public as to whether these soybean sublines posed a plant pest risk. The comments were to have been received by APHIS on or before April 29, 1997. APHIS received no comments on the subject petition during the designated 60-day comment period.

Analysis

Sublines G94-1, G94-19, and G168 have been genetically engineered to contain the GmFad2-1 gene, which causes a coordinate silencing of itself and the endogenous GmFad2-1 gene. Suppression of the GmFad2-1 gene in developing soybeans prevents the addition of a second double bond to oleic acid, resulting in a greatly increased oleic acid content only in the seed. Oil from this seed contains an abundance of monosaturated oleic acid (82-85 percent), a reduced concentration of polysaturated fatty acids, and lower palmitic acid content. While the subject soybean sublines also contain the GUS and Amp marker genes, tests indicate that these genes are not expressed in the soybean plants. The added genes were introduced into meristems of the elite soybean line A2396 by the particle bombardment method, and their expression is controlled in part by gene sequences from the plant pathogens *Agrobacterium tumefaciens* and cauliflower mosaic virus.

The subject soybean sublines have been considered regulated articles under APHIS' regulations in 7 CFR part 340 because they contain gene sequences derived from plant pathogens. However, evaluation of field data reports from field tests of these soybeans conducted under APHIS notifications since 1995 indicates that there were no deleterious effects on plants, nontarget organisms, or the environment as a result of the environmental release of sublines G94-1, G94-19, and G168.

Determination

Based on its analysis of the data submitted by Dupont and a review of other scientific data and field tests of the subject soybeans, APHIS has determined that sublines G94-1, G94-19, and G168: (1) Exhibit no plant pathogenic properties; (2) are no more likely to become weeds than soybean lines developed by traditional breeding techniques; (3) are unlikely to increase the weediness potential for any other cultivated or wild species with which they can interbreed; (4) will not harm threatened or endangered species or other organisms, such as bees, that are beneficial to agriculture; and (5) will not cause damage to raw or processed agricultural commodities. Therefore, APHIS has concluded that the subject soybean sublines and any progeny derived from hybrid crosses with other nontransformed soybean varieties will be as safe to grow as soybeans in traditional breeding programs that are not subject to regulation under 7 CFR part 340.

The effect of this determination is that Dupont's soybean sublines G94-1, G94-19, and G168 are no longer considered regulated articles under APHIS' regulations in 7 CFR part 340. Therefore, the requirements pertaining to regulated articles under those regulations no longer apply to the field testing, importation, or interstate movement of the subject soybean sublines or their progeny. However,

importation of soybean sublines G94–1, G94–19, and G168 or seeds capable of propagation are still subject to the restrictions found in APHIS' foreign quarantine notices in 7 CFR part 319.

National Environmental Policy Act

An environmental assessment (EA) has been prepared to examine the potential environmental impacts associated with this determination. The EA was prepared in accordance with: (1) The National Environmental Policy Act of 1969, as amended (NEPA) (42 U.S.C. 4321 *et seq.*), (2) regulations of the Council on Environmental Quality for implementing the procedural provisions of NEPA (40 CFR parts 1500–1508), (3) USDA regulations implementing NEPA (7 CFR part 1b), and (4) APHIS' NEPA Implementing Procedures (7 CFR part 372). Based on that EA, APHIS has reached a finding of no significant impact (FONSI) with regard to its determination that Dupont's soybean sublines G94–1, G94–19, and G168 and lines developed from them are no longer regulated articles under its regulations in 7 CFR part 340. Copies of the EA and the FONSI are available upon request from the individual listed under **FOR FURTHER INFORMATION CONTACT.**

Done in Washington, DC, this 14th day of May 1997.

Donald W. Luchsinger,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 97–13115 Filed 5–19–97; 8:45 am]

BILLING CODE 3410–34–P

DuPont Petition 97-008-01p for Determination of Nonregulated Status for Transgenic High Oleic Acid Soybean Sublines G94-1, G94-19, and G-168

Environmental Assessment and Finding of No Significant Impact

May 1997

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture, has prepared an environmental assessment in response to a petition (APHIS Number 97-008-01p) received from DuPont Agricultural Products (DuPont) seeking a determination of non-regulated status for their genetically engineered high oleic acid soybean lines G94-1, G94-19, and G-168 derived from transformation event 260-05 under APHIS regulations at 7 CFR Part 340. Based on the analysis documented in its environmental assessment, APHIS has reached a finding of no significant impact (FONSI) on the environment from the unconfined cultivation and agricultural use of the subject soybean sublines and their progeny.

John H. Payne, Ph.D.
Director
Biotechnology and Scientific Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: MAY 7 1997

Keywords: Soybean; modified oil profile; oleic acid

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I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) in response to a petition (APHIS Number 97-008-01p) from DuPont Agricultural Products (DuPont) of Wilmington, DE seeking a determination of non-regulated status for their transgenic high oleic acid soybean sublines G94-1, G94-19, and G-168, derived from transformation event 260-05, hereafter referred to as sublines G94-1, G94-19, and G-168. These soybean sublines are currently regulated articles under USDA regulations at 7 CFR Part 340. Interstate movements, importations, and field tests of these soybean sublines have been conducted under permits issued or notifications acknowledged by APHIS. DuPont has petitioned APHIS for a determination that these sublines do not present a plant pest risk and, therefore, are no longer regulated articles under regulations at 7 CFR Part 340.

Soybean sublines G94-1, G94-19 and G168 have been developed in an effort to improve the quality of oil derived from soybean seed by increasing their oleic acid content. Sublines G94-1, G94-19 and G168 are homozygous for the coding region of the *GmFad2-1* gene isolated from *Glycine max* in the sense orientation under the control of a seed-specific promoter. The introduction of *GmFad2-1* causes a coordinate silencing, or sense suppression, of itself and the endogenous *GmFad2-1* gene resulting in a soybean whose oil has an oleic acid content that exceeds 80%. Conventional soybeans have an oleic acid content of 24%. The *GmFad2-1* gene encodes for a seed-specific delta-12 desaturase that is involved in the synthesis of polyunsaturated fatty acids in developing seeds. In addition, sublines G94-1, G94-19 and G168 have been genetically modified to contain two other genes, which are present but not expressed in the plant. The beta-glucuronidase (*uidA*) gene from *Escherichia coli*, serves as a reporter gene, allowing the selection of transformed plants in the laboratory. The ampicillin resistance gene, beta-lactamase (*bla*) from *Escherichia coli* is used for selection when the construct is in *E. coli* cells. Neither the *uidA* gene nor the *bla* genes are expressed in the soybean sublines under review. The original transformant also contained the *dapA* gene from the bacterium *Corynebacterium glutanicum*, encoding dihydrodipicolinic acid synthase, expression of which can lead to an increase in free lysine content. The *dapA* gene was inserted independently from the *GmFad2-1* gene and was lost via segregation, and is thus not present in the subject soybean lines. The genes were introduced into the parent soybean line (Asgrow A2396) via the particle bombardment technique that results in direct introduction of genes into the plant genome.

In accordance with APHIS procedures for implementing the National Environmental Policy Act, as amended (NEPA) (7 CFR Part 372), EAs were not prepared before granting permission for individual field trials because these soybean sublines met the eligibility criteria under the notification procedure and the trials met the performance

standards (7 CFR Part 340.3). This EA addresses issues that are of relevance to the unconfined planting of soybean sublines G94-1, G94-19 and G168, and APHIS concludes the following:

1. The sublines exhibit no plant pathogenic properties. Although DNA from pathogenic organisms were used in their development, these soybean plants are not infected by these organisms nor can these plants incite disease in other plants;

2. The sublines are no more likely to become a weed than improved quality soybean varieties which have been developed by traditional breeding techniques. Soybean is not a weed in the U.S., and there is no reason to believe that the introduced genes would enable soybean to become a weed pest;

3. Multiple factors ensure that gene introgression from the subject soybean sublines into wild plants in the United States and its territories is extremely unlikely. Even in other regions, potential gene introgression from these soybeans into wild relatives is not likely to increase the weediness potential of any resulting progeny nor adversely effect genetic diversity of related plants any more than would introgression from traditional soybean varieties;

4. The sublines will not have a significant adverse impact on organisms beneficial to plants or agriculture, or other nontarget organisms, and will not affect threatened or endangered species; and

5. The sublines should not cause damage to raw or processed agricultural commodities.

Therefore, after a review of the available evidence, including that provided by DuPont in its petition as well as other scientific data, and information relating to potential plant pest risk and related environmental impacts of these soybean lines, APHIS believes that soybean sublines G94-1, G94-19, and G-168 will be just as safe to grow as traditionally-bred soybean varieties not subject to regulation under 7 CFR Part 340. APHIS concludes that there will be no significant impact on the human environment if soybean sublines G94-1, G94-19, and G-168 and their progeny are no longer considered regulated articles under 7 CFR Part 340. The determination document is in Appendix A.

II. BACKGROUND

Development of High Oleic Acid Transgenic Soybean Sublines. DuPont has submitted a "Petition for Determination of Non-regulated Status" to the USDA, requesting a determination from APHIS that soybean sublines G94-1, G94-19, and G-168, and any progeny derived from crosses between these lines and other

nonregulated soybean varieties, no longer be considered regulated articles under 7 CFR Part 340.

The relative composition of saturated and unsaturated fatty acids in seeds is a major factor in determining the quality of the seeds' edible oils. Plant derived oils that are high in monounsaturated fatty acids (i.e. 18:1) and low in polyunsaturated fatty acids have improved nutritional characteristics and increased stability (Yadav, 1995). The soybean sublines G94-1, G94-19 and G168 have been developed by DuPont to improve the quality of oil derived from soybean seed by increasing their oleic acid content. The subject sublines are homozygous for a GmFad2-1 cDNA isolated from *Glycine max* in the sense orientation under the control of a seed-specific promoter. The introduction of *GmFad2-1* causes a coordinate silencing, or sense suppression, of itself and the endogenous *GmFad2-1* gene resulting in a soybean whose oil has an oleic acid content that exceeds 80%. Conventional soybeans have an oleic acid content of 24%. The *GmFad2-1* gene encodes for a seed-specific delta-12 desaturase that is involved in the synthesis of polyunsaturated fatty acids in developing seeds. In developing soybeans, a second double bond is added to oleic acid in the delta-12 position by the *GmFad2-1* gene. A second, constitutive Fad2 gene (*GmFad2-2*) is expressed in all tissues of the soybean plant. Suppression of the *GmFad2-1* gene in developing soybeans prevents the addition of a second double bond to oleic acid, resulting in greatly increased oleic acid content only in the seed.

Two plasmids were introduced into meristems of an elite soybean line to obtain the original transformant. One plasmid contained the *GmFad2-1* sense cDNA fused to a seed specific promoter from the soybean beta-conglycinin gene and a terminator from the phaseolin gene from *Phaseolus vulgaris*. This plasmid also contained the beta-glucuronidase (GUS) reporter gene (*uidA*) from *E. coli* with a 35S CaMV promoter and 3'NOS terminator from *Agrobacterium*. The second plasmid contained the *dapA* gene from the bacterium *Corynebacterium glutanicum* which encodes dihydrodipicolinic acid synthase. Expression of *dapA* can lead to an increase in free lysine content of the seed (Falco et al., 1995). Both constructs contained the ampicillin resistance gene, beta-lactamase (*bla*) from *Escherichia coli* with a bacterial promoter, which was used for selection when the construct was in *E. coli* cells.

The original transformed line contained inserts at two loci. At one locus (locus A) the GmFad2-1 construct was causing a suppression of the endogenous *GmFad2-1* gene, resulting in an oleic acid content of greater than 80%. At locus B, GmFad2-1 was over expressing, resulting in a decrease of oleic acid content to approximately 4%. The *dapA* gene, as well as a copy of the GmFad2-1 construct, were integrated at locus B. The *GmFad2-1* gene present at locus B was overexpressing, resulting in an undesired decrease in oleic acid. In subsequent generations, segregation led to the isolation of isogenic lines homozygous for locus A, and lacking locus B, which consistently produced soybean seeds with an oleic acid content of above 80% of the

total fatty acids. Tests indicated that the beta-lactamase gene with a bacterial promoter was not expressed. The GUS gene at locus A is also silent, and therefore no beta-glucuronidase is present in high oleic acid transgenic soybeans. Since neither of the marker genes are expressed, and the *dapA* gene was lost due to segregation in the subject soybean sublines, they are not expected to contribute to the phenotypic characteristics of these plants. All the genes were introduced into the parental soybean line (Asgrow A2396) via the particle bombardment technique that results in direct introduction of genes into the plant genome.

Other indirect effects on seed fatty acid content were also observed in the subject soybean lines, including a very low abundance of linoleic acid, and significantly lower linolenic and palmitic acid contents when compared with the parent line. Trace amounts ($<1\%$) of a linoleic acid isomer were also detected. Pleiotropic effects on seed storage protein composition have also been noted in these soybean sublines. In sublines G94-1, G94-19 and G168 the concentration of beta-conglycinin α and α' subunits has been reduced and replaced with glycinin subunits. This was a result of silencing of the α and α' subunit genes mediated by the beta-conglycinin α' promoter sequence used in the GmFad 2-1 vector. It is anticipated that increasing the content of glycinin (11S) subunits and decreasing the content of beta-conglycinin (7S) subunits will improve the functionality of soy proteins in various foods (Kitamura, 1995).

DuPont submitted its petition after numerous field tests of soybean sublines G94-1, G94-19 and G168 in the United States and Puerto Rico. These field tests have been carried out at approximately 25 sites under APHIS notifications 95-088-08N, 95-107-08N, 95-257-10N, 96-071-18n and 96-115-02N. A field test was also carried out in Chile in the winter of 1995-96. Field trial reports from these tests demonstrate that the transformed sublines had no deleterious effects on plants, did not exhibit weedy characteristics, and had no effect on nontarget organisms or the general environment. This EA examines potential environmental impacts from the unrestricted introduction of the subject sublines. DuPont based its petition, in part, on the data gathered from the field trials that demonstrated no deleterious effects on plants, nontarget organisms, or the environment as a result of these field releases. All field trials were performed under conditions of physical and reproductive confinement. Further discussions of the biology of soybean, as well as of the genetic components of these sublines, are found in the determination document (Appendix A). Because this information is included in Appendix A, it will not be described in detail in the body of this document.

APHIS Regulatory Authority. APHIS regulations under 7 CFR Part 340, which were promulgated pursuant to authority granted by the Federal Plant Pest Act, (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act, (7 U.S.C. 151-164a, 166-167) as amended, regulate the introduction (importation, interstate movement, or

release into the environment) of certain genetically engineered organisms and products. A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation and is also a plant pest, or if there is reason to believe that it is a plant pest. The soybean sublines described in the DuPont petition have been considered to be regulated articles because some noncoding DNA regulatory sequences were derived from the plant pathogens *Agrobacterium tumefaciens* and cauliflower mosaic virus (CaMV).

Section 340.6 of the regulations, entitled "Petition Process for Determination of Nonregulated Status," provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If APHIS determines that the regulated article is unlikely to pose a greater plant pest risk than the unmodified organism from which it is derived, the Agency can grant the petition in whole or in part. Therefore, APHIS permits or notifications would no longer be required for field testing, importation, or interstate movement of that article or its progeny. Normal agronomic practices with these sublines, e.g., cultivation, propagation, movement, and cross-breeding could also be conducted without further APHIS approval.

Food and Drug Administration (FDA) Regulatory Authority. These soybean sublines are also subject to regulation by the Food and Drug Administration (FDA). FDA's 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. Consistent with this 1992 policy, FDA expects developers to consult with the agency on safety and regulatory questions. FDA requests that firms provide a summary of their food (including animal feed) safety and nutritional assessment to the agency and discuss their results with agency scientists prior to commercial distribution. The applicant has stated that they completed consultations with the FDA in early 1997.

III. PURPOSE AND NEED

APHIS has prepared this EA before making a determination on the status of sublines G94-1, G94-19 and G168 as regulated articles under APHIS regulations. The developer of the subjuct soybean sublines, DuPont Agricultural Products, submitted a petition to APHIS requesting that APHIS make a determination that soybean sublines G94-1, G94-19 and G168 no longer be considered as regulated articles under 7 CFR Part 340.

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

IV. ALTERNATIVES

A. No Action.

Under the Federal "no action" alternative, APHIS would not come to a determination that soybean sublines G94-1, G94-19 and G168 are no longer regulated articles under the regulations at 7 CFR Part 340. Permits or acknowledgment of notifications from APHIS would still be required for introductions of these soybean sublines. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from uncontained cultivation of soybean sublines G94-1, G94-19 and G168.

B. Determination that soybean sublines G94-1, G94-19 and G168 are no longer regulated articles.

Under this alternative, soybean sublines G94-1, G94-19 and G168 would no longer be regulated articles under the regulations at 7 CFR Part 340. Permits or acknowledgment of notifications from APHIS would no longer be required for introductions of these soybean sublines or their progeny. A basis for this determination would include a "Finding of No Significant Impact" under the National Environmental Policy Act of 1969, as amended (42 USC 4321 *et seq.*) and the pursuant implementing regulations (40 CFR 1500-1508; 7 CFR Part 1b; 7 CFR Part 372).

V. POTENTIAL ENVIRONMENTAL IMPACTS

This EA addresses potential environmental impacts from a determination that soybean sublines G94-1, G94-19 and G168 should no longer be considered to be regulated articles under APHIS regulations at 7 CFR Part 340. This EA considers the genotypic and phenotypic characteristics of soybean sublines G94-1, G94-19 and G168, and the potential environmental impacts that might be associated with the unconfined cultivation of these soybean sublines, i.e., cultivation without intentional physical and reproductive confinement from other sexually compatible plants.

Additional technical information is included in the determination document appended to this EA, and incorporated by reference. This includes detailed discussions of the biology of soybean, the genetic components used in the construction of soybean

sublines G94-1, G94-19 and G168, and the analyses that lead APHIS to conclude that these soybean sublines have no potential to pose a plant pest risk.

A. Potential impacts based on increased weediness of the subject soybean sublines relative to traditionally bred soybeans

Almost all definitions of weediness stress as core attributes the undesirable nature of weeds from the point of view of humans; from this core, individual definitions differ in approach and emphasis (Baker, 1965; de Wet and Harlan, 1975; Muenscher, 1980). In further analysis of weediness, Baker (1965) listed 12 common weed attributes, almost all pertaining to sexual and asexual reproduction, which can be used as an imperfect guide to the likelihood that a plant will behave as a weed. Keeler (1989) and Tiedje *et al.* (1989) have adapted and analyzed Baker's list to develop admittedly imperfect guides to the weediness potential of transgenic plants; both authors emphasize the importance of looking at the parent plant and the nature of the specific genetic changes.

The parent plant in this petition, *Glycine max*, does not show any especially weedy characteristics. The genus *Glycine* also seems to be essentially devoid of such characteristics and shows no particular weedy (aggressive colonization) tendencies (Hermann, 1962; Lackey, 1981; personal communication, Lackey, 1993; Skvortzov, 1927). The standard texts and lists of weeds give no indication that the cultivated soybean,
G. max, is regarded as a weed anywhere (Holm et al., 1979; Muenscher, 1980; Reed, 1970; Weed Science Society of America, 1989). Only the nearest wild relative of cultivated soybean, *G. soja*, is listed as a common weed in Japan by Holm et al. (1979). However, texts on weeds found in Japan place it neither among the harmful weeds on cultivated lands (Kasahara, 1982), nor among the weeds of pastures and meadows (Nemoto, 1982). In addition, *G. gracilis*, known from Northeast China and described as a weedy form (Lackey, 1981) somewhat intermediate between *G. max* and *G. soja* (Skvortzov, 1927), is not listed in any texts or lists of weeds.

The introduced high oleic acid production trait is unlikely to cause or increase weediness of soybean sublines G94-1, G94-19 and G168. To increase weediness of the soybean plant there would have to be selection pressure (Tiedje et al., 1989; Office of Technology Assessment, 1988). No other variation seen in the subject sublines is indicative of increased weediness. DuPont data, included as part of the administrative record for the petition, show no significant differences between the mean seedling emergence rate for soybean sublines G94-1, G94-19 and G168 and the parental variety. In addition, DuPont field data reports showed no volunteers from seed, regrowth from stubble, or increase in seed dormancy.

B. Potential impacts from outcrossing of the subject soybean sublines to wild relatives

There are no relatives of cultivated soybean in the continental United States. However, some members of the wild perennial species of subgenus *Glycine* may be found in United States territories in the Pacific (Hermann, 1962; Hymowitz and Singh, 1987; Newell and Hymowitz, 1978). The subgenus *Glycine* consists of wild perennial species. Soybeans are almost exclusively self-pollinating plants. Inter-subgeneric hybrids between *G. max* and *Glycine* species have been obtained only through *in vitro* seed culture (reviewed by Hymowitz et al., 1992, and Hymowitz and Singh, 1987). Hybrids from such crosses have generally been sterile, and further progeny have only been obtained with extreme difficulty. The formation of hybrids between soybean sublines G94-1, G94-19 and G168 and *Glycine* species in nature is, therefore, highly unlikely.

Crosses between the annuals *G. max* and *G. soja* in the subgenus *Soja* can be made easily. The latter species is found in China, Korea, Japan, Taiwan, and the former USSR. However, even if both species are found growing together in any United States territory, flower development in cultivated soybeans leads to a high percentage of self-fertilization (Carlson and Lersten, 1987; McGregor, 1976), and no competitive advantage would be conferred on any hybrid progeny in the absence of sustained selection pressure.

Even if wild *Glycine* populations were near sites of commercial soybean production, it is highly unlikely that pollen from soybean sublines G94-1, G94-19 and G168 would fertilize the wild relatives because soybeans are: (1) not wind-pollinated; and (2) almost completely self-pollinated. Certified Seed Regulations (7 CFR 201.76) recognize this low probability of cross-pollination in the safeguards set up for Foundation, Registered, and Certified seed. For Foundation Seed, the most stringent category in the Certified Seed Regulations, soybeans are permitted to be grown close to the nearest contaminating source (i.e. other soybean cultivars), as long as the distance is adequate to prevent mechanical mixing. Even if cross-pollination occurred, there would be no significant impacts because any potential effect of the high oleic acid content trait would not alter the weediness potential of the wild soybean.

C. Potential impact on nontarget organisms including beneficial organisms such as bees and earthworms, and threatened or endangered organisms.

Consistent with its statutory authority and requirements under NEPA, APHIS evaluated the potential for the subject soybean sublines and plant products derived from these sublines to have damaging or toxic effects directly or indirectly on nontarget organisms. This includes those that are recognized as beneficial to

agriculture and those that are recognized as threatened or endangered in the United States.

There is no reason to believe that deleterious effects or significant impacts on nontarget organisms, including beneficial organisms, would result from the cultivation of soybean sublines G94-1, G94-19 and G168. The enzyme and genetic material that confers high oleic acid production in the transgenic sublines is normally present in soybeans and is not known to have any toxic properties. Field observations of soybean sublines G94-1, G94-19 and G168 revealed no negative effects on nontarget organisms, suggesting that the relatively higher levels of the oleic acid in the tissues of these sublines are not toxic to organisms. The lack of known toxicity of oleic acid suggests no potential for deleterious effects on beneficial organisms such as bees and earthworms.

The indirect metabolic alterations that occur in the subject soybean sublines, including the production of trace amounts (<1%) of a linoleic acid isomer, and the reduction in seed storage protein beta-conglycinin α and α' subunits and increase in glycinin subunits should have no negative impact on nontarget organisms. The linoleic acid isomer found in the subject soybean sublines is prevalent in the fatty acids of butterfat, beef and mutton tallow, partially hydrogenated vegetable oils, human milk and mango pulp. Antinutritional factors present in traditionally bred soybeans include trypsin inhibitors, phytic acid and the oligosaccharides raffinose and stachyose. In sublines G94-1, G94-19 and G168, the levels of all of these components were similar to those found in traditionally bred soybeans.

APHIS has not identified any other potential mechanisms for deleterious effects on beneficial organisms. In addition, there is no reason to believe that the presence of soybean sublines G94-1, G94-19 and G168 would have an effect on any threatened or endangered species in the United States.

D. Potential Impact on Processed Agricultural Commodities.

Consistent with its statutory authority which defines plant pests as those organisms which cause direct or indirect damage to plants and plant products, APHIS evaluated whether the soybean sublines G94-1, G94-19 and G168 might indirectly harm plant products such as some agricultural commodities. Analysis of the components and processing characteristics of these soybean sublines reveal no differences in any component that could have an indirect plant pest effect on any processed plant commodity.

E. Consideration Of Potential Environmental Impacts Associated With The Cultivation Of High Oleic Acid Soybean Sublines Outside The United States

In accordance with Executive Order 12114, January 4, 1979, entitled "Environmental effects abroad of major federal actions," APHIS has also considered potential environmental impacts associated with the cultivation of soybean sublines G94-1, G94-19 and G168 outside the United States and its territories.

Our analysis of the biology of soybean leads to the conclusion that the cultivation of soybean sublines G94-1, G94-19 and G168 anywhere in the world will not have an adverse impact on the environment. The subject soybean sublines show no significant differences from its parent line, in all avenues investigated, except for its production of high levels of oleic acid.

Several factors contribute to the conclusion that there should be no environmental impacts in foreign countries from the cultivation of these soybean sublines or their progeny.

Any international trade in the subject soybean sublines subject to this determination would be fully subject to national and regional phytosanitary standards promulgated under the International Plant Protection Convention (IPPC) of the Food Agricultural Organization. IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (98 countries as of December 1992). The treaty, administered by a Secretariat housed with the Food and Agriculture Organization in Rome, came into effect on April 3, 1952, and establishes standards to facilitate the safe movement of plant materials across international boundaries. Plant biotechnology products are fully subject to national legislation and regulations or regional standards and guidelines promulgated under the IPPC. The IPPC also has led to the creation of Regional Plant Protection Organizations (RPPOs) to facilitate regional harmonization of phytosanitary standards.

Issues that may relate to commercialization of agricultural commodities produced through biotechnology are being addressed in international fora. APHIS has played a leading role in working toward harmonization of biosafety and biotechnology guidelines and regulations included within the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the United States. NAPPO's Biotechnology Panel advises NAPPO on biotechnology issues as they relate to plant protection.

APHIS participates regularly in biotechnology policy discussions at fora sponsored by the European Union and the Organization for Economic Cooperation and

Development. In addition, APHIS periodically holds discussions on biotechnology regulatory issues with other countries. APHIS also assists in the development of biotechnology guidelines and regulations and has interacted with governments around the world in this matter, including those in regions where soybean originated or is cultivated in significant quantities. APHIS has participated in numerous conferences intended to enhance international cooperation on safety in biotechnology and has sponsored several workshops on safeguards for planned introductions of transgenic crops (crucifers, maize, wheat, potatoes, rice, tomatoes, and sorghum), most of which have included consideration of international biosafety issues.

In addition to the assurance provided by the analysis leading APHIS to a finding of no significant impact for the introduction of this soybean variety, it should be noted that all national and international regulatory authorities and phytosanitary regimes that apply to introductions of new soybean cultivars internationally apply equally to those covered by this determination.

VI. CONCLUSIONS

APHIS has evaluated the information provided by DuPont in its petition as well as other scientific data, and information relating to potential plant pest risk and related environmental impacts of the soybean sublines G94-1, G94-19 and G168. After careful analysis of the available information, APHIS has identified no significant impact to the environment from a determination that the subject sublines should no longer be considered regulated articles under the regulations at 7 CFR Part 340. Thus, the proper alternative is to approve the petition so that soybean sublines G94-1, G94-19 and G168 would have a nonregulated status when grown in the United States and its territories. This conclusion is based on factors discussed herein and in the determination included as Appendix A, as well as the following factors:

1. Neither the desaturase gene, its product, the associated marker genes, nor the regulatory sequences confer on the subject soybean sublines or their progeny any plant pest characteristic. Although DNA from pathogenic organisms were used in their development, these soybean plants are not infected by these organisms nor can these plants incite disease in other plants.

2. In nature, chromosomal genetic material from plants can only be transferred to another sexually compatible flowering plant by cross-pollination. There are no other sexually compatible species of soybeans in nature in the United States and its territories.

3. The gene that confers overproduction of oleic acid will not provide sublines G94-1, G94-19 and G168 or their progeny with any measurable selective advantage over nontransformed soybean plants in their ability to disseminate or to become established

in the environment. There is no reason to believe that these soybean sublines exhibit any increased weediness relative to that of traditional varieties or the unmodified parental lines.

4. There is no reason to believe that the use of the subject soybean sublines or their progeny in agriculture will have a significant impact on any beneficial organisms in the environment or on any threatened or endangered species.

5. The subject soybean sublines will not cause damage to raw or processed agricultural commodities.

Therefore, APHIS concludes that soybean sublines G94-1, G94-19, and G-168 derived from transformation event 260-05 will be just as safe to grow as nontransgenic soybean that are not subject to regulation under 7 CFR Part 340, and that there should be no significant impact on the human environment if the soybean sublines G94-1, G94-19, and G-168 were no longer considered to be regulated articles under its regulations (7 CFR Part 340).

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X. APPENDIX A: Determination of 97-008-01p

APPENDIX A

DETERMINATION OF NONREGULATED STATUS FOR HIGH OLEIC ACID TRANSGENIC SOYBEAN SUBLINES G94-1, G94-19, and G-168 DERIVED FROM TRANSFORMATION EVENT 260-05

Petitioner: DuPont Agricultural Products
Petition Number: 97-008-01p

United States Department of Agriculture

Animal and Plant Health Inspection Service
Biotechnology and Scientific Services
Riverdale, Maryland

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Appendix A

I. SUMMARY

APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA), (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act (PQA), (7 U.S.C. 151-164a, 166-167) as amended, regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. An organism is no longer subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. Section 340.6 of the regulations, entitled, "Petition for Determination of Nonregulated Status," provides that a person may petition the agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated.

On January 8, 1997, the Animal and Plant Health Inspection Service (APHIS) received a petition from the DuPont Agricultural Products (DuPont) requesting a determination that high oleic acid soybean sublines G94-1, G94-19, and G-168 (hereafter referred to as sublines G94-1, G94-19, and G-168) derived from transformation event 260-05 do not pose a plant pest risk and therefore, should no longer be considered regulated articles. On February 28, 1997, APHIS announced the receipt of the DuPont petition in the *Federal Register* (62 FR 9155-9156, Docket No. 96-098-1), seeking comments from the public. The public comment period ended on April 29, 1997. In the *Federal Register* notice, APHIS indicated its role in the process of reviewing the DuPont petition and the role of the Food and Drug Administration (FDA).

The Animal and Plant Health Inspection Service (APHIS), on reviewing the DuPont petition 97-008-01p, has concluded that the high oleic acid soybean sublines G94-1, G94-19, and G-168 do not present any plant pest risk, and are, therefore, determined to no longer be considered regulated articles under its regulations at 7 CFR 340. As such, the applicant is no longer required to obtain a permit or notify APHIS for the unrestricted introduction and movement of soybean sublines G94-1, G94-19, and G-168 into the environment within the continental United States and its territories. Importation of soybean sublines G94-1, G94-19, and G-168 still will remain regulated according to Foreign Quarantine Notice regulations at 7 CFR 319.

The soybean sublines G94-1, G94-19 and G168 have been developed in an effort to improve the quality of oil derived from soybean seed by increasing their oleic acid content. The sublines G94-1, G94-19 and G168 are homozygous for the coding region of the *GmFad2-1* gene isolated from *Glycine max* under the control of a seed-specific promoter. The introduction of *GmFad2-1* causes a coordinate silencing, or sense suppression, of itself and the endogenous *GmFad2-1* gene resulting in a soybean whose oil has an oleic acid content that exceeds 80%. Conventional soybeans have an oleic acid content of 24%. The *GmFad2-1* gene encodes for a seed-specific delta-12 desaturase that is involved in the synthesis of polyunsaturated fatty acids in developing seeds. The subject soybean sublines also contain the reporter gene (*uidA*) beta-glucuronidase (GUS) from *Escherichia coli* and the ampicillin resistance gene (*bla*) from *E. coli* that encodes for beta-lactamase. Tests indicated that the beta-lactamase gene was not expressed, as expected since it is under the control of a bacterial promoter. The GUS gene is also silent, and therefore

no β-glucuronidase is present in soybean sublines G94-1, G94-19, and G-168. These introduced genes also have accompanying DNA regulatory sequences that modulate their expression. All the genes were introduced into the parental soybean line (Asgrow A2396) via the particle bombardment technique that results in direct introduction of genes into the plant genome. The soybean sublines G94-1, G94-19, and G-168 are considered regulated articles because they contain regulatory sequences from the plant pests *Agrobacterium tumefaciens* and cauliflower mosaic virus (CaMV).

The original transformant (event 260-05) contained a second plasmid that included a gene (*dapA*) from *Corynebacterium glutanicum* encoding the enzyme dihydrodipicolinic acid synthase, which was intended to increase lysine content in the seed. This plasmid was integrated along with another copy of the GmFad2-1 construct at a site in the genome (Locus B) distinct from the locus responsible for the increased oleic acid content. At Locus B, GmFad2-1 was over expressing, resulting in an undesired decrease of oleic acid content to approximately 4%. In subsequent generations, segregation led to the isolation of isogenic sublines lacking locus B and the *dapA* gene, which consistently produced soybean seeds with an oleic acid content of above 80% of the total fatty acids.

APHIS has determined that soybean sublines G94-1, G94-19, and G-168 do not present a plant pest risk and will no longer be considered regulated articles, under APHIS regulations at 7 CFR Part 340. The Agency decision is based on an analysis of data provided to APHIS by DuPont as well as other scientific data relating to the potential plant pest risk of soybean sublines G94-1, G94-19, and G-168. From our review, we have determined that these sublines: (1) exhibit no plant pathogenic properties; (2) are no more likely to become a weed than their non-engineered parental varieties; (3) are unlikely to increase the weediness potential for any other cultivated plant or native wild species with which they can interbreed; (4) will not cause damage to raw or processed agricultural commodities, and (5) are unlikely to harm organisms, such as bees and earthworms, that are beneficial to agriculture, or threatened and endangered species.

The potential environmental impacts associated with this determination have been examined in accordance with regulations and guidelines implementing the National Environmental Policy Act of 1969, as amended (42 USC 4321 *et.seq.)* and pursuant implementing regulations (40 CFR 1500-1508, 7 CFR Part 1b; 7 CFR Part 372). An Environmental Assessment (EA) was prepared and a Finding of no Significant Impact (FONSI) was reached by APHIS for the determination that soybean sublines G94-1, G94-19, and G-168 derived from transformation event 260-05 are no longer regulated articles under its regulations at 7 CFR Part 340.

The body of this document consists of two parts: (1) background information which provides the regulatory framework under which APHIS has regulated the field testing, interstate movement, and importation of the subject sublines, as well as a summary of comments provided to APHIS on its proposed action; and (2) analysis of the key factors relevant to APHIS' decision that soybean sublines G94-1, G94-19, and G-168 do not present a plant pest risk.

II. BACKGROUND

A. APHIS Regulatory Authority

APHIS regulations at 7 CFR 340, which were promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA), (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act (PQA), (7 U.S.C. 151-164a, 166-167) as amended, regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. Under these regulations, a genetically engineered organism is deemed a regulated article if either 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 APHIS has reason to believe that the genetically engineered organism presents a plant pest risk. The FPPA gives the U.S. Department of Agriculture (USDA) the authority to regulate plant pests and other articles to prevent direct or indirect injury, disease, or damage to plants and plant products. In addition, the PQA provides an additional level of protection by enabling USDA to regulate the importation and movement of nursery stock and other plants that may harbor injurious pests.

Before the introduction of a regulated article, a person is required under Section 340.0 of the regulations to either (1) notify APHIS in accordance with Section 340.3 or (2) obtain a permit in accordance with Section 340.4. Introduction under notification (Section 340.3) requires that the introduction meets specified eligibility criteria and performance standards. The eligibility criteria impose limitations on the types of genetic modifications that qualify for notification, and the performance standards impose limitations on how the introduction may be conducted. Under Section 340.4, a permit is granted for a field trial when APHIS has determined that the conduct of the field trial, under the conditions specified by the applicant or stipulated by APHIS, does not pose a plant pest risk.

An organism is not subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated to present no plant pest risk. Section 340.6 of the regulations, entitled "Petition for Determination of Nonregulated Status," provides that a person may petition the agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If the agency determines that the regulated article does not present a risk of introduction or dissemination of a plant pest, the petition will be granted, thereby allowing for unregulated introduction of the article in question. A petition may be granted in whole or in part.

Soybean sublines G94-1, G94-19, and G-168 have been considered "regulated articles" for field testing under Part 340.0 of the regulations because certain noncoding regulatory sequences were derived from the plant pathogens *Agrobacterium tumefaciens* and CaMV. APHIS believes it is prudent to provide assurance prior to commercialization that organisms, such as these soybean sublines, that are developed in part from plant pest sequences, do not present any potential plant pest risk. Such assurance may aid the entry of new plant varieties into commerce or into

breeding and development programs. The decision by APHIS that soybean sublines G94-1, G94-19, and G-168 are no longer regulated articles is based in part on evidence provided by DuPont concerning the biological properties of the these sublines and their similarity to other soybean varieties grown using standard agricultural practices for commercial sale or private use.

The fact that APHIS regulates genetically engineered organisms having plant pest components does not carry with it the presumption that the presence of part of a plant pest makes a whole plant pest or that plants or genes are pathogenic (McCammon and Medley, 1990). The regulations, instead, are based on the premise that when plants are developed using biological vectors from pathogenic sources, transforming material from pathogenic sources, or pathogens as vector agents, that they should be evaluated to assure that there is not a plant pest risk. For each field test, APHIS performs a review that allows a verification of the biology and procedures used, assesses the degree of uncertainty and familiarity and allows the identification of any predictable hazards. The overall aim of APHIS regulations in the Code of Federal Regulations at 7 CFR Part 340 is to allow for the safe testing of genetically engineered organisms under an appropriate level of oversight and to enable any issues of potential or hypothetical risks to be addressed early enough in the development of the new organisms for the safe utilization of the technology in agriculture.

A certification that a genetically engineered organism does not present a plant pest risk means that there is reasonable certainty that the organisms cannot directly or indirectly cause disease, injury, or damage either when grown in the field, or when stored, sold, or processed. This approach is considerably broader than a narrow definition of plant pest risk arising from microbial or animal pathogens, including insect pests. Other traits, such as increased weediness, and harmful effects on beneficial organisms, such as earthworms and bees, clearly come under what is meant by direct or indirect plant pest risk. In APHIS regulations at 7 CFR Part 340, a "plant pest" is defined as: "Any living stage (including active and dormant forms) of insects, mites, nematodes, slugs, snails, protozoa, or other invertebrate animals, bacteria, fungi, other parasitic plants or reproductive parts thereof; viruses, or any organisms similar to or allied with any of the foregoing; or any infectious agents or substances, which can directly or indirectly injure or cause disease or damage in or to any plants or parts thereof, or any processed, manufactured, or other products of plants."

A determination that an organism does not present a plant pest risk can be made under this definition, especially when there is evidence that the plants under consideration:(1) exhibit no plant pathogenic properties; (2) are no more likely to become a weed than their non-engineered parental varieties; (3) are unlikely to increase the weediness potential for any other cultivated plant or native wild species with which the organism can interbreed; (4) do not cause damage to processed agricultural commodities; and (5) are unlikely to harm other organisms, such as bees, that are beneficial to agriculture, or threatened and endangered species. Evidence presented by DuPont bears on all of these topics. In addition, because the DuPont petition seeks a determination regarding soybean sublines G94-1, G94-19, and G-168, it should be established

that there is a reasonable certainty that any new soybean varieties bred with these sublines will exhibit plant pest properties not substantially different from any observed for soybeans in traditional breeding programs or as seen in the development of the subject soybean sublines.

B. FDA Regulatory Authority

The Federal Food, Drug and Cosmetic Act (FFDCA) (21 U.S.C. 301 *et.seq.*) provides FDA with authority to ensure the safety and wholesomeness of all food(s), other than meat and poultry. The FDA policy statement concerning the regulation of foods derived from new plant varieties, including genetically engineered plants, was published in the Federal Register on May 29, 1992 (57 FR 22984-23005). Regulatory oversight for the safety of any food or feed products derived from transgenic soybean sublines G94-1, G94-19, and G-168 is under the jurisdiction of the FDA.

III. PUBLIC COMMENTS

APHIS received no comments on DuPont's petition during the designated 60-day comment period that ended April 29, 1997.

IV. PROPERTIES AND ANALYSIS OF HIGH OLEIC ACID SOYBEAN SUBLINES

A brief discussion of the biology of soybean and its cultivation practices follow in the next section to help inform the subsequent analysis.

A. Biology and Cultivation of Soybean:

Soybean (*Glycine max*) is primarily grown for edible vegetable oil and high protein food supplement for livestock. Other fractions and derivatives of the seed have substantial economic importance in a wide range of industrial, food, pharmaceutical, and agricultural products (Smith and Huyser, 1987). Soybean is the third largest crop grown in the United States and is grown in 29 states. The principal soybean producing States are Illinois, Iowa, Missouri, Minnesota, Indiana, and Ohio (Jewell, 1988). The United States, Brazil, China, and Argentina account for over 90 percent of world soybean production (Jewell, 1988).

The cultivated soybean plant is a branched, frost-sensitive (Johnson, 1987) annual that is grown in the United States as a monoculture of row crops for sale to off-farm processors. Generally each field is planted as pure sublines, although blends of two or more sublines are sometimes planted (Johnson, 1987). Clean tillage has been the traditional method of field preparation, but recently no tillage and reduced tillage systems have become more common. Irrigation is not usually practiced (VanDoren and Reicosky, 1987). A complex and sophisticated system of cultivars, agricultural implements, agricultural chemicals, and processing techniques have been developed for the crop.

The genus *Glycine* is divided into two subgenera, *Glycine* and *Soja*. The first consists of twelve wild perennial species (Hymowitz et al., 1992) that are primarily distributed in Australia, South Pacific Islands, Philippines, and Taiwan (Newell and Hymowitz, 1978). The subgenus *Soja* consists of three annual species from Asia, *G. max, G. soja,* and *G. gracilis*. The first species is the cultivated soybean, the second species is the wild form of the soybean, and the third species is referred to as the "weedy" form of the soybean (Lackey, 1981).

Cultivated soybean is essentially self-pollinated (Carlton and Lersten, 1987; McGregor, 1976). The anthers mature in the bud and shed their pollen directly onto the stigma of the same flower, thus ensuring a high degree of self-pollination. Cross-pollination is generally very low and various studies have shown it to be from 0.03 to 3.62% (McGregor, 1976, Woodworth, 1992). Caviness (1970) showed that honey bees are responsible for the occasional cross-pollination and that thrips are ineffective pollinators. Soybean plants are virtually pure breeding homozygous sublines, although manual cross-pollination is practiced routinely in breeding programs (Fehr, 1987). Certified Seed Regulations (7CFR Part 201.67-201.78) recognize the unlikelihood of cross-pollination in the standards they set for the production of Foundation, Registered, and Certified seed. For Foundation seed, the most stringent category in the Certified Seed Regulations, soybeans are permitted to be grown zero distance from the nearest contaminating source (i.e., other soybean cultivars), as long as the distance is adequate to prevent mechanical mixing.

Cultivated soybean is sexually compatible only with members of the genus *Glycine* (Hymowitz and Singh, 1987). Cultivated soybean is the only member of the genus *Glycine* that grows both in the United States and its territories and is sexually compatible with cultivated soybean, with the exception of specialized research collections maintained under scientific care and scrutiny.

Soybean plants are annuals and do not survive vegetatively in the cultivated fields of the United States from one growing season to the next (Hymowitz and Singh, 1987). Survival from one season to the next is by seed. Volunteers are seldom seen when cultivated soybean is grown in the United States. Since soybeans do not retain high germination rates and vigor for long periods, fresh, properly grown and handled seed is required for commercial varieties each growing season (TeKrony et al, 1987).

B. Plant Pest Risk Assessment and the Determination:

Based on information on the biology of soybean, data presented by DuPont and scientific data on other topics relevant to a discussion of plant pest risk, APHIS concluded the following regarding the properties of soybean sublines G94-1, G94-19 and G-168.

1. **Neither the introduced genes, their products, nor the added regulatory sequences controlling their expression presents a plant pest risk in the soybean sublines.**

The high oleic acid soybean sublines G94-1, G94-19 and G-168 were obtained by transforming

the elite soybean line A2396, by the method of particle bombardment, with two plasmids pBS43 and pML102 that together contain the *GmFad 2-1*, *dapA*, *uidA* and *bla* genes. The soybean sublines G94-1, G94-19 and G-168 were derived from transformation event 260-05 to express GmFad2-1 cDNA in the sense orientation under the control of a seed-specific promoter.

Plasmid pBS43 contains three gene expression cassettes, each with appropriate regulatory elements such as promoters and terminators. Plasmid pBS43 contains the *GmFad2-1* sense cDNA from *Glycine max* fused to a seed specific promoter sequence from the α'-subunit of soybean beta-conglycinin gene and a terminator from the 3' region of the phaseolin gene from *Phaseolus vulgaris*. The *GmFad2-1* gene encodes for a seed-specific delta-12 desaturase that is involved in the synthesis of polyunsaturated fatty acids in developing seeds. The seed specific expression of GmFad2-1 leads to a coordinate gene silencing of the sense GmFad2-1 cDNA and the endogenous GmFad2-1. This "sense suppression" effectively turns off the gene in question leading to greatly reduced levels of the delta-12 desaturase and increased oleic acid content only in the seed. A second Fad2 gene (*GmFad2-2*), which remains unaffected, is expressed in all soybean tissue and is responsible for the synthesis of polyunsaturated fatty acids for cell membranes. The pBS43 plasmid contains the beta-glucuronidase (GUS) reporter gene (*uidA*) from *E. coli* with a 35S CaMV constitutive promoter (Kay et al., 1987) and *nos* (nopaline synthase) 3'-terminator region from *Agrobacterium*. The beta-glucuronidase encoded by the *uidA* gene enables a colorimetric assay in the laboratory to identify plant tissues which contain and express the gene. Plasmid pBS43 also contains the ampicillin resistance gene, beta-lactamase (*bla*) from *Escherichia coli* with a bacterial promoter, which was used for selection when the construct was in *E. coli* cells. The *bla* gene does not possess the proper DNA sequences to promote its expression in plants. While the GUS and *bla* genes are present in soybean sublines G94-1, G94-19 and G-168, DuPont has provided evidence that these reporter/marker genes are not expressed in these sublines.

The genes that impact on lipid biosynthesis are from soybean and only increase the amount of oleic acid in the seed, a constituent which is present in the parent cultivar. These genes and the increased oleic acid do not present a plant pest risk.

Dupont's intention was to produce transgenic soybeans with increased lysine in their meal fraction and reduced polyunsaturates in their oil fraction. To increase seed lysine content, the original transformant (event 260-05) contained a second plasmid, pML102, composed of two gene expression cassettes. The first cassette encodes the beta-lactamase (*bla*) gene from *E. coli* for bacterial transformation selection on ampicillin, as described above for pBS43. The plasmid pML102 also contains a *Corynebacterium glutanicum* gene (*dapA*) encoding the enzyme dihydrodipicolinic acid synthase. Expression of *dapA* can lead to an increase in free lysine content in the seed (Falco et al., 1995). Expression of the high lysine phenotype is encoded by a seed specific gene expression cassette. The soybean Kunitz trypsin inhibitor 3 (KTi3) promoter allows high level seed expression of the *dapA* gene. No portions of the coding sequence of Kunitz trypsin inhibitor are present. A chloroplast transit peptide sequence from the small subunit of ribulose bisphosphate carboxylase of soybean was cloned onto the 5' end of the *dapA*

gene (Berry-Lowe et al. 1982). This fragment encodes a plant chloroplast transit peptide that directs the protein into the chloroplast where lysine biosynthesis is carried out. In pML102 the transit peptide sequence was cloned onto the *dapA* gene to make a translational fusion. The soybean KTi3 transcriptional terminator, derived from the region beyond the 3' end of the soybean KTi3 gene (Jofuku & Goldberg, 1989) was fused to the *dapA* gene to ensure appropriate termination of transcription.

The plasmids were introduced in the soybean tissue by particle gun method of transformation (Sanford, 1990), also referred to as the biolistic method. In this method plant tissues are bombarded with particles that are coated with DNA, with the result that particles are able to penetrate the cell wall and the cell membrane and deliver the DNA to the interior of the cell. Particles are typically tungsten or gold with a diameter of 0.2 to 4.0 microns. DNA introduced in this way generally has been shown to be incorporated into the nucleus (Christou et al., 1988; Hain et al. 1985).

DuPont has analyzed the physical structure of the integrated genetic material in soybean sublines G94-1, G94-19 and G-168 using Southern blot analysis. From the observed hybridization patterns it was concluded that the transformed plasmid, pBS43, integrated at two different loci in the soybean genome. At one locus (Locus A) the GmFad 2-1 construct silenced the endogenous GmFad 2-1 gene, resulting in a lack of detectable GmFad 2-1 mRNA, decreased desaturase activity, and an elevation of seed oleic acid content to about 85% (compared with about 20% in elite soybean varieties). At Locus A there were two copies of pBS43. On the DNA hybridization blot this was seen as two cosegregating bands. At Locus B, the GmFad 2-1 was over-expressed, decreasing the oleic acid content to about 4%. At locus B there was also a single copy of pBS43. The pML102 construct containing the *Corynebacterium dapA* gene was only integrated at locus B.

Since the *dapA* gene integrated at locus B was accompanied by an unwanted over-expression of GmFad2-1, it was necessary to select for segregants that lacked this locus, In subsequent generations, segregation led to the isolation of isogenic sublines (G94-1, G94-19 and G-168) homozygous for locus A, but lacking locus B, which consistently produced soybean seeds with an oleic acid content of above 80% of the total fatty acids.

DuPont has presented evidence in its petition that the *GmFad2-1* genes are stably integrated into the soybean genome of soybean sublines G94-1, G94-19 and G-168. As integrated pieces of plant chromosomes, introduced foreign DNA is subject to the same rules governing chromosomal rearrangements and gene stability as other plants. Southern blot analyses of the R1 through R4 generations of the subject soybean sublines clearly demonstrate that there are two copies of the *GmFad2-1* gene, and that they are transmitted to offspring in a stable Mendelian manner. Evidence was also presented that the *dapA* gene, as a part of locus B, had been lost through selective segregation and is not present in soybean sublines G94-1, G94-19 and G-168.

The introduction of the vector DNA does not present a plant pest risk in soybean sublines G94-1,

G94-19 and G-168. The vectors used to transfer the genes for high oleic acid production into the soybean chromosomes, pBS43 and pML102, are derivatives of *Escherichia coli* plasmid pUC19. They do not contain any Tumor-Inducing sequences of the (Ti) plasmid of *A. tumefaciens*. For maintenance and replication of the plasmid in *E. coli*, the GmFad 2-1 transcriptional unit described above was cloned into plasmid pGEM-9z(-) (Promega Biotech, Madison WI, USA). This is a basic ampicillin resistance plasmid derived from pBR322. This plasmid contains the complete β-lactamase (*bla*) gene (Sutcliffe, 1979) for bacterial selection on the antibiotic ampicillin. This prokaryotic gene allows selection of transformed *E. coli* during laboratory recombinant DNA steps and is not expressed in the transformed plants. It contains its own *E. coli* regulatory sequences. Besides genes impacting seed fatty acid biosynthesis, the subject soybean sublines have also been transformed with two selectable marker genes, neither of which has any pathogenic properties. The transgenic soybeans will likely be crushed for oil production and the resulting soybean cake may be used as animal feed. There has been some concern that the *bla* gene may be transmitted horizontally (nonsexually, for example through gene exchange in the food chain) to bacteria that cause diseases. Even if it was to occur, it would not create any novel gene combinations or mechanisms of antibiotic resistance that are not already widespread in nature. At a recent conference of fourteen scientists and international food policy experts (including representatives of the World Health Organization and the Food and Agricultural Organization) the participants concurred that the use of the ampicillin marker gene in corn constitutes an insignificant to near zero risk of causing ampicillin resistance complications in either animals or humans (Foundation for Nutritional Advancement, 1996).

There is no published evidence for the existence of any mechanism, other than sexual crossing of compatible *Glycine* species, by which these genetic sequences can be transferred to other organisms. Comparative analyses of numerous gene sequences from microorganisms and plants have never, to our knowledge, yielded any published evidence of strong inter-kingdom gene homologies that would be indicative of recent or frequent gene exchanges between plants and microorganisms, except for *Agrobacterium*-mediated gene transfers. Movement of genes from plants to microorganisms may have occurred over evolutionary time (Carlson and Chelm, 1986; Wakabayashi et al., 1986; Doolittle et al, 1990). A single report (Bryngelsson et al., 1988) has suggested that plant DNA can be taken up by a parasitic fungus, but no evidence has ever been forthcoming that such DNA uptake has resulted in the frequent transfer of a functional DNA sequence. Even if a rare plant-to-microbe gene transfer were to take place, there is no reason to believe that such a transfer of any of the sequences would pose any plant pest risk. We conclude that concerns regarding DNA transfer from soybean sublines G94-1, G94-19 and G-168 to microorganisms are, at best, highly putative and speculative.

Despite the presence of certain pathogen-derived sequences in the genome of soybean sublines G94-1, G94-19 and G-168, no crown gall or CaMV disease symptoms were observed by DuPont in any of these sublines during greenhouse or field studies. Furthermore, DuPont provides evidence that expression of the introduced gene does not result in disease symptoms or the synthesis of products toxic to other organisms. DuPont also has monitored field tests of soybean sublines G94-1, G94-19 and G-168 to verify that the severity of any disease or insect infestation

of the transgenic plants did not differ from that of the parental line. No difference in disease and insect susceptibility was observed at any field test site where the subject sublines were tested in the United States and Puerto Rico. Thus, the introduced regulatory sequences do not confer a plant pest risk.

C. High Oleic Acid Soybean Sublines Have No Significant Potential To Become Weeds.

APHIS evaluated whether soybean sublines G94-1, G94-19 and G-168 are any more likely to become weeds than nontransgenic control soybean lines. Baker (1965) developed a list of attributes most commonly found in many weeds. Most definitions of weediness stress the undesirable nature of weeds from the point of view of humans; individual definitions differ in approach and emphasis (Baker, 1965). Baker (1965) defines a plant as a weed if, in any specified geographical area, its populations grow entirely or predominantly in situations markedly disturbed by man (without, of course, being deliberately cultivated). He also described the ideal characteristics of weeds as including the following: discontinuous germination and long-lived seeds; rapid seedling growth; rapid growth to reproductive stage; long continuous seed production; self-compatibility, but not obligatory self-pollination or apomixis; if outcrossing, use of wind or an unspecialized pollinator; high seed number under favorable conditions; high germination rates, and seed production under a wide range of environmental conditions; high tolerance or plasticity of climatic and edaphic variation; special adaptations for dispersal; good competitiveness achieved through, for example, allelochemicals or choking growth; and, if perennial, then exhibiting vigorous vegetative reproduction, brittleness either at the lower nodes or of rhizomes or rootstocks, and having the ability to regenerate from severed rootstocks.

Soybean does not possess characteristics of plants that are notably successful weeds. It is an annual crop and is considered to be a highly domesticated, well-characterized crop plant that is not persistent in undisturbed environments without human intervention. The parental soybean line is not considered a weed, and introduction of the oil modification trait should not impart any new weedy characteristics. Since the *dapA* gene and selectable marker genes *uidA* and the *bla* gene introduced into the soybean sublines G94-1, G94-19 and G-168 are not expressed in the plant, they are not expected to contribute to the plant's phenotype, including the plant's ability to become a weed. The subject soybean sublines are likely to be grown mostly in areas that are currently under soybean cultivation, i.e., in typical growing regions for the crop.

DuPont has designed experiments and collected data from field trials that support the contention that the soybean sublines G94-1, G94-19 and G-168 have no potential to become successful weeds. Data provided in the petition indicate that the applicant has not observed any significant changes in the number of seeds produced, germination characteristics, final stand, over-wintering capability, or pathogen susceptibility.

D. High Oleic Acid Soybean Sublines Will Not Increase The Weediness Potential Of Any Other Plant With Which They Can Breed or Adversely Impact Biodiversity

The only wild species that cross with the cultivated soybean are members of the genus *Glycine*. Soybean is not reported to cross with any extra-generic relatives (Hymowitz and Singh, 1987). Some members of the wild perennial species of subgenus *Glycine* may be found in United States territories in the Pacific (Hermann, 1962; Hymowitz and Singh, 1987; Newell and Hymowitz, 1978); however, there are no known reports of successful natural hybridization between cultivated soybean and the wild perennial species. Hybridization is known only in *vitro* culture, i.e., under human intervention, and hence the probability of natural gene transfer is very low. Even when hybridization is achieved, the F1 plants obtained are generally sterile. Only the nearest wild relative of cultivated soybean, *G. soja*, is listed as a common weed in Japan by Holm et al (1979). However, texts on weeds found in Japan place it neither among the harmful weeds on cultivated lands (Kasahara, 1982), nor among the weeds of pastures and meadows (Nemoto, 1982). Although natural hybridization is known to occur between cultivated soybean and the wild, annual species *G. soja* (Kwon et al, 1972), the latter is not found in the United States or its territories. *G. soja* is found in China, Korea, Japan, Taiwan, and Russia. However, even if both species are found growing together in any United States territory, flower development in cultivated soybeans leads to a high percentage of self-fertilization (Carlson and Lersten, 1987; McGregor, 1976), and no competitive advantage would be conferred on any hybrid progeny in the absence of sustained selection pressure.

Cultivated soybeans are almost completely self-pollinated, with hybridization reported generally at less than 1%. Should movement of genetic material take place to any receptive plants, and high oleic acid production transferred, no competitive advantage would be conferred on the progeny. In agricultural areas such plants would be controlled by normal agronomic practices.

Because commercial soybeans are almost exclusively self-pollinating and the seed is sold commercially, crosses unaided by humans between the subject soybean sublines and another plant are highly unlikely. Therefore, there is very little likelihood that the soybean sublines G94-1, G94-19 and G-168 will negatively impact the biodiversity of other plant species in the United States or elsewhere. Although limited by high self-pollination rates, outcrossing of these high oleic acid soybean sublines to wild or weedy relatives (primarily *G. soja*) would be possible in China, Korea, Japan, Taiwan, and Russia. Our analysis of the biology of soybean and its relatives leads us to predict that the environmental impacts of cultivation of soybean sublines G94-1, G94-19 and G-168 anywhere in the world would be no different from such impacts attributable to similar varieties produced with traditional breeding techniques.

Even if an outcrossing event involving pollen from the subject soybean sublines did occur, there is no reason to believe that the progeny would be any more weedy than progeny from crosses resulting from pollination by other traditionally bred soybean varieties. The minor genotypic differences observed in soybean sublines G94-1, G94-19 and G-168 are not expected to confer a selective advantage that would increase their weediness potential relative to other

commercial soybean varieties, nor would they be expected to increase the weediness potential if introgressed into other sexually compatible plants.

We note also that any international traffic in soybean sublines G94-1, G94-19 and G-168 would be fully subject to national and regional phytosanitary standards promulgated under the International Plant Protection Convention (IPPC). The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (101 countries as of September 1994). The treaty, administered through the United Nations Food and Agriculture Organization, came into force on April 3, 1952. It establishes standards to facilitate the safe movement of plant materials across international boundaries.

Plant biotechnology products are fully subject to national legislation and regulations, or regional standards and guidelines promulgated under the IPPC. The vast majority of IPPC signatories have promulgated, and are now administering, such legislation or guidelines. Japan in particular has in place a regulatory process that would require a full evaluation of the transgenic soybeans before they could be introduced into their environment. Our decision in no way prejudices regulatory action in any country. The IPPC has also led to the creation of regional plant protection organizations such as the North American Plant Protection Organization (NAPPO) whose member countries are the U.S., Canada, and Mexico. Our trading partners are kept informed of our regulatory decisions through NAPPO, and other fora. It should also be noted that all the existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new soybean varieties internationally apply equally to the transgenic soybeans covered by this analysis.

E. High Oleic Acid Soybean Sublines Will Not Cause Damage To Raw Or Processed Agricultural Commodities.

Information provided by DuPont regarding the components and processing characteristics of soybean sublines G94-1, G94-19 and G-168 revealed no differences in any component that could have a direct or indirect plant pest effect on any raw or processed plant commodity. DuPont has been in direct consultation with the Food and Drug Administration to assure that both oil and meal from these sublines are safe for human and animal consumption. DuPont completed consultations with the FDA regarding the soybean sublines G94-1, G94-19 and G-168 in early 1997.

Conventional breeding techniques (mutational breeding) have been used to produce soybean plants with an elevated oleic acid content (Kinney, 1994). However the environmental instability of the high oleic phenotype is a major drawback of these plants. A typical high oleic soybean produced by conventional techniques, such as DuPont's HO7-9, yields an oil with widely variable oleic acid content (35-55% of total fatty acids). The high oleic transgenic soybeans sublines G94-1, G94-19 and G-168 yield an oil with oleic acid consistently in the 82-85% range, irrespective of the locality or weather conditions. Of all high oleic soybean lines, the transgenic

soybean lines are clearly superior.

The subject high oleic acid soybean sublines will be grown in a manner similar to that of traditionally bred soybeans, except that they will be grown in a identity preserved manner so that they can be stored and processed separately from regular soybean lines. This will allow high oleic acid soybean oil to be segregated from traditional soybean oil.

Soybean oil is currently the predominant plant oil in the world, and is used in a wide variety of food applications. Soybean oil derived from the high oleic acid soybean sublines would be superior to that produced from conventional soybean varieties in terms of both physical and food use functionality. Untreated, commodity soybean oil is rich in polyunsaturated fatty acids, which are oxidatively unstable, making it unusable for many food applications (Frankel, 1980). Reducing levels of polyunsaturated fatty acids by selective hydrogenation tends to increase the abundance of monounsaturated oleic acid while also producing substantial quantities of the trans isomer of oleic acid and other trans isomers. There is wide consensus that substituting harmful saturated fats with unsaturated oils is desirable (American Society of Clinical Nutrition, 1996). If this can be done without introducing trans fatty acids or increased amounts stearic acid into the diet, then the unresolved issues surrounding their consumption become less of a concern.

The high oleic acid soybean sublines yield a more stable oil containing 82-85% oleic acid, a greatly reduced concentration of polyunsaturated fatty acids, and a lower palmitic acid content. The stearic acid content of this oil is less than 4%. The increased stability obviates the need for an additional chemical hydrogenation processing step. Consumption of fried foods cooked in high oleic soybean oil instead of hydrogenated vegetable oil would significantly decrease a person's daily intake of trans fatty acids. Most likely the high oleic soybean oil also would be preferred over animal-derived cooking fats, such as lard, which contain greater amounts of saturated fatty acids as well as cholesterol.

F. High Oleic Acid Soybean Sublines Are Not Harmful To Beneficial, Threatened or Endangered Organisms.

There is no reason to believe that soybean sublines G94-1, G94-19 and G-168 will harm beneficial threatened or endangered organisms. The enzyme and genetic material that confers high oleic acid production in the transgenic sublines is normally present in soybeans and is not known to have any toxic properties. Field observations of the subject soybean sublines revealed no negative effects on nontarget organisms, suggesting that the relatively lower levels of desaturase enzyme and increased levels of oleic acid in the seed of the soybean sublines G94-1, G94-19 and G-168 are not toxic to beneficial organisms. Since the high oleic acid trait in these soybean sublines is produced by sense suppression and no new protein is produced in the seed or plant material from soybean sublines G94-1, G94-19 and G-168, there is no potential for exposing humans, animals or other organisms to any new protein components. Knowledge of this enzyme's mode of action, and the lack of known toxicity for this protein suggest no potential for deleterious effects on beneficial organisms, such as bees and earthworms. Oleic acid is a

natural constituent of diet of animals and humans and should not cause any harmful effects other than those known to be caused by high fat diet. APHIS has not identified any other potential mechanisms for deleterious effects on beneficial organisms.

The indirect metabolic alterations that occur in soybean sublines G94-1, G94-19 and G-168, including the production of trace amounts (<1%) of a linoleic acid isomer, and the reduction in seed storage protein beta-conglycinin α and α′ subunits and increase in glycinin subunits should have no negative impact on nontarget organisms. The alteration in seed storage protein composition was the result of a silencing of the α and α′ subunit genes mediated by the beta-conglycinin α′ promoter sequence used in the GmFad 2-1 vector. The linoleic acid isomer found in soybean sublines G94-1, G94-19 and G-168 is prevalent in the fatty acids of butterfat, beef and mutton tallow, partially hydrogenated vegetable oils, human milk and mango pulp. Other changes in the oil from these sublines versus the parent elite line include very low abundance of linoleic acid, approximately 1%, and significantly lower linolenic and palmitic acid contents when compared with the parent elite. These changes are not expected to have any impact on nontarget organisms. Antinutritional factors present in traditionally bred soybeans include trypsin inhibitors, phytic acid and the oligosaccharides raffinose and stachyose. In soybean sublines G94-1, G94-19 and G-168 the levels of all of these components were similar to those found in traditional soybeans. There is also no reason to believe that the *uidA* or *bla* genes in these sublines would have any impact on non-target organisms since these genes do not encode infectious agents and the genes are not expressed in the plant.

V. CONCLUSION

APHIS has determined that soybean sublines G94-1, G94-19 and G-168 derived from transformation event 260-05 developed by DuPont that have been field tested under APHIS authority will no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Permits or notifications under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of soybean sublines G94-1, G94-19 and G-168 or their progeny. Importation of soybean sublines G94-1, G94-19 and G-168 or seeds capable of propagation are still, however, subject to the restrictions found in the Foreign Quarantine notice regulations at 7 CFR Part 319 just as applies to any other importation of soybean seeds. This determination has been made based on data collected from these approved field trials, laboratory analyses and literature references presented herein which demonstrate that:

1. Soybean sublines G94-1, G94-19 and G-168 exhibit no plant pathogenic properties. Although DNA from pathogenic organisms were used in their development, these soybean plants are not infected by these organisms nor can these plants incite disease in other plants.

2. Soybean sublines G94-1, G94-19 and G-168 are no more likely to become a weed than improved quality soybean varieties which have been developed by traditional breeding techniques. Soybean is not a weed in the U.S., and there is no reason to believe that the introduced genes would enable soybean to become a weed pest.

3. Multiple factors ensure that gene introgression from soybean sublines G94-1, G94-19 and G-168 into wild plants in the United States and its territories is extremely unlikely. Even in other regions, potential gene introgression from soybean sublines G94-1, G94-19 and G-168 into wild relatives is not likely to increase the weediness potential of any resulting progeny nor adversely effect genetic diversity of related plants any more than would introgression from traditional soybean varieties.

4. Soybean sublines G94-1, G94-19 and G-168 will not have a significant adverse impact on organisms beneficial to plants or agriculture, or other nontarget organisms, and will not affect threatened or endangered species.

5. Soybean sublines G94-1, G94-19 and G-168 should not cause damage to raw or processed agricultural commodities.

APHIS has also concluded that there may be new varieties bred from the soybean sublines G94-1, G94-19 and G-168; however, if such varieties are developed they are unlikely to exhibit new plant pest properties, i.e., properties substantially different from any observed for high oleic acid soybean already field tested, or those observed for soybeans developed from traditional breeding.

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