DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 99-002-2]

University of Saskatchewan; Availability of Determination of Nonregulated Status for Flax Genetically Engineered for Tolerance to Soil Residues of Sulfonylurea Herbicides

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public of our determination that the University of Saskatchewan's flax line designated as CDC Triffid, which has been genetically engineered for tolerance to soil residues of sulfonylurea herbicides, is no longer considered a regulated article under our regulations governing the introduction of certain genetically engineered organisms. Our determination is based on our evaluation of data submitted by the University of Saskatchewan in its petition for a determination of nonregulated status and our 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 19, 1999.

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 to facilitate entry into the reading room.

FOR FURTHER INFORMATION CONTACT: Dr. James White, Biotechnology and Biological Analysis, PPQ, APHIS, 4700 River Road Unit 147, Suite 5B05, Riverdale, MD 20737-1236; (301) 734-

5940. 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: kay.peterson@usda.gov.

SUPPLEMENTARY INFORMATION:

Background

On December 1, 1998, the Animal and Plant Health Inspection Service (APHIS) received a petition (APHIS Petition No. 98–335–01p) from the Crop Development Centre (CDC) of the University of Saskatchewan (CDC/Saskatchewan) of Saskatchewan, Saskatoon, Canada, seeking a determination that a flax (*Linum usitatissimum* L.) line designated as CDC Triffid, which has been genetically engineered for tolerance to residues of sulfonylurea herbicides in soil, does not present a plant pest risk and, therefore, is not a regulated article under APHIS' regulations in 7 CFR part 340.

On March 4, 1999, APHIS published a notice in the **Federal Register** (64 FR 10442-10443, Docket No. 99–002–1) announcing that the CDC/Saskatchewan petition had been received and was available for public review. The notice also discussed the role of APHIS, the Environmental Protection Agency, and the Food and Drug Administration in regulating the subject flax line and food products derived from it. In the notice, APHIS solicited written comments from the public as to whether the CDC Triffid flax line posed a plant pest risk. The comments were to have been received by APHIS on or before May 3, 1999. APHIS received no comments on the subject petition during the designated 60-day comment period.

Analysis

The CDC Triffid flax line has been genetically engineered to contain a modified acetolactate synthase (*als*) gene derived from *Arabidopsis thaliana*. The *als* gene encodes a modified acetolactate snythase enzyme that extends to root tissues the reported natural ability of flax to withstand sulfonylurea herbicides. The subject flax line also contains and expresses the nopaline synthase (*nos*) gene derived from *Agrobacterium tumefaciens* and the neomycin phosphotransferase-II (*nptII*) gene derived from *Escherichia coli*. The *nos* and *nptII* genes were used as selectable markers during the plant transformation process. Expression of the added genes is controlled in part by gene sequences from the plant pathogen *A. tumefaciens*, and the *A. tumefaciens* method was used to transfer the added genes into the parental Norlin commercial flax variety.

The CDC Triffid flax line has been considered a regulated article under APHIS' regulations in 7 CFR part 340 because it contains gene sequences derived from a plant pathogen. However, evaluation of data from field tests and site monitoring conducted in Canada indicates that there were no deleterious effects on plants, nontarget organisms, or the environment as a result of the environmental release of the CDC Triffid flax line.

Determination

Based on its analysis of the data submitted by CDC/Saskatchewan and a review of other scientific data and field tests of the subject flax line, APHIS has determined that the CDC Triffid flax line: (1) Exhibits no plant pathogenic properties; (2) is no more likely to become a weed than flax varieties developed by traditional plant breeding; (3) is unlikely to increase the weediness potential for any sexually compatible cultivated or wild species; (4) will not harm nontarget organisms, including threatened or endangered species or organisms that are recognized as beneficial to the agricultural ecosystem; and (5) will not cause damage to raw or processed agricultural commodities. Therefore, APHIS has concluded that the subject flax line and any progeny derived from hybrid crosses with other flax varieties will be as safe to grow as flax in traditional breeding programs that is not subject to regulation under 7 CFR part 340.

The effect of this determination is that CDC/Saskatchewan's CDC Triffid flax line is no longer considered a regulated article under APHIS' regulations in 7 CFR part 340. Therefore, the requirements pertaining to regulated articles under those regulations no longer apply to the subject flax line or its progeny. However, importation of the CDC Triffid flax line 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 (NEPA), as amended (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 CDC/Saskatchewan's CDC Triffid flax line and lines developed from it 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 21st day of May 1999.

Craig A. Reed,

Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 99–13515 Filed 5–26–99; 8:45 am]

BILLING CODE 3410–34–P

USDA/APHIS Petition 98-335-01p for Determination of Nonregulated Status for the Transgenic Flax Line CDC Triffid Tolerant to Sulfonylurea Residues in the Soil

Finding of No Significant Impact

May 1999

The Animal and Plant Health Inspection Service (APHIS) of the U. S. Department of Agriculture has prepared an environmental assessment before issuing a determination of nonregulated status for a genetically engineered flax line designated CDC Triffid. This flax line has been modified to provide enhanced tolerance to sulfonylurea herbicide residues in soil. APHIS received a petition from the University of Saskatchewan's Crop Development Centre seeking a determination that CDC Triffid should no longer be a regulated article under APHIS regulations at 7 CFR Part 340. APHIS has conducted an extensive review of the petition and supporting documentation, as well as other relevant scientific information, to reach its determination that this flax line should no longer be considered a regulated article. APHIS concludes that CDC Triffid: (1) exhibits no plant pathogenic properties; (2) is no more likely to become a weed than traditionally bred flax varieties; (3) is unlikely to increase the weediness potential of any sexually compatible cultivated or wild species; (4) will not harm nontarget organisms, including organisms beneficial to the agricultural ecosystem, and threatened or endangered species; and (5) will not adversely impact raw or processed agricultural commodities. Based upon the analysis documented in this environmental assessment, APHIS has reached a finding of no significant impact (FONSI) on the environment from this determination.

Rebecca A. Bech
Assistant Director
Scientific Services
Plant Protection and Quarantine
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date:
MAY 19 1999

I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has prepared an Environmental Assessment (EA) in response to a petition (APHIS Number 98-335-01p) from the University of Saskatchewan's Crop Development Centre (CDC) in Canada seeking a determination of nonregulated status for the transgenic flax line CDC Triffid. CDC Triffid has been engineered with genes to enhance the tolerance of the plant to sulfonylurea herbicide residues in the soil. The developer of CDC Triffid seeks a determination that this flax line does not pose a plant pest risk and therefore should not be considered a regulated article under APHIS regulations found at 7 CFR Part 340.

CDC Triffid was developed to be cultivated in rotation with crops, particularly wheat and barley, that are treated with sulfonylureas to control broadleaf weeds, providing an alternative to continued cropping or summer-fallowing. CDC Triffid was engineered to be tolerant to sulfonylurea residues in the soil by transforming plant tissue of the flax cultivar Norlin with a modified acetolactate synthase (*als*) gene from *Arabidopsis thaliana*. Two selectable marker genes, neomycin phosphotransferase-II (*npt*II) from *Eschericia coli* and nopaline synthase (*nos*) from *Agrobacterium tumefaciens*, were also introduced and are expressed in the plant. The *als* and *nos* genes are under the control of their native regulatory sequences; the *npt*II gene is under the control of the *nos* regulatory sequences from *Agrobacterium tumefaciens*. Ampicillin and spectinomycin antibiotic resistance selectable marker genes from *E. coli* were introduced into the plant but are not expressed. The genes were introduced via a well characterized *Agrobacterium*-mediated transformation protocol.

This EA specifically addresses the potential for impacts to the environment through the use in agriculture of CDC Triffid. After a review of the available evidence, APHIS believes that CDC Triffid flax will be just as safe as nontransgenic flax that are typically grown and which are not subject to regulation under 7 CFR Part 340. This includes crosses between CDC Triffid and any other flax plant that is not regulated or that has been deregulated under 7 CFR Part 340.

II. INTRODUCTION

A. Development of flax line CDC Triffid tolerant to sulfonylurea residues in the soil

Although the sulfonylurea (SU) class of herbicides are popular with cereal farmers because they give good weed control in low doses, some classes of SU can take a long time to break down in some soil types (especially those with low moisture, low pH and

low temperature). This requires the farmers either to continuous crop to cereals or to summer fallow while the SU residue deteriorates. CDC Triffid provides cereal farmers with an alternative to those practices by allowing them to rotate cereal crops with a broadleaf crop. This may be a more environmentally and agronomically sustainable alternative because it can limit soil erosion associated with summer fallowing and the depletion of nutrients and build up of pathogens associated with continuous cropping.

Ordinary flax is moderately tolerant to SU application, but the roots are sensitive to residual SU in the soil. CDC Triffid was engineered to be tolerant to SU residues in the soil by transforming plant tissue of the flax cultivar Norlin with a modified acetolactate synthase (*als*) gene from *Arabidopsis thaliana*. Two selectable marker genes, neomycin phosphotransferase-II (*npt*II) from *Eschericia coli* and nopaline synthase (*nos*) from *Agrobacterium tumefaciens*, were also introduced and are expressed in the plant. The *als* and *nos* genes are under the control of their native regulatory sequences; the *npt*II gene is under the control of the *nos* regulatory sequences from *Agrobacterium tumefaciens*. Ampicillin and spectinomycin antibiotic resistance selectable marker genes from *E. coli* were introduced into the plant but are not expressed. The genes were introduced via a well characterized *Agrobacterium*-mediated transformation protocol. This protocol has been widely used for over a decade for introducing various genes of interest directly into plant genomes.

CDC Triffid was authorized by the Canadian Food Inspection Agency (CFIA) for field testing under confined conditions in the Canadian provinces of Saskatchewan, Manitoba, and Alberta from 1989 to 1995. It was evaluated extensively to confirm that it exhibits the desired agronomic characteristics and does not present a plant pest risk. In 1998, CDC Triffid was authorized by CFIA for unconfined release into the environment (Decision Document 98-24).

B. APHIS regulatory authority

APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to authority granted by the Federal Plant Pest Act as amended (7 U.S.C. 150aa-150jj) and the Plant Quarantine Act as amended (7 U.S.C. 151-164a, 166-167) regulate the introduction (importation, interstate movement, release into the environment, or any attempt thereat) 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. 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. This CDC Triffid flax line has been considered a regulated article because it contains genetic material from a

plant pathogen, and the vector agent used to deliver the plasmid vector is a plant pathogen.

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 therefore should no longer be regulated. If APHIS determines that the regulated article is unlikely to present a greater plant pest risk than the unmodified organism, the Agency can approve the petition in whole or in part. As such, APHIS permits would no longer be required for field testing, importation, or interstate movement of the non-regulated article or its progeny.

C. EPA and FDA regulatory authority

This genetically engineered flax line may also be subject to regulation by other agencies.

The Environmental Protection Agency (EPA) is responsible for the regulation of pesticides under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) as amended (7U.S.C. 136 *et seq.*). FIFRA requires that all pesticides be registered for use on specific crops prior to distribution or sale. Residue tolerances for pesticides are established by the EPA under the Federal Food, Drug and Cosmetic Act (FFDCA) as amended (21 U.S.C. 301 *et seq.*), and the Food and Drug Administration (FDA) enforces the tolerances set by the EPA under FFDCA. Sulfonylureas are currently not registered for use on flax in the United States or in Canada. However, this CDC Triffid flax line was developed to be tolerant to sulfonylurea residues in the soil when grown in rotation with cereal crops treated with sulfonylureas, and was not developed to be treated with sulfonylurea.

The FDA policy statement concerning regulation of products derived from new plant varieties, including those that are genetically engineered, was published in the Federal Register on May 29, 1992, and appears at 57 FR 22984-23005. The University of Saskatchewan's Crop Development Centre has successfully concluded its consultation with FDA on this CDC Triffid flax line.

III. PURPOSE AND NEED

APHIS has prepared this EA before making a determination on the status of this CDC Triffid flax line as a regulated article under APHIS regulations (7 CFR Part 340). This EA was prepared in compliance with the National Environmental Policy Act (NEPA) of 1969 as amended, (42 USC 4321 *et seq.*) and the implementing regulations and procedures (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 this CDC Triffid flax line is not a regulated article under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would still be required for introductions of this flax line. APHIS might choose this alternative if there were insufficient evidence to demonstrate the lack of plant pest risk from uncontained cultivation of this flax line.

B. Determination that CDC Triffid is No Longer a Regulated Article.

Under this alternative, this CDC Triffid flax line would no longer be a regulated article under the regulations at 7 CFR Part 340. Permits issued or notifications acknowledged by APHIS would no longer be required for introductions of this flax line or its progeny.

V. POTENTIAL ENVIRONMENTAL IMPACTS

If APHIS were to choose the Federal "no action" alternative and not come to a determination that CDC Triffid is not a regulated article (Alternative A), the potential environmental impact would relate to the restricted use of this flax line by the growers. In order to grow CDC Triffid, growers would have to meet the conditions of permit or notification to prohibit persistence of this flax line in the environment by physical or reproductive confinement. Growers who produce flax on a commercial scale would find these conditions difficult, or impossible, to meet. The use of CDC Triffid in cereal crop rotation as an alternative to summer fallowing or continuous cropping to cereals would be limited by the conditions of the regulations. Rotation with a broadleaf crop may be a more environmentally and agronomically sustainable alternative because it can limit soil erosion associated with summer fallowing and the depletion of nutrients and build up of pathogens associated with continuous cropping.

The remaining potential impacts to be addressed in this EA are those that pertain to the use of CDC Triffid in the absence of confinement (Alternative B). Additional technical information is included in the determination document appended to this EA, including detailed discussion of the genetic components used in the construction of CDC Triffid.

Potential for the transformation process or the introduced genetic material to cause CDC Triffid to exhibit plant pathogenic properties

CDC Triffid is unlikely to exhibit plant pathogenic properties due to the transformation process or the introduced genetic material. CDC Triffid has been considered a regulated article because it has the nopaline synthase (*nos*) gene with its native promoter and terminator from the plant pathogen *Agrobacterium tumefaciens* C58 (the causal agent of crown gall disease), and because the *Eschericia coli* neomycin phosphotranferase-II gene (*npt*II) for kanamycin antibiotic resistance is regulated by the *nos* regulatory elements from *Agrobacterium tumefaciens*, and *Agrobacterium tumefaciens* was the vector agent used in the transformation process. No other components of the transformation process were derived from a plant pathogen. Because the genes that cause crown gall disease were removed from the Ti (tumor-inducing) plasmid in *Agrobacterium tumefaciens*, the flax plant does not develop crown gall disease. Once inserted into the chromosome of the plant, the introduced genes are maintained by the plant as any other genes. *Npt*II is not derived from a plant pathogen and the *nos* regulatory elements from *Agrobacterium tumefaciens* do not cause it to exhibit plant pathogenic properties. The *nos* gene codes for the expression in CDC Triffid of nopaline synthase, an enzyme which catalyzes the synthesis of nopaline by the plant. Nopaline is utilized by wild-type soil-borne *Agrobacterium tumefaciens* strains as a carbon and nitrogen source. It does not cause plant disease. Many plant species (i.e. soybeans and cotton) produce nopaline naturally (Christou et al., 1986).

Potential impacts based on the relative weediness of CDC Triffid to traditionally bred flax varieties

Traditionally bred flax (*Linum usitatissimum* L.) is known to escape cultivation and is found in fields and along roadsides throughout the United States and in southern Canada (Gleason and Cronquist, 1991), and is considered a common weed in these places in the United States by Holm et al. (1979). *L. usitatissimum* is not considered a weed in agriculture by the Weed Science Society of America (1992) nor is it on the list of noxious weed species distributed by the Federal Government (7 CFR Part 360). Flax is a poor biological competitor with little persistence or invasiveness (Government of Canada Regulatory Directive Dir94-10; 1994). Other than the introduced sulfonylurea tolerance trait, CDC Triffid is substantially equivalent to the parental plant Norlin and other traditionally bred flax varieties. The introduced trait for tolerance to SU soil residue in CDC Triffid is not expected to cause CDC Triffid to become a serious weed.

Although CDC Triffid may volunteer in the field, because flax is such a poor competitor it is not expected to result in yield reduction to the successive crop. However, it can be controlled if necessary by other herbicides registered for use on traditionally bred flax varieties (e.g. dichlorprop or dicamba). Neither CDC Triffid nor traditionally bred flax volunteers can be controlled by sulfonylureas, since both are tolerant to 'over the top' application of these herbicides, as stated by the petitioner. Field tests by the University of Saskatchewan's Crop Development Centre to study CDC Triffid volunteers in soils

with and without SU residues demonstrated that it is not more persistent or invasive than traditionally bred flax and survivability is similar to the parental cultivar Norlin. CDC Triffid is not expected to pose a more serious problem as a volunteer in the field than traditionally bred flax varieties.

Potential impacts from outcrossing of CDC Triffid to wild relatives

CDC Triffid is unlikely to increase the weediness of any wild relatives. Because flax is almost entirely self-pollinated, hybridization with any other *Linum* species is unlikely (Dillman, 1938; Beard and Comstock, 1980). If the unintended movement of the sulfonylurea tolerance trait from CDC Triffid to other *Linum* species does occur in low frequency, it should not have a significant impact relative to hybridization between those species and traditionally bred flax varieties, especially if sulfonylurea is not applied and sulfonylurea residue is not present in the soil where the hybrids grow. *L. rigidum* and *L.sulcatum*, the only compatible species in the flax growing region, are not weeds and are not likely to become weeds if hybridization occurs (Holm et al., 1979; Muenscher, 1980; Lorenzi and Jeffery, 1987). Hybrids growing in agricultural fields can be eliminated with other herbicides used on traditional flax volunteers if necessary.

Potential impact on threatened or endangered species or nontarget organisms including beneficial organisms

There is no reason to believe that deleterious effects or significant impacts on nontarget organisms, including beneficial organisms, would result from the cultivation of CDC Triffid. The acetolactate synthase (*als*) gene and its regulatory elements which enhance the sulfonylurea tolerance in this flax line come from another plant species, *Arabidopsis thaliana*, and has no known adverse effect on threatened and endangered species or nontarget organisms. There is no indication that it is toxic to organisms. The University of Saskatchewan's CDC demonstrated that the only anti-nutritional component of linseed flax, cyanogenic glycoside, was present in both Norlin and CDC Triffid in similar quantities. Field observations of CDC Triffid in Canada revealed no negative effects of this line on nontarget organisms.

CDC Triffid also produces nopaline due to expression of the nopaline synthase (*nos*) gene from *Agrobacterium tumefaciens* C58. Many plant species (i.e. soybeans and cotton) produce nopaline naturally (Christou et al., 1986). Soil-borne wild type *Agrobacterium tumefaciens* strains utilize this compound as a carbon and nitrogen source. Nopaline produced by CDC Triffid in soils, like that produced by other nopaline producing plant species, may provide an environment for enrichment of this soil bacterium. Many dicotyledonous plants and some gymnosperms are susceptible to infection by *Agrobacterium tumefaciens*. Monocotyledonous plants, such as small grain cereal crops, are not susceptible to infection by this bacterium. The University of

Saskatchewan's Crop Development Centre did an experiment in 1995 to compare the performance of a dicot crop on soil cultivated the previous year with CDC Triffid versus on soil cultivated the previous year with the non-transgenic parent Norlin, and found no difference in plant counts of the dicot following either line, nor were any crown galls present to indicate infection by *Agrobacterium tumefaciens*. As with all flax varieties, cultivation of CDC Triffid for more than one growing season is not encouraged, to prevent the buildup of other root diseases such as *Melampsora lini* (Government of Canada Regulatory Directive Dir94-10, 1994). As with other nopaline producing crop species, rotation of CDC Triffid with monocots (e.g. cereals) is a sensible management practice.

CDC Triffid also contains the *npt*II gene, which confers kanamycin resistance and was used as a selectable marker gene in the development of this flax line. This protein has been reviewed for safe human consumption by FDA (Internet addresses http://vm.cfsan.fda.gov/~lrd/biotechm.html, see: Backgrounder on Biotechnology, and Guidance for Industry). The application of kanamycin to this transgenic flax line when grown on a commercial scale is highly unlikely and would require additional Federal government review.

Potential damage to raw or processed agricultural commodities

Information provided by University of Saskatchewan's Crop Development Centre regarding the processing characteristics of CDC Triffid and studies cited in the petition revealed no differences in any component that could have a direct or indirect plant pest effect on any raw or processed commodity. The genetically engineered modifications of this flax line should not affect any raw or processed commodity.

VI. CONCLUSIONS

APHIS has reviewed the information provided by the University of Saskatchewan's Crop Development Centre and other sources in evaluating the flax line CDC Triffid. After careful analysis of the available information, APHIS concludes that CDC Triffid:

(1) exhibits no plant pathogenic properties;

(2) is no more likely to become a weed than traditionally bred flax varieties;

(3) is unlikely to increase the weediness potential of any sexually compatible cultivated or wild species;

(4) will not harm nontarget organisms, including organisms beneficial to the agricultural ecosystem, and threatened or endangered species; and

(5) will not adversely impact raw or processed agricultural commodities.

VII. REFERENCES

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APPENDIX A

DETERMINATION OF NONREGULATED STATUS FOR TRANSGENIC FLAX LINE CDC TRIFFID TOLERANT TO SULFONYLUREA HERBICIDE RESIDUES IN THE SOIL

United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection and Quarantine
Scientific Services
Riverdale, Maryland

APPENDIX A

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I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS) on reviewing the University of Saskatchewan's Crop Development Centre (CDC) petition 98-335-01p has concluded that the flax line designated as CDC Triffid, which is tolerant to sulfonylurea residues in the soil, does not present a plant pest risk, and therefore is determined to be no longer regulated articles under regulations at 7 CFR Part 340. The applicants are no longer required to obtain a permit or notify APHIS for the unrestricted introduction of CDC Triffid and its progeny lines into the environment within the continental United States and its territories. Exportation of such lines still will remain regulated according to Foreign Quarantine Notice regulations at 7 CFR Part 319.

The University of Saskatchewan's CDC petition was submitted to APHIS on December 1, 1998. On March 4, 1999, APHIS announced the receipt of this petition in the *Federal Register* (64 FR 10442-10443, Docket Number 99-002-1) seeking comments from the interested public. The public comment period ended on May 3, 1999. This petition sought a determination of nonregulated status for the flax line CDC Triffid and its progeny lines from the regulations at 7 CFR Part 340. In the *Federal Register* notice, APHIS indicated its role in the process of reviewing the petition and the roles of the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) in regulating this flax line.

CDC Triffid was developed to be cultivated in rotation with crops, particularly wheat and barley, that are treated with sulfonylureas to control broadleaf weeds, providing an alternative to continued cropping or summer-fallowing. CDC Triffid was engineered to be tolerant to sulfonylurea residues in the soil by transforming plant tissue of the flax cultivar Norlin with a modified acetolactate synthase (*als*) gene from *Arabidopsis thaliana*. Two selectable marker genes, neomycin phosphotransferase-II (*npt*II) from *Eschericia coli* and nopaline synthase (*nos*) from *Agrobacterium tumefaciens*, were also introduced and are expressed in the plant. The *als* and *nos* genes are under the control of their native regulatory sequences; the *npt*II gene is under the control of the *nos* regulatory sequences from *Agrobacterium tumefaciens*. Ampicillin and spectinomycin antibiotic resistance selectable marker genes from *E. coli* were introduced into the plant but are not expressed. The genes were introduced via a well characterized *Agrobacterium*-mediated transformation protocol.

APHIS regulations at 7 CFR Part 340, which were promulgated pursuant to the authority granted by the Federal Plant Pest Act (FPPA) as amended (7 U.S.C. 150aa-jj) and the Plant Quarantine Act (PQA) as amended (7 U.S.C. 151-164a, 166-167) regulate the introduction (importation, interstate movement, release into the environment, or any attempt thereat) of certain genetically engineered organisms and products. An organism is not subjected to the regulatory oversight 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 the submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated under 7 CFR Part 340.

If the Agency determines that the regulated article does not present a risk of direct or indirect injury or damage to plants or plant products, the petition would be approved, thereby allowing for unregulated introduction of the article in question.

The flax line CDC Triffid and its progeny have been considered "regulated articles" under 7 CFR Part 340 because they have been genetically engineered to contain components or DNA sequences from organisms considered to be plant pathogens, and which are on the list of regulated articles (organisms). In this instance, the well-known plant pathogen *Agrobacterium tumefaciens*, and DNA sequences from it, have been used to create CDC Triffid, rendering it to be a regulated article. Field tests of CDC Triffid were authorized by the Canadian Food Inspection Agency (CFIA) under confined conditions in the Canadian provinces of Saskatchewan, Manitoba, and Alberta from 1989 to 1995.

Based on an analysis of data provided to APHIS by the University of Saskatchewan's Crop Development Centre, as well as other scientific data, the agency concluded that CDC Triffid: (1) exhibits no plant pathogenic properties; (2) is no more likely to become a weed than traditionally bred flax varieties; (3) is unlikely to increase the weediness potential of any sexually compatible cultivated or wild species; (4) will not harm nontarget organisms, including organisms beneficial to the agricultural ecosystem, and threatened or endangered species; and (5) will not adversely impact raw or processed agricultural commodities.

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 and procedures (40 CFR 1500-1508, 7 CFR Part 1b; 7 CFR Part 372).

The body of this document consists of two parts: (1) background information which provides the regulatory framework under which APHIS had considered CDC Triffid a regulated article for field testing, interstate movement, and importation, 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 CDC Triffid does not present a plant pest risk.

II. BACKGROUND

A. APHIS regulatory authority.

APHIS regulations at 7 CFR part 340, which were promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA) as amended (7 U.S.C. 150aa-150jj) and the Plant Quarantine Act (PQA) as amended (7 U.S.C. 151-164a, 166-167), regulate the introduction (importation, interstate movement, release into the environment, or any attempt thereat) of certain genetically engineered organisms and products. Under this regulation, 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 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.

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 approved 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 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. 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 approved, thereby allowing for unregulated introduction of the article in question. A petition may be approved in whole or in part.

B. EPA and FDA regulatory authority

CDC Triffid is currently subject to regulations administered by the EPA and the FDA as described in the Environmental Assessment. University of Saskatchewan's Crop Development Centre has successfully completed their consultation process with FDA on food and feed safety for this transgenic flax line.

C. Rationale for developing flax line CDC Triffid tolerant to sulfonylurea soil residues

Sulfonylurea (SU) herbicides applied to cereal crops give good broadleaf weed control in low doses, but some classes of SU can take a long time to break down in some soil types (especially those with low moisture, low pH, and low temperature). This requires the farmers either to continuous crop to cereals or else to summer fallow while the SU residue deteriorates. Ordinary flax is moderately tolerant to SU application, but the roots are sensitive to residual SU in the soil. CDC Triffid was engineered to be tolerant to SU residues in the soil, so it provides cereal farmers with an alternative to continuous cropping or summer fallowing by allowing them to rotate cereal crops with a broadleaf crop. This may be a more environmentally and agronomically sustainable alternative because it can limit soil erosion associated with summer fallowing and the depletion of nutrients and build up of pathogens associated with continuous cropping. In 1998, CDC Triffid

was authorized by the Canadian Food Inspection Agency (CFIA) for unconfined release into the environment (Decision Document 98-24).

III. PUBLIC COMMENTS

APHIS received no comments from the public in response to the Federal Register notice on this petition.

IV. ANALYSIS OF THE PROPERTIES OF FLAX Line CDC Triffid TOLERANT TO SULFONYLUREA HERBICIDE RESIDUES IN THE SOIL

A. The Transformation Process And Introduced Genetic Material Do Not Cause CDC Triffid To Exhibit Plant Pathogenic Properties

CDC Triffid was developed by transformation of the flax line Norlin using the disarmed *Agrobacterium tumefaciens* Ti-plasmid vector pGV3850. The vector contained the T-DNA region of an *Agrobacterium tumefaciens* plasmid from which oncogenic regions had been deleted. DNA sequences included in the T-DNA region, inserted into and expressed in the flax plant, are the acetolactate synthase (*als*) gene from *Arabidopsis thaliana* with its native regulatory sequences, two copies of the nopaline synthase (*nos*) gene with its native regulatory sequences from *Agrobacterium tumefaciens*, and the neomycin phosphotransferase-II gene (*npt*II) for kanamycin antibiotic resistance from *Eschericia coli* with the *nos* regulatory sequences from *Agrobacterium tumefaciens*. Also included in the T-DNA region, inserted but not expressed in the plant, are the spectinomycin and ampicillin resistance genes under prokaryote regulation from *E. coli*, the left border and two copies of the right border sequences of the *Agrobacterium tumefaciens* T-DNA region, and portions of HindIII fragments 10 and 23, with no known open reading frames, from the flanking regions of the right and left borders of the Ti-plasmid vector (Zambryski et al., 1983).

CDC Triffid was developed as an inbred of the initial transformant. Data were presented in the petition by the University of Saskatchewan's Crop Development Centre on Southern DNA restriction analyses conducted on eighth generation self-pollinated seed of CDC Triffid. The results show that there are two insertions of the complete T-DNA in CDC Triffid at different loci, that the transferred DNA does not include functional bacterial DNA outside the T-DNA, and that the T-DNA is stable over several seed generations. Mendelian inheritance studies on the segregation of the sulfonylurea tolerance trait also indicate that there were two loci of insertion.

CDC Triffid has been considered a regulated article because it has the nopaline synthase (*nos*) gene with its native promoter and terminator from the plant pathogen *Agrobacterium tumefaciens* C58 (the causal agent of crown gall disease), the *npt*II gene is regulated by the *nos* regulatory elements from *Agrobacterium tumefaciens*, and *Agrobacterium tumefaciens* was the vector agent used in the transformation process. Because the genes that cause crown gall disease were

removed from the Ti (tumor-inducing) plasmid in *Agrobacterium tumefaciens*, the flax plant does not develop crown gall disease. Once inserted into the chromosome of the plant, the introduced genes are maintained by the plant as any other genes. *Npt*II is not derived from a plant pathogen and the *nos* regulatory elements from *Agrobacterium tumefaciens* do not cause it to exhibit plant pathogenic properties. There were two copies of the *nos* gene in the T-DNA region introduced into CDC Triffid. The *nos* gene codes for the expression in CDC Triffid of nopaline synthase, an enzyme which catalyzes the synthesis of nopaline by the plant. Nopaline is utilized by wild-type soil-borne *Agrobacterium tumefaciens* strains as a carbon and nitrogen source. It does not cause plant disease. Many plant species (i.e. soybeans and cotton) produce nopaline naturally (Christou et al., 1986).

The introduced acetolactate synthase (*als*) gene, with its native regulatory elements from *Arabidopsis thaliana*, is homologous to endogenous *als* in flax. The gene product of *als* is an intermediate enzyme in the biosynthetic pathway for the amino acids leucine, valine, and isoleucine. The *als* gene used in CDC Triffid is modified from the original *Arabidopsis als* by the substitution of a single base change, resulting in a single amino acid change in the protein (Haughn et al., 1988). Enzyme assays demonstrate that *als* activity is higher, as expected, in CDC Triffid than in the parental line Norlin. Although enzyme activity is higher, there is no significant difference between CDC Triffid and Norlin for the three relevant amino acids. The higher enzyme activity does not appear to adversely affect growth (as determined by agronomic parameters) or metabolism (as determined by the amino acid profile). Laboratory assays and field tests demonstrated that the *als* gene from *Arabidopsis* allows CDC Triffid to be grown in soil with sulfonylurea residues. Aside from this desired affect, CDC Triffid is substantially equivalent to Norlin. The *als* gene does not have any plant pathogenic properties and does not make CDC Triffid a plant pathogen.

Ampicillin and spectinomycin resistance genes from *E. coli* were also introduced into CDC Triffid. These genes, used for selection of transformed bacteria, are under prokaryotic regulation and therefore are not expressed in the transformed flax plant.

The transformation process and the introduced genes, their products, and the added regulatory sequences controlling their expression do not cause CDC Triffid to exhibit plant pathogenic properties.

B. CDC Triffid Is No More Likely To Become A Weed Nor Increase The Weediness Potential Of Any Other Plant With Which It Can Breed.

The biology of flax is thoroughly described in the Government of Canada Regulatory Directive Dir94-10 (1994). *Linum usitatissimum* L. is commonly known as flax, flaxseed, or linseed. It is the only member of the genus grown as a crop, although some *Linum* species have limited ornamental use. *Linum usitatissimum* grows throughout the temperate regions of the world,

either for oil or for fibre or both. Flax is grown generally as an oil crop in the United States (James J. Hammond, North Dakota State University, personal communication), in North Dakota primarily and in parts of South Dakota and Minnesota (USDA Agricultural Statistics 1995-96). Although uncertain, it is widely accepted that linseed flax originated in the area east of the Mediterranean Sea towards India because of the diverse forms found in the area (Zeven and Zhukovsky, 1975). The most likely progenitor is *L. angustifolium*, but other species such as *L. bienne* Mill. may have contributed some germplasm (Lay and Dybing, 1989). Oil type flax was grown in southwestern Asia, while the fibre types were developed primarily in the Mediterranean. Lay and Dybing (1989) suggest that selection for annual plants with indehiscent or partially dehiscent seed-bearing capsules has resulted in genotypes suitable for modern agriculture.

Traditionally bred flax (*Linum usitatissimum* L.) is known to escape cultivation and is found in fields and along roadsides throughout the United States and in southern Canada (Gleason and Cronquist, 1991), and is considered a common weed in these places in the United States by Holm et al. (1979). *L. usitatissimum* is not listed as a weed in agriculture by the Weed Science Society of America (1992) nor is it on the list of noxious weed species distributed by the Federal Government (7 CFR Part 360). Flax is a poor biological competitor with little persistence or invasiveness (Government of Canada Regulatory Directive Dir94-10; 1994). Other than the introduced sulfonylurea tolerance trait, CDC Triffid is substantially equivalent to the parental plant Norlin and other traditionally bred flax varieties. The introduced trait for tolerance to SU soil residue in CDC Triffid is not expected to cause CDC Triffid to become a serious weed.

Although CDC Triffid may volunteer in the field, because flax is such a poor competitor it is not expected to result in yield reduction to the successive crop. However, it can be controlled if necessary by a number of other herbicides registered for use on traditionally bred flax varieties (e.g. dichloprop and dicamba). Neither CDC Triffid nor traditionally bred flax volunteers can be controlled by sulfonylureas, since both are tolerant to 'over the top' spray application of these herbicides. Field tests by the University of Saskatchewan's Crop Development Centre to study CDC Triffid volunteers in soils with and without SU residues demonstrated that it is not more persistent or invasive than traditionally bred flax and survivability is similar to the parental cultivar Norlin.

Flax is predominately self-pollinated. As the flower opens, the anthers come together and form a cap over the stigma. The pollen is viable for only a few hours, from the time of anther dehiscence until about the time the petals dehisce, between four and seven hours. Although the "sticky" pollen is not easily transferred by insects, outcrossing can occasionally (less than 2%) result from insect activity (Beard and Comstock, 1980). One study based on morphological evidence of natural outcrossing in flax reported a range in outcrossing from 0-5% among genotypes (Dillman, 1938). There is no isolation distance required for flax foundation seed in the Certified Seed Regulations (7 CFR 201.76), other than that necessary to avoid mechanical mixing of seed.

Three species related to flax are found in the flax growing region of the US: *L. lewisii* (2n=18), *L. rigidum* (2n=30), and *L. sulcatum* (2n=30). Although compatibility and natural hybridization between *L.usitatissimum* (2n=30) and these species has not been reported, it is possible with *L. rigidum* and *L. sulcatum*, since all other species of *Linum* with a ploidy level of 2n=30 crossed with *L.usitatissimum* have been compatible (James J. Hammond, North Dakota State University, personal communication; Gill and Yermanos, 1967; Seetharam, 1972). *L. rigidum* and *L. sulcatum* are not listed as weeds in any of the major weed references (Holm et al., 1979; Muenscher, 1980; Lorenzi and Jeffery, 1987) or by the Weed Science Society of America (1992). Two *Linum* species listed by Holm et al. (1979) as weeds in the US, *L. catharticum* (2n=16) found in eastern US and *L. neomexicanum* found in southwest US, are not found in the flax growing region nor has cross compatibility between these species and *L. usitatissimum* been reported. (Distribution information is primarily from the USDA Natural Resources Conservation Service National PLANTS Database website: http://plants.usda.gov /plantproj/ plants/ plntmenu.html).

Because flax is almost entirely self-pollinated, hybridization with any other *Linum* species is unlikely. If the unintended movement of the sulfonylurea tolerance trait from CDC Triffid to other *Linum* species does occur in low frequency, it should not have a significant impact relative to hybridization between those species and traditionally bred flax varieties, especially if sulfonylurea is not applied and sulfonylurea residue is not present in the soil where the hybrids grow. *L. rigidum* and *L.sulcatum*, the only compatible species in the flax growing region, are not weeds and are not likely to become weeds if hybridization occurs. Hybrids growing in agricultural fields can be eliminated with other herbicides used on traditional flax volunteers if necessary.

CDC Triffid is no more likely to become a weed nor increase the weediness potential of any other flax plant with which it can breed.

C. CDC Triffid Is Unlikely To Harm Nontarget Organisms, Including Beneficial, Threatened Or Endangered Organisms.

There is no reason to believe that deleterious effects or significant impacts on nontarget organisms, including beneficial organisms or threatened and endangered species, would result from the cultivation of CDC Triffid. The acetolactate synthase (*als*) gene and its regulatory elements which enhance the sulfonylurea tolerance in this flax line come from a plant, *Arabidopsis thaliana*, and has no known adverse effect on threatened and endangered species or nontarget organisms. There is no indication that it is toxic to organisms. Field observations of CDC Triffid in Canada revealed no negative effects on nontarget organisms.

The only potentially harmful characteristic naturally produced in flax is the presence of small amounts of cyanogenic glycosides. A typical range for Canadian cultivars is 6.0 to 9.2 mg/g of seed (Bhatty, 1993; Oomah et al., 1992). An analyses by the University of Saskatchewan's CDC

comparing cyanogenic glycoside content of CDC Triffid with the parental line Norlin showed no significant difference between the two. CDC Triffid gave a reading of 7.2 (0.24) mg/g of seed compared to 7.0 (0.15) mg/g in Norlin.

CDC Triffid also produces nopaline due to expression of the nopaline synthase (*nos*) gene from *Agrobacterium tumefaciens* C58. Many plant species (i.e. soybeans and cotton) produce nopaline naturally (Christou et al., 1986). Soil-borne wild type *Agrobacterium tumefaciens* strains utilize this compound as a carbon and nitrogen source. Nopaline produced by CDC Triffid in soils, like that produced by other nopaline producing plant species, may provide an environment for enrichment of this soil bacterium. Many dicotyledonous plants and some gymnosperms are susceptible to infection by *Agrobacterium tumefaciens*. Monocotyledonous plants, such as small grain cereal crops, are not susceptible to infection by this bacterium. The University of Saskatchewan's Crop Development Centre did an experiment in 1995 to compare the performance of a dicot crop on soil cultivated the previous year with CDC Triffid versus on soil cultivated the previous year with the non-transgenic parent Norlin, and found no difference in plant counts of the dicot following either line, nor were any crown galls present to indicate infection by *Agrobacterium tumefaciens*. As with all flax varieties, cultivation of CDC Triffid for more than one growing season is not encouraged, to prevent the buildup of other root diseases such as *Melampsora lini* (Government of Canada Regulatory Directive Dir94-10, 1994). As with other nopaline producing crop species, rotation of CDC Triffid with monocots (e.g. cereals) is a sensible management practice.

CDC Triffid also contains the *npt*II gene, which confers kanamycin antibiotic resistance and was used as a selectable marker gene in the development of this flax line. This protein has been reviewed for safe human consumption by FDA (Internet addresses http://vm.cfsan.fda.gov/~lrd/biotechm.html, see: Backgrounder on Biotechnology, and Guidance for Industry). The application of kanamycin to this transgenic flax line when grown on a commercial scale is highly unlikely and would require additional Federal government review.

D. CDC Triffid Will Not Cause Damage To Raw Or Processed Agricultural Commodities.

Information provided by University of Saskatchewan's Crop Development Centre regarding the processing characteristics of CDC Triffid revealed no differences in any component that could have a direct or indirect plant pest effect on any raw or processed commodity. Agronomic performance of CDC Triffid has been studied and published in McHughen and Rowland, 1991; McHughen and Holm, 1991; McHughen and Holm, 1995; and McHughen et al., 1997. All agronomic parameters measured, including seed yield, time to maturity, plant height, seed weight, oil content, protein, etc., were all not significantly different than the parental Norlin. The genetically engineered modifications of this flax line should not affect any raw or processed commodity.

VI. CONCLUSION

APHIS has determined that CDC Triffid developed by University of Saskatchewan's Crop Development Centre will no longer be considered a regulated article 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 CDC Triffid or its progeny. This determination has been made based on data collected from the field trials, laboratory analyses and literature references presented herein which demonstrate that:

1. CDC Triffid exhibits no plant pathogenic properties. Although DNA from plant pathogens were used in their development, these plants are not infected nor can they incite disease in other plants.

2. CDC Triffid is no more likely to become a weed than traditionally bred flax varieties. Flax is a poor biological competitor and is not considered a serious weed in agriculture.

3. CDC Triffid is unlikely to increase the weediness potential of any sexually compatible cultivated or wild species. Because of the biology of flax, outcrossing is unlikely. If outcrossing should occur in low frequency, it would not have a significant impact relative to hybridization with traditionally bred flax varieties.

4. CDC Triffid will not harm organisms beneficial to the agricultural ecosystem, and threatened or endangered species. None of the genetic material introduced into CDC Triffid make it toxic to organisms, nor would cause it to have a significant impact on nontarget organisms.

5. CDC Triffid will not cause damage to raw or processed agricultural commodities. CDC Triffid is substantially equivalent to the parental line Norlin and should pose no new risks of damage to this commodity.

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Date: MAY 19 2000

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