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DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 93-148-2]

Availability of Determination of Nonregulated Status of Monsanto Co., Genetically Engineered Soybean Line

AGENCY: Animal and Plant Health Inspection Service, USDA.
ACTION: Notice of determination.

SUMMARY: The Animal and Plant Health Inspection Service (APHIS) is announcing the issuance of a determination that glyphosate-tolerant soybean line 40-3-2 does not present a plant pest risk and should therefore no longer be a regulated article under regulations at 7 CFR part 340. APHIS' determination has been made in response to a petition received on September 15, 1993, from Monsanto Co. of St. Louis, MO, seeking such a determination. This notice also announces the availability of the determination, which provides the basis for the ruling, as well as the availability of an environmental assessment of this action.

EFFECTIVE DATE: This ruling is effective May 18, 1994.

ADDRESSES: The determination, the environmental assessment, the Monsanto Co. submission, and written comments received in response to our December 6, 1993, notice published in the Federal Register 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 access to this room are requested to call ahead on (202) 690-2817.

FOR FURTHER INFORMATION CONTACT: Ms. Shirley P. Ingebritsen, Regulatory Analyst, Biotechnology, Biologics, and Environmental Protection, APHIS, USDA, room 850 Federal Building, 6505 Belcrest Road, Hyattsville, MD 20782, (301) 436-7601. For a copy of the determination or the environmental assessment, please write or call Ms. Kay Peterson at this same address and telephone number.

SUPPLEMENTARY INFORMATION: On December 6, 1993, the Animal and Plant Health Inspection Service (APHIS) published a notice in the Federal Register (58 FR 64287-64288, Docket No. 93-148-1), announcing receipt of a petition from the Monsanto Co. of St. Louis, MO, that requested a determination on the reguintory status of glyphosate-tolerant soybean (GTS) line 40-3-2. This notice also indicated APHIS' role, as well as those of the United States Environmental Protection Agency and the Food and Drug Administration, in the regulation of GTS line 40-3-2, food products derived from it, and the herbicide glyphosate that may be used in conjunction with it, if a new label for the herbicide is approved. This notice further announced that the petition was available for public review and invited written comments on whether such soybeans present a plant pest risk, to be submitted on or before February 4, 1994.

Comments

APHIS received a total of 33 comments on the Monsanto petition from farm cooperatives, farmers, a food company, national and State soybean associations, a science and technology policy organization, seed companies and affiliated research organizations, a State agricultural commodity commission for soybeans, State departments of agriculture, universities, and a university agricultural experiment station. With a single exception, the comments were favorable to the petition. APHIS has provided a discussion of the comments in the determination document, which is available upon request from the individual listed under FOR FURTHER INFORMATION CONTACT.

GTS line 40-3-2 is described by Monsanto as soybeans containing a Roundup Ready™ gene, and any progeny derived from crosses between GTS line 40-3-2 and traditional soybean varieties. The Roundup Ready™ gene contained in GTS line 40-3-2 is a single insert of DNA comprised of the enhanced 35S promoter derived from cauliflower mosaic virus, the chloroplast transit peptide coding sequence from *Petunia hybrida* fused to the 5-

enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene derived from *Agrobacterium* sp. strain CP4, and the nopaline synthase 3' terminator from *A. tumefaciens*. Glyphosate, the active ingredient in Roundup® herbicide, controls weeds due to the inhibition of the enzyme EPSPS. GTS line 40–3–2 soybeans express an EPSPS enzyme tolerant to glyphosate, thereby conferring tolerance to Roundup® herbicide.

GTS line 40–3–2 contains components from organisms that are known plant pathogens, i.e., the bacterium *A. tumefaciens* and cauliflower mosaic virus. GTS line 40–3–2 has, therefore, been a regulated article under 7 CFR part 340, and was field tested in 1991, 1992, and 1993 in accordance with APHIS regulations. APHIS' determination that GTS line 40–3–2 does not present a plant pest risk is based on an analysis of data and information provided by Monsanto, as well as other relevant published scientific data and comments received by APHIS concerning the potential plant pest risk of the soybean line. From this review, we have determined that the GTS line 40–3–2: (1) Exhibits no plant pathogenic properties; (2) is no more likely to become a weed than its nonengineered parental variety; (3) is unlikely to increase the weediness potential for any other cultivated plant or native wild species with which the organism can interbreed; (4) will not cause damage to processed agricultural commodities; and (5) is unlikely to harm other organisms, such as bees and earthworms, that are beneficial to agriculture. In addition, we have determined that there is no reason to believe that new progeny bred from GTS line 40–3–2 will exhibit new plant pest properties, i.e., properties substantially different from any observed for the field tested GTS line 40–3–2, or those observed for soybeans in traditional breeding programs.

The effect of this determination is that GTS line 40–3–2 will no longer be considered a regulated article under APHIS regulations at 7 CFR part 340. Permits and notifications under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of those soybean lines or their progeny. Agronomic practices involving the GTS line 40–3–2, e.g., cultivation, propagation, movement, and cross-breeding with other nonregulated soybean lines, can now be conducted without APHIS' approval. Importation of GTS line 40–3–2 and seeds capable of propagation is still, however, subject to the restrictions found in the Foreign Quarantine Notice regulations at 7 CFR part 319. Variety registration and/or seed certification for individual soybean lines carrying the Roundup Ready™ gene may involve future actions by the U.S. Plant Variety Protection Office and State Seed Certification officials.

The potential environmental impacts associated with this determination have been examined in accordance with: (1) The National Environmental Policy Act (NEPA) of 1969 (42 USC 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 Guidelines Implementing NEPA (44 FR 50381–50384, August 28, 1979, and 44 FR 51272–51274, August 31, 1979). An environmental assessment was prepared and a finding of no significant impact was reached by APHIS for the determination that GTS line 40–3–2 is no longer a regulated article under its regulations at 7 CFR part 340.

Done in Washington, DC, this 18th day of May 1994.

William S. Wallace,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 94–12625 Filed 5–23–94; 8:45 am]

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United States Department of Agriculture

Animal and Plant Health Inspection Service

APHIS-USDA Petition 93-258-01 for Determination of Nonregulated Status for Glyphosate-Tolerant Soybean Line 40-3-2

Environmental Assessment and Finding of No Significant Impact

May 1994

Finding of No Significant Impact

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 P93-258-01) received from Monsanto Agricultural Company regarding the status of glyphosate-tolerant soybean (GTS) line 40-3-2 under APHIS regulations at 7 CFR Part 340. The plants have been engineered with a gene that confers tolerance to the herbicide glyphosate. Based upon the analysis documented in its environmental assessment, APHIS has reached a finding of no significant impact on the environment from the unconfined, agricultural use of GTS line 40-3-2 and its progeny.

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Acting Director
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Animal and Plant Health Inspection Service
U.S. Department of Agriculture

Date: MAY 19 1994

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Appendix:

I. Summary

The Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agricuture (USDA), has prepared an Environmental Assessment (EA) in response to a petition (APHIS Number P93-258-01) from Monsanto Company regarding glyphosate-tolerant soybean (GTS) line 40-3-2. Monsanto seeks a determination that GTS line 40-3-2 does not present a plant pest risk and should therefore no longer be a regulated article under regulations at 7 CFR Part 340. GTS line 40-3-2 has been genetically engineered to express a gene that confers tolerance to the herbicide glyphosate.

Monsanto submitted its petition after the completion of field tests of GTS line 40-3-2 at 54 sites in 19 States and Puerto Rico, conducted since 1991 under 9 APHIS permits. Field trial reports from these tests demonstrate no deleterious effects on plants, nontarget organisms, or the environment. Field tests were also conducted in Argentina and Costa Rica in accordance with national regulatory requirements. Additional trials of line 40-3-2 were conducted in the United States and Puerto Rico under permit and notification during the 1993 growing season. All field trials were performed under conditions of physical and reproductive confinement.

An Environmental Assessment (EA) was prepared prior to granting each of the permits for a field trial using GTS line 40-3-2. The EAs for the previous introductions of line 40-3-2 addressed plant pest risk issues relative to the conduct of field trials under physical and reproductive confinement. This EA specifically addresses the potential for impacts to the human environment through the use in agriculture of GTS line 40-3-2. The U.S. Environmental Protection Agency (EPA) has authority over the potential uses of the herbicide glyphosate (Roundup®) in conjunction with GTS line 40-3-2.

APHIS has considered the information provided by Monsanto in its petition as well as other scientific data and comments received from the public relating to the potential plant pest risk of GTS line 40-3-2. A thorough evaluation of the potential for significant impact to the human environment through the unconfined, agricultural use of GTS line 40-3-2 has brought APHIS to a Finding of No Significant Impact (FONSI). This conclusion is based upon (1) the nature of the genetic modification; (2) the fact that soybean has no weedy relatives with which it can interbreed in the United States and its territories; and (3) the fact this modification will not increase the weediness of the soybeans or negatively effect any nontarget organisms, including beneficials. In conjunction with the FONSI, APHIS has made the determination that line 40-3-2 and its progeny have no potential to pose a plant pest risk, and is, therefore, no longer a regulated article. Our documentation of that determination is attached as Appendix A.

II. Introduction

This EA examines potential environmental impacts from the unrestricted introduction of GTS line 40-3-2. GTS line 40-3-2 has been extensively field tested under permit by Monsanto since 1991. The genetic material introduced into this line has been discussed in detail in EAs prepared for field tests under 19 APHIS permits: 91-018-01, 91-151-01, 92-007-01, 92-007-02, 92-015-01, 92-037-02, 92-037-06, 92-041-01, 92-055-01, 92-335-01, 92-350-01, 92-359-01, 93-011-03, 93-011-04, 93-12-05, 93-012-06, 93-012-07, 93-026-01, and 93-078-01. Trials under the first 9 permits were completed prior to the submission of the petition, and Monsanto has based its petition, in part, on the data acquired from these trials. Field tests under the 9 permits that are encompassed by the petition took place at approximately 54 sites in the following 19 states and Puerto Rico: Alabama, Arkansas, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Michigan, Mississippi, Missouri, Nebraska, North Carolina, Ohio, Puerto Rico, South Carolina, Tennessee, and Virginia. Field trial reports from these tests demonstrate no deleterious effects on plants, nontarget organisms, or the environment as a result of these releases. Field tests were also conducted in Argentina and Costa Rica in accordance with national regulatory requirements. Additional trials of GTS line 40-3-2 were conducted in the United States and Puerto Rico under permit and notification during the 1993 growing season. 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 GTS line 40-3-2 are found in APHIS' Determination of Nonregulated Status. Because this information is included as Appendix A, it will not be described in detail in the body of this document.

Prior to issuing a permit for a field release, APHIS analyzes the potential impacts associated with the proposed introduction, and prepares an environmental assessment which documents the analysis in accordance with regulations and guidelines implementing the National Environmental Policy Act of 1969 (42 USC 4331 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; 44 FR 50381-50384; and 44 FR 51272-51274). APHIS also evaluates the potential for significant impact to the human environment from its determination of nonregulated status.

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 transgenic soybean plants described in the Monsanto petition have been considered regulated articles because noncoding DNA regulatory sequences are derived from plant pathogens.

III. Purpose and Need

The purpose of this EA is to ascertain whether the approval of a petition submitted to USDA/APHIS for the determination of nonregulated status of GTS line 40-3-2, which will allow the unconfined introduction of the article, will have a significant impact on the environment. A petition was submitted to APHIS pursuant to regulations codified in 7 CFR Part 340 entitled "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There is Reason to Believe Are Plant Pests." The regulations govern the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. An organism is not 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 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 the agency determines that the regulated article does not present a risk of introduction or dissemination of a plant pest, the petition would be granted, thereby allowing for unregulated introduction of the article in question. Permits under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of that article or its progeny. Normal agronomic practices with it, e.g., cultivation, propagation, movement, and cross-breeding could then be conducted without APHIS' approval.

Effects associated with the potential uses of the herbicide glyphosate (Roundup®) in conjunction with GTS line 40-3-2 are outside the scope of APHIS' analysis. APHIS' determination does not constitute authorization to use glyphosate on GTS line 40-3-2. The EPA has authority over the use in the environment of pesticidal substances, including herbicides, under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA); specifically, EPA has jurisdiction over registration of glyphosate for use on soybean. Approval by EPA of a particular label condition for a pesticide is granted when, under the specified conditions of use, it will not generally cause adverse effects. EPA considers both human health and safety as well as nontarget effects of both the herbicide and its breakdown products in making a decision on registration of an herbicide. The potential issuance by EPA of a new label for use of the herbicide glyphosate on GTS line 40-3-2 and the determination by APHIS regarding the line itself are independent decisions, under consideration by different agencies, based on distinct regulations under unrelated legal authorities.

IV. Alternatives

In the course of preparing the environmental assessment for Monsanto's petition, APHIS considered the following three alternatives: (1) deny the petition, so that GTS line 40-3-2 would continue to be regulated under 7 CFR Part 340; (2) approve the petition, with geographical limitations; and (3) approve the petition, so that GTS line 40-3-2 would have a nonregulated status when grown in the United States and its territories. Based on the biology of soybean, the nature of the genetic change, data and information presented by Monsanto, scientific literature, and information and comment provided by the public, APHIS could find no basis for denying the petition (Alternative 1), or for imposing geographical limitations on the use of GTS line 40-3-2 (Alternative 2).

V. Affected Environment and Potential Environmental Impacts

Potential impacts to be addressed in this EA are those that pertain to the use of GTS line 40-3-2 in the absence of confinement.

Potential impacts based on increased weediness of GTS line 40-3-2 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, *G. max*, does not show any especially weedy characteristics. The genus *Glycine* also seems to be essentially absent of such characteristics and to show no particular weedy aggressive tendencies (Hermann, 1962; Lackey, 1981; personal communication, Lackey; 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 relevant introduced trait, glyphosate tolerance, is unlikely to increase weediness of this soybean. Glyphosate would not be applied on GTS line 40-3-2 for the purpose of controlling the soybean itself, but rather for controlling unrelated weeds in the field. To increase weediness of the soybean plant there would have to be selection pressure on line 40-3-2 (Tiedje et al., 1989; Office of Technology Assessment, 1988) in association with glyphosate use. Because glyphosate will not affect the survival of GTS line 40-3-2 and because *G. max* is not itself weedy, this type of selection pressure does not now and is unlikely ever to exist. Even if such glyphosate-tolerant weedy plants did exist, glyphosate treatment would not be the control method of choice; many other methods of control would be readily available.

No other variation seen in GTS line 40-3-2 is indicative of increased weediness. Monsanto's data from greenhouse studies, included as part of the administrative record for the petition, show no significant differences between the mean germination rate percentage for GTS line 40-3-2 and the parental variety. In addition, Monsanto's field reports show no obvious increase in volunteers from seed, regrowth from stubble, or increase in seed dormancy.

Potential impacts from outcrossing of GTS line 40-3-2 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. Intersubgeneric 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). First generation hybrids from such crosses have generally been sterile, and further progeny have only been obtained with extreme difficulty. The formation of hybrids between GTS line 40-3-2 and *Glycine* species in nature is highly unlikely.

Interspecific crosses between the annuals *G. max* and *G. soja* in subgenus *Soja* can be easily made. 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 glyphosate use.

Cultivated soybean is the only member of the genus *Glycine* that appears both to grow and be sexually compatible with cultivated soybean in the United States and its territories. Even if non-agricultural land containing any wild *Glycine* populations were near sites of commercial soybean production it is highly unlikely that pollen from GTS line 40-3-2 would fertilize the wild relative, 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 zero distance from 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, APHIS believes, because any potential effects of the trait would not alter the weediness of the wild soybean in the absence of sustained glyphosate use. Therefore, the presence of an occasional glyphosate-tolerant relative of GTS line 40-3-2 should pose no significant impact to the environment.

Potential impact on nontarget organisms, including beneficial organisms such as bees and earthworms

There is no reason to believe that deleterious effects or significant impacts on nontarget organisms, including beneficial organisms, would result from the cultivation of GTS line 40-3-2. The enzyme that confers glyphosate tolerance in this soybean line is normally expressed in soybeans and is not known to have any toxic properties. Field observations of line 40-3-2 revealed no negative effects on nontarget organisms, suggesting that the relatively higher levels of the protein in the tissues of line 40-3-2 are not toxic to beneficial organisms. The lack of known toxicity for this protein suggests no potential for deleterious effects on beneficial organisms such as bees and earthworms. The high specificity of the enzyme for its substrates makes it unlikely that the introduced enzyme would metabolize endogenous substrates to produce compounds toxic to beneficial organisms. 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 GTS line 40-3-2 would have an effect on any threatened or endangered species in the United States.

Consideration of potential environmental impacts associated with the cultivation of GTS line 40-3-2 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 GTS line 40-3-2 outside the United States and its territories.

Our analysis of the biology of soybean leads to the conclusion that the cultivation of GTS line 40-3-2 anywhere in the world will not have an adverse impact on the environment. The GTS line 40-3-2 shows no significant differences from its parent line, A5403, in all avenues investigated, save for its production of an EPSPS enzyme that is not inhibited by the glyphosate herbicide.

Several factors contribute to the conclusion that there should be no impacts abroad from cultivation of this soybean line or its progeny.

Any international traffic in the soybeans subject to this determination 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 (98 countries as of December, 1992). The treaty, now administered by a Secretariat housed with the Food and Agriculture Organization in Rome, came into force 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 vast majority of IPPC signatories have promulgated, and are now adminstering, such legislation or guidelines. The IPPC has also led to the creation of Regional Plant Protection Organizations (RPPOs) to facilitate regional harmonization of phytosanitary standards.

Issues that may relate to commercialization of particular agricultural commodities produced through biotechnology are being addressed in international fora. APHIS has played a role in working toward harmonization of biosafety and biotechnology guidelines and regulations included within the RPPO for our region, 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 bilateral or quadrilateral discussions on biotechnology regulatory issues with other countries, most often Canada and Mexico. APHIS also acts as a consultant for the development of biotechnology guidelines and regulations, and has interacted with governments around the world in this manner, including those in regions where soybean originated or is cultivated in significant quantities (e.g., China, Japan, Korea, Association of South East Asian Nations member States, India, Pakistan, African States, and more). We have participated in numerous conferences intended to enhance international cooperation on safety in biotechnology, and sponsored several workshops on safeguards for planned introductions of transgenic

crops (crucifers, maize, wheat, potatoes, rice, tomatoes) most of which have included consideration of international biosafety issues.

In the course of these wide-ranging studies and interactions, APHIS has not identified any impacts on the environment that might be relevant to GTS line 40-3-2 or follow from the unconfined cultivation of this soybean line in the United States and its territories, or abroad. 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 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 this determination.

VI. Conclusions

In accordance with the requirements of NEPA, APHIS has considered the potential for significant impact on the environment of a proposed action, i.e, reaching the determination that GTS line 40-3-2 has no potential to pose a plant pest risk and should no longer be considered a regulated article under the regulations at 7 CFR Part 340. After careful analysis of the available information, APHIS concludes that its proposed action should not have a significant impact on the environment, and that the proper alternative is to approve the petition, so that GTS line 40-3-2 would have a nonregulated status when grown in the United States and its territories. APHIS has identified no factors that would suggest any likelihood of impacts to the environment of the United States and its territories. While isolated environments, such as are found in Hawaii, Puerto Rico, or in territories or possessions of the United States, have fragile ecologies that have frequently been damaged through human intervention, APHIS has determined that in these environments GTS line 40-3-2 will have impacts no different from traditional soybean varieties that are not subject to petition requirements under 7 CFR Part 340 before they enter agriculture. This conclusion is based on factors discussed herein or in the determination included as appendix A, as well as the following factors:

1. A gene that confers tolerance to the herbicide glyphosate has been inserted into a soybean chromosome in soybean line 40-3-2. In nature, chromosomal genetic material from plants can only be transferred to another sexually compatible flowering plant by cross-pollination. There are no other species sexually compatible in nature with cultivated soybean in the United States and its territories.

2. Neither the gene that confers tolerance to the herbicide glyphosate, nor the gene product, nor its associated regulatory sequences, confers on GTS line 40-3-2 or its progeny any plant pest characteristic.

3. In nature, the gene that confers tolerance to the herbicide glyphosate will not provide GTS line 40-3-2 or its 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 GTS line 40-3-2 exhibits any increased weediness relative to that of traditional varieties.

4. There is no reason to believe that the use of GTS line 40-3-2 or its progeny in agriculture will lead to an increase in weediness in any plant with which it can successfully interbreed.

5. There is no reason to believe that the use of GTS line 40-3-2 or its progeny in agriculture will have a significant impact on any beneficial organisms in the environment, or on any threatened or endangered species.

VII. Literature Cited

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VIII. Preparers and Reviewers

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Appendix

Response to Monsanto Petition P93-258-01 for Determination of Nonregulated Status for Glyphosate Tolerant Soybean Line 40-3-2

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I. Summary

The Animal and Plant Health Inspection Service (APHIS) has determined, based on a review of scientific data and information, that glyphosate-tolerant soybean (GTS) line 40-3-2 does not present a plant pest risk and is therefore no longer a regulated article under its regulations at 7 CFR Part 340. As a result of this determination, approval under those regulations will no longer be required from APHIS for planting, importation, or interstate movement of GTS line 40-3-2 or its progeny. (Importation of GTS line 40-3-2 [and nursery stock or seeds capable of propagation] is still, however, subject to the restrictions found in the Foreign Quarantine Notice regulations at 7 CFR Part 319.) Variety registration and/or seed certification for individual soybean lines carrying the Roundup Ready™ gene may involve future actions by the U.S. Plant Variety Protection Office and State Seed Certification officials.

APHIS' determination has been made in response to a petition from Monsanto Company of St. Louis, Missouri, received on September 15, 1993. The petition seeks a determination from APHIS that GTS line 40-3-2 and its progeny do not present a plant pest risk and are therefore not regulated articles. On December 6, 1993, APHIS announced receipt of the Monsanto petition in the Federal Register (58 FR 64287-64288) and stated that the petition was available for public review. APHIS also indicated its role, as well as those of the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA), in regulation of GTS line 40-3-2, food products derived from it, and the herbicide glyphosate that may be used, if a new label for the herbicide is approved, in conjunction with it. APHIS invited written comments on whether GTS line 40-3-2 poses a plant pest risk, to be submitted on or before February 4, 1994.

GTS line 40-3-2 soybeans have been described by Monsanto as soybeans containing a Roundup Ready™ gene, and any progeny derived from crosses between GTS line 40-3-2 and traditional soybean varieties. The Roundup Ready™ gene contained in GTS line 40-3-2 is a single insert of DNA comprised of the enhanced 35S promoter derived from cauliflower mosaic virus, the chloroplast transit peptide coding sequence from *Petunia hybrida* fused to the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene derived from *Agrobacterium* sp. strain CP4, and the nopaline synthase 3' terminator from *A. tumefaciens*. Glyphosate, the active ingredient in Roundup® herbicide, controls weeds via the inhibition of the enzyme EPSPS. GTS line 40-3-2 soybeans express an EPSPS enzyme tolerant to the herbicide glyphosate, thereby conferring tolerance to Roundup® herbicide. Each of the introduced sequences will be discussed in detail in Section IV of this determination.

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 not subject to the regulatory requirements of Part 340 when it is demonstrated not to present a plant pest risk. 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 the agency determines that the regulated article does not present a risk of introduction or dissemination of a plant pest, the petition would be granted, thereby allowing for unregulated introduction of the article in question.

GTS line 40-3-2 has been considered a "regulated article" because it contains components from organisms that are known plant pathogens, i.e., the bacterium *Agrobacterium tumefaciens* and cauliflower mosaic virus. Field testing of GTS line 40-3-2 has been conducted with APHIS approval since 1991. Monsanto submitted its petition after the completion of field tests of GTS line 40-3-2 under 9 APHIS permits. These permitted field tests took place at approximately 54 sites in 19 states and Puerto Rico. All field trials were performed under conditions of physical and reproductive confinement.

APHIS has determined that GTS line 40-3-2 does not present a plant pest risk and will no longer be considered a regulated article under APHIS regulations at 7 CFR Part 340. The Agency's decision is based on an analysis of data provided to APHIS by Monsanto as well as other scientific data and comments received from the public relating to the potential plant pest risk of GTS line 40-3-2. Monsanto provided both general and specific information and data from field testing of GTS line 40-3-2. From our review, we have determined that GTS line 40-3-2: (1) exhibits no plant pathogenic properties; (2) is no more likely to become a weed than their non-engineered parental varieties; (3) is 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 processed agricultural commodities; and (5) is unlikely to harm other organisms, such as bees and earthworms, that are beneficial to agriculture. APHIS has also concluded that there is a reasonable certainty that new progeny varieties bred from GTS line 40-3-2 will not exhibit new plant pest properties, i.e., properties substantially different from any observed for the field tested line 40-3-2, or those observed for soybean in traditional breeding programs.

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 (42 USC 4331 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; 44 FR 50381-50384; and 44 FR 51272-51274). An Environmental Assessment (EA) was prepared and a Finding of No Significant Impact (FONSI) was reached by APHIS

for the determination that GTS line 40-3-2 is no longer a regulated article under its regulations at 7 CFR Part 340. The EA and FONSI are available from APHIS upon written request.

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 GTS line 40-3-2, 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 GTS line 40-3-2 does not present a plant pest risk.

II. Background

USDA Regulatory Authority

APHIS regulations, 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. A genetically engineered organism is deemed a regulated article either if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in section 340.2 of the regulations and is also a plant pest; if it is unclassified; or if APHIS has reason to believe that the genetically engineered organism presents a plant pest risk.

Prior to the introduction of a regulated article, a person is required under § 340.1 of the regulations to either (1) notify APHIS in accordance with § 340.3 or (2) obtain a permit in accordance with § 340.4. Introduction under notification (§ 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 § 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.

The FPPA gives USDA authority to regulate plant pests and other articles to prevent direct or indirect injury, disease, or damage to plants, plant products, and crops. The PQA provides an additional level of protection by enabling USDA to regulate the importation and movement of nursery stock and other plants which may harbor injurious pests or diseases, and requires that they be grown under certain conditions after importation. For certain genetically engineered organisms, field testing may be required to verify that they exhibit the expected biological properties, and

to demonstrate that although derived using components from plant pests, they do not possess plant pest characteristics.

An organism is not 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 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 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.

GTS line 40-3-2 has been considered a "regulated article" for field testing under Part 340.0 of the regulations because the noncoding regulatory sequences were derived from the plant pathogens *A. tumefaciens* and cauliflower mosaic virus.

APHIS believes it prudent to provide assurance prior to commercialization that organisms, such as GTS line 40-3-2, that are derived in part from plant pest sequences, do not pose 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 GTS line 40-3-2 is no longer a regulated article is based in part on evidence provided by Monsanto concerning the biological properties of line 40-3-2 and its similarity to other varieties of soybean grown using standard agricultural practices for commercial sale or private use. GTS line 40-3-2 has been field tested under 19 permits (91-018-01, 91-151-01, 92-007-01, 92-007-02, 92-015-01, 92-037-02, 92-037-06, 92-041-01, 92-055-01, 92-335-01, 92-350-01, 92-359-01, 93-011-03, 93-011-04, 93-12-05, 93-012-06, 93-012-07, 93-026-01, and 93-078-01). Trials under the first 9 permits were completed prior to the submission of the petition, and Monsanto has based its petition, in part, on the data acquired from these trials. The 9 completed field tests took place at approximately 54 sites in the following 19 states and Puerto Rico: Alabama, Arkansas, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Michigan, Mississippi, Missouri, North Carolina, Nebraska, Ohio, Puerto Rico, South Carolina, Tennessee, and Virginia. Field trial reports from these tests show no deleterious effects on plants, nontarget organisms, or the environment as a result of these releases. Field tests were also conducted in Argentina and Costa Rica in accordance with national regulatory requirements. Additional trials of line 40-3-2 were also conducted in the United States and Puerto Rico under permit and notification during the 1993 growing season.

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. The regulations instead have the premise that

when plants are developed using biological vectors from pathogenic sources, transforming material from pathogenic sources, or pathogens are used as vector agents, that they should be evaluated to assure that there is not a plant pest risk (McCammon and Medley, 1990). For each 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 aims of APHIS regulations in the Code of Federal Regulations at 7 CFR Part 340 are 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 to allow for the safe utilization of the technology in agriculture.

A certification that an organism does not present a plant pest risk means that there is reasonable certainty that the organism 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, are clearly subsumed within 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." Lack of plant pest risk may be concluded when there is evidence that the plant under consideration: (1) exhibits no plant pathogenic properties; (2) is no more likely to become a weed than its non-engineered parental varieties; (3) is unlikely to increase the weediness potential for any other cultivated plant or native wild species with which the organism can interbreed; (4) does not cause damage to processed agricultural commodities; and (5) is unlikely to harm other organisms, such as bees, that are beneficial to agriculture. Evidence has been presented by Monsanto that bears on all of these topics. In addition, because the Monsanto petition seeks a determination regarding GTS line 40-3-2, it should be established that there is a reasonable certainty that any new soybean varieties bred from GTS line 40-3-2 will exhibit plant pest properties not substantially different from any observed for soybeans in traditional breeding programs or as seen in the development of GTS line 40-3-2.

Oversight by Other Federal Agencies

The EPA regulates the use of pesticide chemicals, including herbicides, in the environment. Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 et seq.), EPA has the authority to regulate the testing, sale, distribution, use, storage, and disposal of pesticides. Before a pesticide may be sold, distributed, or used in the United States, it must be registered under FIFRA Section 3. For a pesticide that is already registered, the use of the pesticide on a new crop plant (i.e., use on a crop for which the pesticide is not already registered) requires EPA approval of an amendment to the registration. In determining whether to approve the new use of the pesticide, EPA considers the possibility of adverse effects to human health and the environment from the new use. Under the Federal Food, Drug and Cosmetic Act (FFDCA) (21 U.S.C. 201 et seq.), EPA also has responsibility for establishing tolerances for pesticide residues on food or feed. Roundup® (glyphosate) is currently registered as an herbicide for use on a number of crop plants, including soybean. However, any new use of the herbicide on soybean would require the approval by EPA of an amendment to the registration under FIFRA and a tolerance review under FFDCA.

The FFDCA provides FDA with authority to ensure the safety and wholesomeness of all food(s), other than meat and poultry. FDA's 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 GTS line 40-3-2 is under the jurisdiction of the FDA.

III. Summary and Response to Comments

Summary and Analysis of Comments

APHIS received 33 comments on the Monsanto petition from the following: farm cooperatives (4); farmers (8); a food company (1); national and state soybean associations (3); a science and technology policy organization (1); seed companies and affiliated research organizations (6); a State agricultural commodity commission for soybeans (1); state departments of agriculture (4); universities (4); and a university agricultural experiment station (1). With one exception, the comments were favorable to the petition.

Potential Benefits of GTS Line 40-3-2

A majority of the commenters stressed the potential environmental and economic benefits of a GTS variety. The herbicide glyphosate is described by many respondents as "environmentally friendly" and "soil and water friendly," and as "efficient in time and money" for the farmer. Claims

were also made for the herbicide's safety to humans and other mammals. Over half the respondents cited the additional benefits of a GTS variety for farmers using the "no-till" method because of the broad spectrum of weeds controlled by a single application of the herbicide. Several commenters claimed that use of a GTS variety would reduce the need for pre-emergence herbicides as well as the total amount of herbicide used, while flexibility in weed-control options would be increased.

Plant Pest Risk Issues

Approximately one-half the respondents addressed "plant pest risk" issues such as the weediness potential of a GTS variety and the potential for the development of glyphosate-resistant weeds. As evidence that the GTS does not present a plant pest risk the respondents cited the following: absence of weedy properties of soybean; absence of weedy relatives of soybean in the United States; and poor overwintering ability of domesticated soybean varieties. Weed scientists commented that despite widespread use of glyphosate for 20 years, there have been no known cases of the development of glyphosate-resistant weeds.

Fully one-third of the respondents reported on the agronomic characteristics of the Monsanto GTS line 40-3-2 based on their personal experience with the line in field tests. Several of these respondents made the general observation that the GTS line 40-3-2 developed "as well as" or no differently from genotypes not carrying the Roundup Ready™ gene. One respondent reported that the transgenic and nontransgenic plants showed "the same" germination rates and survival characteristics, that disease incidence and severity for brown stem rot and pod and stem blight were similar, and that feeding damage by bean leaf beetle was "not different." On the basis of two years of testing the GTS line 40-3-2, a university weed scientist reported no evidence of pathogenicity and no change in weed or insect populations for the transgenic variety.

Comment Unfavorable to the Petition

One comment letter stated that USDA should not approve the Monsanto petition or any other petition until the federal government has revised its oversight program for transgenic crops at the commercialization stage. The revisions requested include the establishment of standardized assessment and data collection schemes for consideration of risks of transgenic crops to ecosystems in the United States and world-wide, with particular attention to centers of diversity for food and fiber crops. The commenter also expressed the view that development of herbicide-tolerant crops (HTC) should not be encouraged because they increase farmers' dependence on chemical herbicides.

Response to Comments

APHIS has found the range of perspectives reflected in the comments on the Monsanto petition to be helpful in the determination process. Because APHIS specifically requested comments on whether Monsanto's GTS line 40-3-2 presents a plant pest risk, the several comment letters containing observations of the subject transgenic plants during multiyear field tests were of particular interest. These observations are corroborated by the field data reports submitted by Monsanto demonstrating growth patterns for the GTS line 40-3-2 similar to the non-transformed variety. Detailed comments from weed scientists on the absence of weediness potential of soybean and the lack of potential for the development of glyphosate-resistance in weeds also supplement the information presented in the petition and in the scientific literature. Similar views were expressed by State officials based on their knowledge of these transgenic plants.

The comment letters expressing enthusiasm for a glyphosate-tolerant soybean variety based on projected economic or environmental benefits would be useful for providing a view of marketing potential for the GTS line, or a risk-benefit analysis. However, APHIS is primarily concerned with plant pest risk issues in evaluating a petition. Similarly, comments about the relative environmental safety of glyphosate are confirmed by data available about the herbicide, but these kinds of judgements will be made by the EPA in the course of the agency's review under the FIFRA.

In response to the single comment letter urging revision of the Federal approach to oversight for commercialization for transgenic crop plants, APHIS continues to welcome a dialogue on specific issues that can be addressed under its authorities. APHIS shares the respondent's expressed concerns about weediness potential and threats to centers of diversity of any transgenic crop plant. However, in the case of soybean, all the information available from a wide variety of sources, including botanists, agronomists, crop specialists, and growers, supports the conclusion that cultivated soybean is not a weed, and there are no weedy relatives of soybean in the United States or its territories. Further, the fact that soybean is largely self-pollinated limits the outcrossing potential for transgenic or nontransgenic soybean. The limited potential for cross-pollination is evident in certified seed regulations for Foundation seed, which permit zero distance between different soybean cultivars in the field. APHIS believes that the characteristics of soybean virtually eliminate concerns about threats to centers of diversity from the nontransgenic and the transgenic GTS variety. These issues are discussed in more detail in Section IV of the determination document.

In answer to the contention that all transgenic plants should be subject to a standardized set of tests, APHIS believes that the case-by-case assessment of risk employed under 7 CFR Part 340 is a far more effective approach because attention can be centered on scenarios with some probability of occurrence. Scientific issues of concern to Federal regulatory

agencies such as APHIS are addressed through USDA's "Biotechnology Risk Assessment Research Grants Program," which specifically targets issues surrounding the ecological impacts of genetically modified organisms.

Finally, the negative respondent suggests that assessments be made of changing herbicide use patterns resulting from use of HTC, and that HTC development should be discouraged in favor of sustainable weed control methods. While these issues exceed APHIS' authority, the agency regularly shares information and comment with the EPA on these and related matters.

IV. Analysis of the Properties of GTS Line 40-3-2

Brief discussions of the biology of soybean and soybean cultivation practices follow in the next section to help inform the subsequent analysis. This information is expanded in subsequent sections when it is relevant in addressing particular issues with respect to GTS line 40-3-2.

Biology and Cultivation of Soybean

Soybean, *Glycine max*, is primarily grown for edible vegetable oil and the high-protein feed supplements 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 grows between about one meter above ground level and two meters below ground level. In the United States it is grown as a monoculture of row crops for sale to off-farm processors. Generally each field is planted as pure lines, although blends of two or more lines 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 (Van Doren and Reicosky, 1987). A complex and sophisticated system of cultivars, agricultural implements, agricultural chemicals, and processing techniques has 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 (Carlson 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 thus virtually pure breeding homozygous lines, although manual cross-pollination is practiced routinely in breeding programs (Fehr, 1987). Certified Seed Regulations (7 CFR (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*. Soybean crosses with members of subgenus *Glycine* only imperfectly, requiring extreme technical assistance. Soybean does not cross with any extrageneric relatives (Hymowitz and Singh, 1987). Cultivated soybean is the only member of the genus *Glycine* that both grows 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 United States from one growing season to the next (Hymowitz and Singh, 1987). Survival from one season to the next is by seed; however, 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).

To reach its determination the GTS line 40-3-2 does not present a plant pest risk, APHIS has analyzed not only public comments and basic information on the biology of soybean but also data presented by Monsanto and scientific data on other topics relevant to a discussion of plant pest risk. Based on the data, APHIS has arrived at a series of conclusions regarding the properties of GTS line 40-3-2.

Neither the Introduced Genes, Their Products, nor the Added Regulatory Sequences Controlling Their Expression Presents a Plant Pest Risk in the GTS Line 40-3-2

The introduction of the vector DNA does not present a plant pest risk in GTS line 40-3-2. The vector system used to transfer the Roundup Ready™ gene into the soybean nuclear genome, PV-GMGT04, is a derivative of a high copy *Escherichia coli* plasmid pUC19 (Viera and Messing, 1987), and does not contain any sequences from the natural tumor-inducing (Ti) plasmid system used by the plant pathogenic bacterium *Agrobacterium tumefaciens* for plant infection and gene transfer (Zambryski, 1988). (*A. tumefaciens* is the causal agent of a plant disease called crown gall.) The vector system was introduced by a relatively new technique for the transformation of plant cells, the method of particle acceleration, also referred to as the biolistic, microprojectile bombardment, or particle gun method of transformation (reviewed by Sanford, 1990). In this method plant tissues are bombarded with heavy particles that are coated with DNA, with the result that particles are able to penetrate the barriers of 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 has been shown generally to be incorporated into the nucleus (Christou et al. 1988; Hain et al. 1985). Monsanto has presented evidence in Table V.1 of its petition that the Roundup Ready.™ gene in GTS line 40-3-2 is transmitted through mitosis and meiosis in a Mendelian fashion, i.e., in a fashion consistent with integration of the added material into nuclear chromosomal DNA. As integrated pieces of plant chromosomes, introduced foreign DNA is subject to the same rules governing chromosomal rearrangements and gene stability as other plant genes.

Monsanto has analyzed the physical structure of the integrated genetic material in GTS line 40-3-2. (See Figures V.1 through V.7 in Volume I of the petition.) This analysis revealed that all of the vector PV-GMGT04 DNA was not present in the plant's genome. Polymerase chain reaction (PCR) and Southern analyses indicate that the soybean genome contains a single insert of DNA derived from PV-GMGT04, and that this insert contains all or a portion of the enhanced 35S promoter derived from cauliflower mosaic virus, the chloroplast transit peptide coding sequence from *Petunia hybrida*, the 3-enolpyruvylshikimate-5-phosphate synthase (EPSPS) gene derived from *Agrobacterium* sp. strain CP4, and all or a portion of the nopaline synthase 3' terminator from *A. tumefaciens*.

The introduced coding regions do not confer a plant pest risk. The soybean plants have been transformed with the Roundup Ready™ gene, a gene comprised of the chloroplast transit peptide (CTP) coding sequence from *Petunia hybrida* EPSPS and the EPSPS gene isolated from *Agrobacterium* sp. strain CP4. Agrobacteria occur almost worldwide in soils and in the rhizosphere of plants. This strain is not known to cause disease in animals or plants. The CTP coding sequence is fused to the 5' end of the

CP4 EPSPS gene. It targets the enzyme to the chloroplast, the site of both EPSPS localization and activity, and action of the herbicide glyphosate. The enzyme EPSPS is in the shikimate pathway for aromatic amino acid biosynthesis (tyrosine, tryptophan, and phenylalanine) in plants, including soybeans, and microorganisms, and is thus ordinarily present in food derived from plant sources. EPSPS catalyzes the formation of 5-enolpyruvylshikimate-3-phosphate from shikimate-3-phosphate and phosphoenolpyruvate (PEP). The substrate 5-enolpyruvylshikimate-3-phosphate is an intermediate in the aromatic amino acid pathway. This pathway is not present in mammals. There is no reason to believe that this gene or its protein product could impart any capability to a GTS line 40-3-2 to cause disease or damage to any other plant.

The herbicide glyphosate kills plants via the inhibition of the enzyme EPSPS. Inhibition of EPSPS directly arrests aromatic amino acid synthesis, which, in turn, results in the reduction of protein synthesis and inhibition of growth. Glyphosate tolerance can be conferred to plants and microbes by either overproduction of EPSPS or the use of glyphosate-tolerant EPSPSs. The CP4 EPSPS is insensitive to the herbicide. Genes from numerous EPSPS's have been cloned, and active site domains are conserved among them (Padgette et al., 1991). EPSPS's from a number of bacteria exhibit tolerance to glyphosate (Schultz et al., 1985). The CP4 EPSPS represents one of many different EPSPS's found in nature.

The introduced regulatory sequences do not confer a plant pest risk. Both of the regulatory sequences fused to the Roundup Ready™ gene are derived from organisms that are on the list of regulated articles. Specifically, 3' transcription termination and polyadenylation sequences from the nopaline synthase (*nos)* gene from the Ti plasmid pTiT37 (Fraley et al., 1983) are derived from *A. tumefaciens*, and the enhanced *35S* promoter region is derived from the cauliflower mosaic virus (CaMV) (Odell et al., 1985) and contains a duplication of the *35S* promoter's enhancer region (Kay et al., 1987). All or portions of these regulatory sequences are introduced into the soybean genome. (See Chapter V.B.2, Volume I, of the petition.) None of these sequences causes any plant or animal disease, is the source of pathogenicity in its host, or encodes any polypeptide product.

Despite the presence of certain pathogen-derived sequences in the GTS line 40-3-2 genome, no crown gall or CaMV disease symptoms were observed by Monsanto in any GTS line 40-3-2 cotton plants during greenhouse or field studies. Furthermore, Monsanto provides evidence that expression of the introduced gene does not result in disease symptoms or the synthesis of products toxic to other organisms. Monsanto has also monitored GTS line 40-3-2 field trials 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 the 40 sites where GTS line 40-3-2 was tested in the United States and Puerto Rico. In addition, greenhouse studies did not

reveal any differences in the sensitivity of line 40-3-2 to soybean cyst nematode as compared to A5403, the parent line.

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. A certain amount of information can be found in the scientific literature (e.g., Carlson and Chelm, 1986; Wakabayashi et al., 1986; Doolittle et al., 1990) that provides a suggestion that transfer of genes from plants to microorganisms may have occurred over evolutionary time, i.e., in the eons since the various times of divergence between the kingdoms. 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 GTS line 40-3-2 to microorganisms are, at best, highly speculative.

GTS Line 40-3-2 Has No Significant Potential to Become a Weed

Baker (1965) developed a list of attributes most commonly found in many weeds. Soybean possesses few of the 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 line A5403 is not considered a weed, and introduction of the glyphosate tolerance trait should not impart any new weedy characteristics. GTS line 40-3-2 is likely to be grown mostly in areas that are currently under soybean cultivation, i.e., in typical growing regions for the crop.

Monsanto has designed experiments and collected data from greenhouse and field trials that support the contention that the glyphosate tolerant soybean has little potential to become a serious or successful weed. Data provided in the petition indicate clearly that the applicant has not observed any significant changes in the number of seeds produced, germination characteristics, final stand, overwintering capability, or pathogen susceptibility. Monsanto observed a small increase in the plant height of line 40-3-2 when compared with the parental line. However, this difference is still within the range spanned by traditional soybean cultivars and presents nor apparent cause for concern. GTS line 40-3-2 should not increase the weediness of the parent plant, *G. max*.

GTS Line 40-3-2 Will Not Increase the Weediness Potential of Any Other Plant With Which it Can Interbreed

The only wild species that cross with the cultivated soybean are members of the genus *Glycine*. Soybean is not reported to cross with any extragenic 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* seed culture, i.e., under extreme technical assistance, 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.

Cultivated soybeans are almost completely self-pollinated, with hybridization reported generally at less than 1%. Honey bees are responsible for the occasional cross-pollination, while thrips are ineffective pollination vectors (Caviness, 1970). Thus, soybean can be grown adjacent to other soybean cultivars as long as the distance between cultivars is adequate to prevent mechanical mixing.

Should movement of genetic material take place to any receptive plants, and glyphosate tolerance be transferred, no competitive advantage would be conferred, because glyphosate is not used with these plants when they are found in non-agricultural areas. In agricultural areas, such plants would be controlled by normal agronomic practices.

GTS Line 40-3-2 Will Not Cause Damage to Processed Agricultural Commodities

Information provided by Monsanto regarding the components and processing characteristics of GTS line 40-3-2 revealed no differences in any component that could have a direct or indirect plant pest effect on any processed plant commodity. Monsanto evaluated the effects of the genetic modifications on GTS line 40-3-2 by performing compositional analyses on soybean seeds from line 40-3-2 and the parental control line grown at 10 sites within the United States and Puerto Rico. Components measured were protein, fat, crude fiber, ash, carbohydrate, and moisture. These measured components all fell within those reported in the literature despite some statistically significant differences between the transgenic and control line. In the petition Monsanto states additional

soybean quality data were collected on line 40-3-2 and the control A5403. Additional data were collected for amino acids, fatty acids, stachyose, raffinose, trypsin inhibitor, phytate, phytoestrogens, and lectins. Monsanto indicates that levels of antinutritional factors normally found in soybean, such as stachyose, raffinose, phytates and trypsin inhibitors, are essentially equivalent to those found in the parental line, A5403.

GTS Line 40-3-2 Will Not be Harmful to Beneficial Organisms, Including Bees

There is no reason to believe that deleterious effects on beneficial organisms could result from the cultivation of GTS line 40-3-2. The protein EPSPS is normally expressed in soybeans and is not known to have any toxic properties. Monsanto has provided data to show that the expression levels of the CP4 EPSPS in soybean seeds and leaves correspond to 0.024-0.029% and a maximum of 0.085% fresh weight of the soybean seed and leaf weight, respectively. In the detection assay used the level of expression of the native enzyme in the parental control was below detection thresholds. Field observations of GTS line 40-3-2 revealed no negative effects on nontarget organisms suggesting that the relatively higher levels of EPSPS in the tissues of the line are not toxic to beneficial organisms. Knowledge of this protein'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. The high specificity of EPSPS for its substrates shikimate-3-phosphate and PEP makes it unlikely that the CP4 EPSPS would metabolize endogenous substrates to produce compounds toxic to beneficial organisms. APHIS has not identified any other potential mechanisms for deleterious effects on beneficial organisms.

IV. Conclusions

APHIS has determined that GTS line 40-3-2 field will no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Permits under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of line 40-3-2 or its progeny. (Importation of GTS line 40-3-2 [and nursery stock or seeds capable of propagation] is still, however, subject to the restrictions found in the Foreign Quarantine Notice regulations at 7 CFR Part 319.) This determination has been made based on an analysis which revealed that this soybean line: (1) exhibits no plant pathogenic properties; (2) is no more likely to become a weed than its non-engineered parental variety; (3) is unlikely to increase the weediness potential for any other cultivated plant or native wild species with which the organisms can interbreed; (4) will not cause damage to processed agricultural commodities; and (5) is unlikely to harm other organisms, such as bees, that are beneficial to agriculture. APHIS has also concluded that there is a reasonable certainty that new progeny varieties bred from GTS line 40-3-2 will not exhibit new plant pest properties, i.e., properties substantially different from any observed for the field tested line 40-3-2, or those observed for soybean in traditional breeding programs.

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Date: MAY 19 1994

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