USDA-APHIS
Environmental Assessment

In response to permit application (06-363-103r), received from SemBioSys, Inc. for a field-test to produce human proinsulin (line 4438-5A) in genetically engineered safflower (*Carthamus tinctorius*) seeds

U.S. Department of Agriculture

Animal and Plant Health Inspection Service

Biotechnology Regulatory Services

TABLE OF CONTENTS

I. SUMMARY

The Animal and Plant Health Inspection Service of the United States Department of Agriculture (USDA-APHIS), has prepared an Environmental Assessment (EA) in response to a request for a permit (APHIS Number 06-363-103r) submitted by SemBioSys Genetics, Inc. (SemBioSys) for a release of genetically engineered (transformed) safflower *(Carthamus tinctorius)*. The genetically engineered (GE) safflower *(Carthamus tinctorius)* was developed to express an oleosin-human proinsulin protein exclusively within its seed. This transformed safflower is currently a regulated article under USDA regulations at 7 CFR Part 340, and as such, any field tests of transformed safflower have to be conducted under a permit issued by APHIS.

II. INTRODUCTION

The World Health Organization (WHO) estimates that more than 180 million people worldwide have diabetes. This number is likely to more than double by 2030. The increase in diabetes worldwide translates into an estimated 8,000 to 11,000 pounds of insulin needed for the year 2005 and is projected to increase to 35,000 pounds by 2010 (http://www.who.int/mediacentre/factsheets/fs312/en/index.html). This demand is expected to grow due to earlier diagnosis, increased incidence based on demographic trends, and consumption and behavioral habits. Insulin use is also expected to increase from new alternative delivery methods, including inhaled insulin devices that require between five and ten times the amount of insulin as injection methods. Existing commercial insulin production methods typically rely on yeast (*Saccharomyces cerevisiae*) or bacteria (*E. coli*) genetically engineered to produce synthetic human insulin. These organisms are grown in large bioreactors, and the insulin is then extracted and purified for final formulation.

SemBioSys believes that safflower-produced insulin will help meet the growing market demands while reducing equipment and manufacturing costs compared to current processes. The transgenic safflower seeds produced in the proposed planting will be ground and used in the development of techniques to extract insulin and is not for commercial production. A technical review of human proinsulin can be found in Appendix IV.

A. USDA Regulatory Authority

APHIS regulates genetically engineered safflower under the authority of the Plant Protection Act of 2000, 7 U.S.C. 7701-7772, and USDA-APHIS regulations under 7 CFR § 340, "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." 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 taxonomic groups listed in the regulation and is also a plant pest, or if there is a reason to believe it is a plant pest. In this submission, safflower has been

genetically engineered using disarmed *Agrobacterium tumefaciens*. *A. tumefaciens* is a plant pest listed under 7 CFR § 340. Thus, the genetically engineered organism in this submission is deemed a regulated article.

B. Food and Drug Administration (FDA) Regulatory Authority

The FDA policy statement concerning regulation of products derived from new plant varieties, including those genetically engineered, was published in the Federal Register on May 29, 1992, and appears at 57 FR 22984-23005. Under this policy, FDA uses what is termed a consultation process to ensure that human food and animal feed safety issues or other regulatory issues (e.g., labeling) are resolved prior to commercial distribution of bioengineered food. This planting is not for commercial distribution, but for development of proinsulin purification techniques.

The Federal Food, Drug and Cosmetics Act (U.S.C. Title 21, Chapter 9), addresses the FDA regulations concerning pharmaceuticals and new drugs (as amended on December 31, 2004). Under these regulations, FDA allows an exemption for investigational new drugs provided required information and protocols have been submitted. SemBioSys has begun the application process needed to apply for an Investigational New Drug (IND) with the FDA in order to commence clinical trials for future dates.

III. PURPOSE AND NEED

The proposed action is for APHIS, Biotechnology Regulatory Services (BRS), to issue a permit for field-testing safflower S-317 in Lincoln County, WA. The safflower is genetically engineered to express, within its seeds, human proinsulin fused to an *Arabidopsis* oleosin. The purpose of this introduction is to obtain a source of seed containing proinsulin to be used to develop an insulin purification process. The permit application was received by APHIS-BRS on December 18, 2006. It was submitted by SemBioSys Genetics, Inc., West Sacramento, CA. The application number is 06-363-103r.

APHIS has prepared this EA before making a determination on whether to allow the planting of transformed safflower under APHIS regulations at the request of SemBioSys. Under regulations in 7 CFR Part 340, APHIS is required to give a determination on the permit application before release into the environment.

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

IV. ALTERNATIVES

Under APHIS regulations, the receipt of a permit application to introduce a genetically engineered organism requires a response from the Administrator:

Administrative action on applications. After receipt and review by APHIS of the application and the data submitted pursuant to paragraph (a) of this section, including any additional information requested by APHIS, a permit shall be granted or denied 7 CFR 340.4(e).

A. No Action: Do not allow planting

For the purposes of this Environmental Assessment, the No Action alternative would be the denial of permit application 06-363-103r. APHIS might choose this alternative if there were evidence to demonstrate a plant pest risk from the cultivation of safflower engineered to express human proinsulin within its seeds.

Under the No Action Alternative, the transgenic safflower plants would not be planted.

B. Issue the Permit: Allow planting with supplemental conditions

In this alternative, issuing this permit would allow the proposed research trial to proceed at a field site in Lincoln County, WA (see Appendix III for additional details). In this alternative, input from the State of Washington and public comment from this environmental assessment would be required. If APHIS chooses this alternative, additional mitigating measures specified within the Supplemental Permit Conditions (Appendix VII) would be required to prevent the persistence of the organism outside the field production area.

Under APHIS regulations, compliance with all mitigating measures is required:

> *Permit conditions.* A person who is issued a permit and his/her employees or agents shall comply with the following conditions (Standard Permit Conditions, Appendix VI), and any supplemental conditions (Supplemental Permit Conditions, Appendix VII) which shall be listed on the permit, as deemed by the Deputy Administrator to be necessary to prevent the dissemination and establishment of plant pests, 7 CFR 340.4(f).

SemBioSys has submitted Standard Operating Procedures (SOP) for this permit [CBI, reviewed by APHIS] detailing protocols for planting, harvesting and storing genetically engineered material to ensure a confined field release. These SOPs follow the requirements set up within the Supplemental Permit Conditions (Appendix VII) and are specific to SemBioSys facilities, equipment and employees. The following mitigation measures also have been incorporated into the permit's experimental procedures:

a. **The field site will be geographically isolated from any commercial safflower production fields or SemBioSys experimental safflower fields, ensuring no cross pollination between the GE safflower and food or feed safflower crops.** The location of the field site has been claimed CBI. Crops surrounding the field site will be barley and wheat with the closest commercial safflower production field expected to be at least 15 miles away. APHIS Supplemental

Permit Conditions (Appendix VII) would require an isolation distance of two miles from any commercial safflower fields. The Association of Official Seed Certifying Agencies (AOSCA) mandates a distance of 1320 ft from contaminating sources for a 99.9% purity of foundation certified safflower seed. The 15 mile distance between the proposed SemBioSys field test site and any commercial safflower fields would exceed the requirements specified by AOSCO and APHIS.

b. **The field test site will be geographically isolated from any sexually compatible wild safflower species to ensure no cross pollination between the GE safflower and any wild safflower species.** There exists about 25 species of wild safflower divided into four sections based on chromosome number (Ashri and Knowles, 1960). Of all the species of wild safflower, cultivated safflower (*C. tinctorius*) is only sexually compatible with the federally listed noxious weed known as wild safflower or jeweled distaff thistle (*C. oxyacanthus*). *C. oxyacanthus* is normally distributed in Afghanistan, Azerbaijan, India, Iran, Iraq, Kyrgyzstan, Pakistan, Tajikistan, Turkmenistan (www.plants.usda.gov). According to the Federal Noxious Weed website (www.invasive.org), Monterey County, CA (southern-central coast) is the only known area where this noxious weed has been found. There are no known wild safflower relatives in Washington State. There is little chance of wild safflower (jeweled distaff thistle) establishing itself in the agriculture area where the SemBioSys field will be located because of the Noxious Weeds Program, a stringent monitoring program set up by APHIS Plant Protection and Quarantine (PPQ).

c. **There are no apiaries within a ten mile radius of the field test site ensuring no pollen flow of safflower by honey bees.** The S-317 safflower cultivar is 85-90% self-pollinating with insects and bees being responsible for the remaining 10-15%. Of insects and bees, honey bees are the primary pollinator for safflower (Eckert, 1962). SemBioSys has provided a list of registered apiaries in Lincoln County. No apiaries are found within a 10 mile radius of the test plot site. Wild bees and pollinating insects are not expected to be present due to insecticides used on adjacent wheat and barley fields to prevent thrips, wireworms, cutworms and other insect pests. Based on the expected absence of beehives in the vicinity of the field trial and the use of insecticides to control insect pests in the area, insect pollination is expected to be less than 10%. (http://www.ipmcenters.org/CropProfiles/docs/wabarley.html, http://www.ipmcenters.org/cropprofiles/docs/IDwheat.html).

d. **The test plot will be surrounded by a 50 ft fallow (bare ground) border to facilitate the detection of any growth of transgenic safflower plants or volunteers that may occur as dedicated equipment is moved throughout the field site**.

If APHIS chooses alternative B, APHIS would require the following safety measures in the Supplemental Permit Conditions (Appendix VII) to promote a confined field release:

a. In addition to any removed transgenic plant material, any non-transgenic plant material removed from the test field plot will be treated as a regulated article. This includes any viable stems, whole plants, seedlings, and seeds.

b. APHIS requires that the field site be at least 2 miles from any commercial safflower production field sites to ensure no outcrossing of the GE safflower with any food or feed safflower crop.

C. Preferred Alternative

APHIS has chosen Alternative B as the preferred alternative based on APHIS' scientific analysis of the permit application. This decision was based on the following points (discussed in depth in the following sections of this EA)

1. The experimental test site is geographically isolated.

2. There are no known wild relatives of safflower in WA.

3. Safflower is not weedy and will not establish itself readily without human intervention.

4. Dedicated equipment will be used during the field test.

5. There are no commercial seed grinding processing plants in Lincoln County and therefore, no commercially produced safflower seed oil products in the area. This ensures the experimental crop will not accidentally be mixed with feed or food sources of safflower.

6. The proinsulin hormone is only found in seeds and not in pollen or elsewhere in plant parts.

7. There are no registered apiaries in a 10 mile radius to add to pollen flow if safflower is unexpectedly within a two mile radius from the test site.

8. APHIS requires all transgenic material to be tracked and labeled after it leaves the test site for further processing, ensuring a chain of custody and prevention of accidental mixing into the food or feed supply chain.

9. Proinsulin lacks biological activity when seeds are ingested.

V. AFFECTED ENVIRONMENT

Safflower is a minor crop of North America and is grown mainly for its seed, which is used as edible oil, meal and birdseed. India produces about half of the world's safflower

each year (430,000 tons) compared to 89,000 tons in the combined United States Great Plains area and southwestern prairies of Canada. In the United States, this annual oilseed crop is adapted primarily to the cereal grain areas of North and South Dakota as well as Montana. In North Dakota, acreage has been concentrated in the western part of the state. It also grows well in the southwestern United States, most notably Arizona and New Mexico (USDA APHIS, 2004).

Traditionally, safflower was grown for its flowers to be used in medicines as well as coloring and flavoring in foods until cheaper aniline dyes came on the market. Safflower oil is used by both food producers and by industry. There are two types of safflower oil with corresponding types of safflower varieties: those high in monounsaturated fatty acid (oleic) and those high in polyunsaturated fatty acid (linoleic). Currently the predominant oil market is for those varieties that produce seed high in oleic acid and very low in saturated fatty acids. High oleic safflower oil is lower in saturates and higher in monounsaturates than olive oil. High oleic safflower is used as a heat stable cooking oil to fry such food items as french fries, chips and other snack items and is also used in cosmetics, food coatings, and infant food formulations. High linoleic safflower oil is also used in human nutrition, but in recent years market demand has drastically shifted from the traditional high linoleic oils to high oleic oil. High linoleic oil is valued as a drying agent in paints and varnishes because of its non-yellowing characteristic. Safflower meal, which is about 24 percent protein and high in fiber, is used as a protein supplement for livestock and poultry feed. The S-317 variety that was transformed by SemBioSys to contain the human proinsulin-oleosin fusion gene is a moderate oleic oil producer (~39% oleic oil production).

Outcrossing between safflower plants has been reported to be anywhere from 0 to 100% (Claassen, 1950); (Knowles, 1980) with an average between 15 and 20% (based on dominant flower-color markers). The S-317 safflower cultivar is 85-90% self-pollinating with bees being primarily responsible for the remaining 10-15% cross pollination.

Wild relatives of cultivated safflower, *Carthamus creticus* and *C. oxyacanthus,* have been reported to occur sporadically in several U.S. states (Kartesz, 2004) and are listed as noxious weeds. *C. creticus* is not sexually compatible with cultivated safflower due to its chromosome number (2n=20 or 4n=44 compared to that of cultivated safflower with a chromosome number of n=12). Although, *C. oxyacanthus* has been reported in California (specifically in Monterey County), this sexually compatible species is rare and has not been detected in Washington. (Kartesz, 2004); (Kiel and Turner, 1993). Noxious weeds are carefully monitored, quarantined, and subject to eradication efforts thereby minimizing the possibility these species will establish. Because they are listed as noxious weeds, there are detailed records of their distribution.

The field site (<1 acre) will be located on private property in Lincoln County, WA. This county contains a mix of agricultural sagebrush-steppe ecosystems with an average rainfall between 7-10 inches per year (www.worldclimate.com). The adjacent agricultural lands will grow barley and wheat. There are no bodies of water (lakes, streams or rivers) within a 5 mile radius of the proposed field test site. Since the human

proinsulin is only expressed in the safflower seeds and not the pollen, any safflower pollen that could be blown far enough away during a rogue wind event will not affect any aquaculture in the area. Wind is also not known to be a significant pollen dispersal agent as the pollen is large, having a mean diameter of 53-56 μm (Berglund et al., 1998).

Safflower grows best with low atmospheric humidity and in deep soils with good internal drainage. It also has good drought and heat tolerance and does not survive in standing water, especially in warm weather (air temperature above 68°F) where it will die in less than two hours (Berglund et al., 1998). Most of the diseases that safflower is susceptible to occur when soil moisture and humidity conditions exceed the optimal. Crop rotation, careful irrigation practices, and planting treated and disease-free seed are important methods for controlling losses from disease.

Large or small animals are unlikely to browse the safflower field sites during plant growth due to the sharp spines that S-317 safflower cultivar produces. Animals would most likely only browse for loose seeds after harvest. Smaller animals such as rodents and rabbits normally would only scavenge for dropped seeds after harvest, as the seed head is tough and difficult to access during maturation compared to the cereal grains that are to be planted in adjacent fields. The transformed safflower seed is of the striped variety, which is associated with an unpalatable color and odor. More palatable cereal grains in nearby fields would more likely be the target of any seed scavenging animals. Another built-in deterrent will be the proposed 50 ft fallow (bare ground) zone around the field test site. Many small seed-scavenging mammals are unwilling to be exposed to predators over such distances in order to reach a food source when there are more accessible food sources nearby such as wheat and barley. Birds rarely feed on standing mature safflower fields due to the tough, tightly held seed heads (Berglund et al., 1998). Birds could scavenge loose seeds after harvest, but do not prefer the striped seed safflower variety (white hulled variety is readily eaten by birds and is sold as birdseed) and would most likely be more attracted to the nearby cereal grain fields SemBioSys contract farmers have not reported any problems with birds during the maturation stage of the safflower plants. The closest migratory pathway for birds is the Pacific Flyway. The territory of this flyway comprises the western Arctic, including Alaska and the Aleutian Islands and the Rocky Mountain and Pacific coast regions of Canada, the United States and Mexico, south to where it becomes blended with other flyways in Central and South America. (http://www.birdnature.com/flyways.html). This flyway does not pass through Lincoln County in WA. (The closest the Pacific Flyway comes to Lincoln County is approximate 125 mi in either easterly or westerly directions.)

Safflower has relatively few insect pests that cause economic damage and the use of insecticides against safflower pests generally is not required. Insect damage to safflower can occur at crop establishment, during seedling and stem growth, and during the bud to flower stage. The most susceptible period likely is the bud to flower stage. Thrips and lygus bugs potentially are the most damaging pests to the plant, but do not injure the seed head other than superficially. The head becomes too tough for more than localized injury and seed loss is rare (http://agric.ucdavis.edu/crops/oilseed/saff11insect.htm). In practice, wireworms and cutworms, which affect stand establishment (germination and

seedling development), and lygus bugs, which migrate from safflower to cotton, are the only insects commonly controlled with pesticides in safflower. Wireworms can reduce stands but can be controlled with Lindane either as a planter box treatment or as a combination with seed treatment fungicides. In cotton growing regions, however, safflower may be sprayed to control lygus bugs as the crop begins to mature, to prevent the migration of those insects to nearby cotton fields. This control is for the sake of the cotton, rather than the safflower. Since no cotton is expected to be planted nearby the safflower field test site, it is unlikely that insecticides will be used at all.

The most serious pests of safflower are weeds. Early weeds may compete with safflower for moisture, sunlight, and nutrients, lowering production and increasing cultivation costs. Heavy infestations of weeds later in the season may interfere with mechanical harvesting. Since safflower often matures before many common weed species, green weed matter taken in by the harvester impairs quality and must be cleaned at the grower's expense.

After harvest, seeds will be shipped to a storage and processing plant owned by SemBioSys in Calgary, Canada following SemBioSys' standard operating procedure [CBI, reviewed by APHIS] and as described in BRS Variance #06-027 to prevent dissemination of genetically engineered plants. All grinding and processing equipment will be dedicated for use only with GE safflower and will not process food or feed from non-transformed safflower.

A more detailed description of the biology of safflower, including genetics and out-crossing is available in Appendix I, page 22.

VI. POTENTIAL ENVIRONMENTAL IMPACTS

1. Potential impacts from gene introgression from GE safflower into its sexually compatible relatives

Safflower genes may escape from the test plots in two ways. The first pathway of escape is by pollen transfer. The second is by movement of propagative material, *i.e*., whole seeds, or by vegetative growth.

Pollen Movement

Pollen gene flow is expected to be limited in safflower for the following reasons:

a) The field site will be at least 2 miles and up to 15 miles from any commercially produced safflower fields.

b) Cultivated safflower is not sexually compatible with plant species outside of the *Carthamus* genus. The only wild relative it could hybridize with is listed as a noxious weed (http://www.invasive.org/browse/subject.cfm?sub=4553) and is not found in Washington State.

c) The S-317 safflower cultivar is 85-90% self-pollinating with insects and honey bees being responsible for the remaining 10-15%. Of insects and bees, honey bees are the primary pollinator for safflower (Eckert, 1962). The average foraging radius of honey bees from the colony is only a few hundred meters in agricultural areas and they typically do not move beyond 1.6 km (1 mi) (Winston, 1987). However, foragers may fly up to 10 km (6.25 mi) and cover a 100 km^2 (38 sq mi) area around the hive (Seeley, 1995), and there is evidence of honey bees flying several kilometers (2-3 mi) between apiaries and to safflower fields (Gary et al., 1977). No apiaries are found within a 10 mile radius of the proposed test plot sites. A caged study was done comparing honey bees to convergent lady beetles (*Hippodamia convergens*), lygus (*Lygus hesperus*), and flower beetle (*Notoxus calcaratus*) as pollinators of safflower. This study showed that honey bees, followed by paper wasps (*Polistes exclamans*; a wasp of the Atlantic seaboard states) were the primary pollinators of safflower while the other insects demonstrated little or no pollinator activity (Levin et al., 1967). Any insect pollinators will not be affected by expression of human proinsulin because it is only expressed in the seed of safflower.

d) Wind is not known to be a significant pollen dispersal agent, most likely due to the pollen's large size (mean diameter of 53-56 μm).

Human proinsulin is not expressed in pollen or any of the vegetative material except seeds (see Figure 1). Because it is not expressed in the pollen, no cumulative effects of human proinsulin are expected if APHIS chooses to allow planting and the pollen falls outside the field test site.

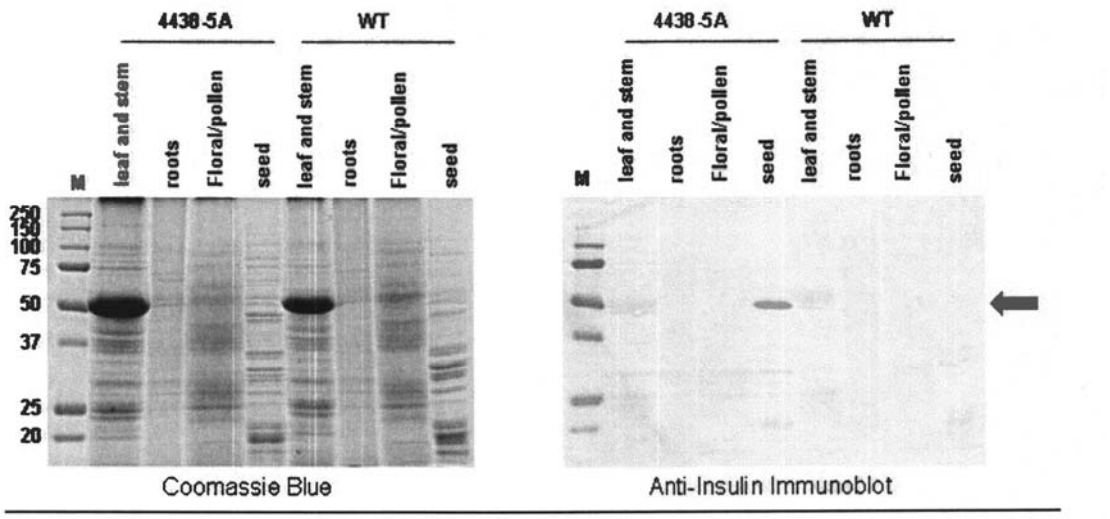

Figure 1. The proinsulin fusion protein accumulates in seed tissue only. 4438-5A refers to the proinsulin fusion safflower line and WT is the non-transgenic safflower.

Seed Movement

Movement of whole seed by animals is one way by which safflower seed is disseminated. For example, rodents may carry seed to new areas where it could become established; however, eaten seed is not expected to survive the digestive processes (USDA APHIS, 2004). Safflower seeds generally lack dormancy and are light sensitive; allowing for monitoring of any volunteers in the short distances that small animals could carry viable seed away from the field site. As mentioned above, the proposed field site will have a 50 ft fallow (bare ground) zone around the field test site. Many small seed-scavenging mammals are unwilling to be exposed to predators over such distances in order to reach a food source when there are more accessible food sources nearby such as wheat and barley. The S-317 cultivar is a spiny variety (comparable to many other spiny thistles) that discourages large animals to browse the fields after flowering. The S-317 safflower also has a striped hull, and is not as palatable (due to odor and color) to birds as the white hulled variety. The nearby fields of wheat and barley would more likely be targeted by scavenging by birds than the maturing safflower field. Due to the large seed size, any seeds eaten by birds will be ground up and digested and are not expected to remain viable after passing through the avian digestive tract.

Any foraging animals that ingest the transgenic safflower seeds will effectively digest the seed and proinsulin protein. Proinsulin is the inactive form of insulin (See Appendix IV for a more detailed review on proinsulin) and, like insulin, has no biological activity when ingested (Crane et al., 1968). Like most peptides and proteins that animals eat as food sources, the seed and proinsulin will be broken into amino acids or small peptides within the stomach before they can be absorbed in the intestine. This breakdown of protein begins in the stomach where hydrochloric acid (HCl) denatures the protein and facilitates the action of pepsin, the major gastric enzyme that splits the peptide bonds. Other proteolytic enzymes (enzymes that break down protein bonds) involved in the gastric process are trypsin, chymotrypsin, carboxypeptidase and elastase (Zeitlin et al., 1999). In simulated digestive studies, active insulin is completely digested in 60 minutes whereas the proinsulin seed fusion protein is completely digested in 15 minutes (SemBioSys, permit application data). In the unlikely event of an exclusive diet of transgenic proinsulin safflower seeds, no effects of human proinsulin are expected on animals due to the digestion process.

Another way that seeds could be dispersed outside the field test site is by human error. In a recent workshop hosted by APHIS dealing with gene confinement issues in genetically engineered crops (USDA APHIS, 2004), one of the more likely mechanisms contributing to the breakdown of confinement and movement of seed was identified as human error, and the most reliable means of preventing this is to maintain and reinforce stringent standard operating procedures. In this study, the applicant will follow detailed SOPs [CBI, reviewed by APHIS] to prevent accidental dispersal of the seeds or plants into the environment. These SOPs detail the chain of custody of the GE safflower material (labeling and record keeping procedures) as well as procedures necessary to clean dedicated equipment used at the field site. All site managers, farmers and technicians have been given a copy of these SOPs and are trained in their implementation. The

SOPs, while CBI, are in keeping with the requirements listed in the Supplemental Permit Conditions (pages 35-41).

The seeds are the only place where the gene of interest (human proinsulin) is expressed. Due to the reasons listed above, APHIS believes this field test will remain contained. Since there are no bodies of water within a 5 mile radius of any of the proposed field test sites, it is unlikely that aquatic animals will be exposed to any human proinsulin should GE safflower seeds be transported outside the field test site despite strict confinement measures. Should APHIS choose Alternative B and allow planting, APHIS has determined that the current permit and supplementary permit conditions will confine the crop and alleviate the risk of gene flow through seed movement and mitigate any cumulative effects of the expressed gene within the seeds themselves.

Vegetative Growth

Safflower is not known to spread vegetatively and propagates only through seed germination. The inclusion of a 50 ft fallow zone (bare ground) around the experimental field sites is primarily to be used as an equipment staging and turn-around area. Any seeds dropped during harvest would germinate in this zone and be easily seen and destroyed. The fallow zone also serves as a deterrent for small animals looking to forage seed from the safflower field sites. Few small animals will cross such a distance and be exposed to predation when there are more easily accessible cereal fields nearby (wheat and barley). No cumulative effects of human proinsulin are expected as volunteers in this area will be monitored and properly destroyed before seed set.

Horizontal Gene Transfer

Transfer and expression of DNA from the plant to bacteria is unlikely to occur. Gebhard and Smalla (Gebhard and Smalla, 1999) and Schlüter *et al.* (Schlüter et al., 1995) have studied transgenic DNA movement to bacteria and although theoretically possible, it occurs at extremely low rates (approximately 1 in 10^{-14}). Many genomes (or parts thereof) have been sequenced from bacteria that are closely associated with plants including *Agrobacterium* and *Rhizobium* (Kaneko et al., 2000). There is no evidence that these organisms contain genes derived from plants. Koonin *et al.* (Koonin et al., 2001) and Brown (Brown, 2003) presented reviews based on sequencing data that revealed horizontal gene transfer occurs occasionally on an evolutionary time scale of millions of years. Even in the unlikely event transfer were to occur, the gene would be poorly expressed, if at all, because transgene promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression. A more detailed description of the transforming method and the genes involved can be found in Appendix II, page 24.

If APHIS chooses the no action alternative (Alternative A), APHIS would not permit the environmental release of this GE safflower. If APHIS chooses Alternative B, no gene introgression is expected due to safflower biology and geographical location of the field test sites.

2. Potential impacts based on the relative weediness of GE safflower

During early stages of growth, cultivated safflower is slow growing and a poor competitor with fast growing weeds. If not controlled, weeds can grow taller than safflower and compete for light, nutrients and moisture and ultimately can cause complete crop losses if left uncontrolled (Dajue and Mündel, 1996). The lack of seed dormancy also decreases the weediness potential of cultivated safflower, and volunteers after harvest are not common. The transgenic safflower plants are no weedier than traditional cultivated safflower; which has limited weediness potential due to its biology (also see Appendix I for a detailed description of safflower biology).

Because cultivated safflower is not described as a weedy species and none of its sexually compatible weedy species are known to be present in Washington State, there would be no weedy impact from allowing the field test for seed increase to occur (Alternative B). If APHIS chooses the no action alternative (Alternative A) there would also be no weed impact from this variety.

3. Potential impact on non-target organisms, including beneficial organisms and threatened or endangered species

No commercially grown safflower for seed oil is grown in WA due to the lack of processing (seed grinding) facilities. Since the proinsulin hormone is only found in the seeds, the only way for the seeds to end up in the food and feed supply would be for the transgenic seeds to be inadvertently shipped to a seed processor. APHIS requires comprehensive procedures and safeguards be implemented by SemBioSys to prevent contamination, release, and dissemination of the regulated article during any shipping activities. After harvest, seeds will be shipped to a storage and processing plant owned by SemBioSys in Calgary, Canada following SemBioSys' standard operating procedure [CBI, reviewed by APHIS] and as described in BRS Variance #06-027. All grinding and processing equipment will be dedicated for use only with GE safflower and will not process food or feed from non-transformed safflower.

Dermal exposure and ingestion are not viable delivery routes of proinsulin. The proinsulin protein is associated exclusively with the endosperm of the seed and not found in the seed coat or other plant parts (see Figure 1, page 11). Inhalation of endosperm seed dust from grinding would be the only way exposure to proinsulin could occur. SemBioSys has reported that each safflower seed contains approximately 66 μg of proinsulin-oleosin fusion protein. The literature has been reported that proinsulin has approximately 10% biological activity of the active hormone, insulin on *in vitro* cell culture (Kitabchi, 1970; Yu and Kitabchi, 1973). The mean weight of 1000 safflower seeds is 32g (about 1 oz) (Camas and Esendal, 2006) and would potentially contain 66 mg of proinsulin. That amount would translate to 6.6 mg of insulin-type activity. It has been reported that one unit of pure insulin is 38.5 μg (Volund, 1993). Therefore, one ounce of dust has the potential to deliver 171 U of insulin activity. Inhaled delivery of insulin has been method recently approved by the FDA (http://www.fda.gov/bbs/topics/news/2006/NEW01304.html). One of the potential problems is that only 10% of insulin delivered nasally or via lungs is transported across

the membranes, requiring large amounts to be inhaled in order to be effective (Hite et al., 2006). Therefore the one ounce of seed dust inhaled will deliver 17.1 U of insulin-type activity from the proinsulin hormone provided all the dust is inhaled and retained. This potential route of exposure will require SemBioSys to provide adequate respiratory protection to workers during the processing of its transgenic safflower seeds. A more detailed description of proinsulin can be found in Appendix IV, page 27.

Safflower has minimal insect pests and none that feed directly on the seed; therefore, APHIS has determined there will be no impact of human proinsulin on insects should Alternative B be chosen. APHIS has determined there will be no impact of human proinsulin on birds or scavenging animals that could possibly ingest seed (refer to Seed Movement section). Due to the lack of ingested toxicity of the protein that will be produced APHIS concludes that if it chooses Alternative B, there will be no significant effect on any native faunal species for Lincoln County.

APHIS has analyzed the direct and indirect effects of Alternative B on federally listed threatened and endangered species and proposed species and designated critical habitat and habitat proposed for designation as required by Section 7 of the Endangered Species Act (ESA) of 1973 (see Appendix V). In conclusion, APHIS has determined that the proposed environmental release described in Alternative B will have no effect on federally listed threatened and endangered species or species proposed for listing, and will have no effect on designated critical habitat or habitat proposed for designation. Likewise, if APHIS chooses the no action alternative (Alternative A) there will be no impact on non-target organisms, and no effect on federally listed threatened and endangered species or species proposed for listing, and will have no effect on designated critical habitat or habitat proposed for designation. Consequently, consultation under Section 7 of the Endangered Species Act with the U.S. Fish and Wildlife Service will not be required.

4. Potential impacts on biodiversity

Analysis of available information indicates that SemBioSys' GE safflower containing the human proinsulin-oleosin fusion gene exhibits no traits that would cause increased weediness in the proposed planting area; nor should it lead to increased weediness of other cultivated safflower or other sexually compatible relatives. Furthermore, it is unlikely to harm non-target organisms common to the agricultural ecosystem or threatened or endangered species recognized by the U.S. Fish and Wildlife Service. There has been no intentional genetic change in these plants to affect their susceptibility to disease or insect damage. Neither the selectable marker gene [CBI, reviewed by APHIS], nor the human proinsulin-oleosin fusion gene is expected to change any plant pest characteristics. There is no reason to believe that weediness or plant pest characteristics are different between the genetically engineered and non-engineered plants. The selectable marker gene [CBI, reviewed by APHIS] is not expected to alter the susceptibility of the transgenic safflower plants to disease or insect damage.

Execution of the prescribed periodic monitoring of the field plots will allow the detection of any unexpected infestation by plant disease organisms or animal pests. SemBioSys, Inc. is required to report any such unanticipated effects to APHIS under the terms of the permit. See 7 CFR § 340.4(f)(10)(ii).

If APHIS chooses either of the alternatives, there would also be no impact on biodiversity.

5. Potential impacts on agricultural practices

No impact on existing agricultural practices is expected if APHIS allows planting (Alternative B). SemBioSys will employ agricultural practices consistent with growing healthy safflower plants. Weeds will be controlled using herbicide applications such as glyphosate both pre-, during, and post-harvest. A description of SemBioSys' agricultural practices can be found in Appendix III, page 26.

No environmental impacts on nearby crops are expected if APHIS chooses to allow planting (Alternative B). No safflower seed production plots are adjacent to the field test area. Barley and wheat are the only crops that will be grown in the adjacent agricultural land. The closest commercial safflower seed production field is expected to be greater than 15 miles away from the closest test plot site and may be no closer than 2 miles from the test plot.

6. Potential impacts on organic farming

The National Organic Program (NOP) administered by USDA's Agricultural Marketing Service (AMS) requires organic production operations to have distinct, defined boundaries and buffer zones to prevent unintended contact with prohibited substances from adjoining land that is not under organic management. Organic production operations must also develop and maintain an organic production system plan approved by their accredited certifying agent. This plan enables the production operation to achieve and document compliance with the National Organic Standards, including the prohibition on the use of excluded methods. Excluded methods include a variety of methods used to genetically modify organisms or influence their growth and development by means that are not possible under natural conditions or processes.

Organic certification involves oversight by an accredited certifying agent of the materials and practices used to produce or handle an organic agricultural product. This oversight includes an annual review of the certified operation's organic system plan and on-site inspections of the certified operation and its records. Although the National Organic Standards prohibit the use of excluded methods, they do not require testing of inputs or products for the presence of excluded methods.

The presence of a detectable residue of a product of excluded methods alone does not necessarily constitute a violation of the National Organic Standards. The unintentional presence of the products of excluded methods will not affect the status of an organic product or operation when the operation has not used excluded methods and has taken

reasonable steps to avoid contact with the products of excluded methods as detailed in their approved organic system plan. Organic certification of a production or handling operation is a process claim, not a product claim.

It is not likely that organic farmers or other farmers will be significantly impacted by the expected planting of this product since this proposed planting is greater than two miles away from any cultivated safflower fields, which is 8 times the distance required to produce certified organic safflower seed of 99.9% purity.

This particular product should not present new and different issues regarding the use of pesticides and other organic cultivation practices. APHIS has considered that gene transfer to cultivated *Carthamus* species in the U.S. would be limited due to its status as a minor North American crop and the geographical distance between the proposed field test sites and any other conventional safflower production fields.

If APHIS chooses either of the proposed alternatives, there would be no impact on organic farmers and their current cultivation practices are unlikely to change.

7. Potential impacts on raw or processed agricultural commodities

The genetically engineered safflower is not being planted for commercial sale or use and therefore, it will not have any opportunity to come into contact with any commercialized raw or processed commodities. Nevertheless, the field test sites will be confined by strict requirements and conditions pursuant to prevent its presence in such commodities.

There would be no impacts on raw or processed agricultural commodities if APHIS chooses the No Action option regarding the genetically engineered safflower (Alternative A). There would also be no impacts from allowing the GE safflower to be planted (Alternative B), should APHIS choose to allow planting with supplemental conditions.

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

Executive Order (EO) 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations," requires Federal agencies to conduct their programs, policies, and activities that substantially affect human health or the environment in a manner so as not to exclude persons and populations from participation in or benefiting from such programs. It also enforces existing statutes to prevent minority and low-income communities from being subjected to disproportionately high and adverse human health or environmental effects.
EO 13045, “Protection of Children from Environmental Health Risks and Safety Risks,” acknowledges that children may suffer disproportionately from environmental health and safety risks because of their developmental stage, greater metabolic activity levels, and behavior patterns, as compared to adults. The EO (to the extent permitted by law and

consistent with the agency's mission) requires each Federal agency to identify, assess, and address environmental health risks and safety risks that may disproportionately affect children. Each alternative was analyzed with respect to EO 12898 and 13045. None of the alternatives are expected to have a disproportionate adverse effect on minorities, low-income populations, or children.

EO 13112, "Invasive Species", states that federal agencies take action to prevent the introduction of invasive species and provide for their control and to minimize the economic, ecological, and human health impacts that invasive species cause. Safflower is a minor crop in the United States. Based on historical experience with safflower and the data submitted by the applicant and reviewed by APHIS, the engineered plant is sufficiently similar in fitness characteristics to other safflower varieties currently grown that it is not expected to have an increased invasive potential.

Executive Order 12114, "Environmental Effects Abroad of Major Federal Actions" requires Federal officials to take into consideration any potential environmental effects outside the U.S., its territories and possessions that result from actions being taken. APHIS has given this due consideration and does not expect a significant environmental impact outside the United States should APHIS choose any of the listed alternatives to permit# 06-363-103r. APHIS has determined that the shipment to the SemBioSys Canadian processing facility after harvest does not pose an environmental risk because of stringent container and shipping requirements for export. Any international traffic of genetically engineered safflower subsequent to a determination of regulated status for GE safflower would be fully subject to national phytosanitary requirements and be in accordance with phytosanitary standards developed under the International Plant Protection Convention (IPPC).

APHIS continues to work toward harmonization of biosafety and biotechnology consensus documents, guidelines and regulations, including within the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the United States and in the Organization for Economic Cooperation and Development. NAPPO has completed three modules of a standard for the *Importation and Release into the Environment of Transgenic Plants in NAPPO Member Countries* (see http://www.nappo.org/Standards/Std-e.html). APHIS also participates in the North American Biotechnology Initiative (NABI), a forum for information exchange and cooperation on agricultural biotechnology issues for the U.S., Mexico and Canada. In addition, bilateral discussions on biotechnology regulatory issues are held regularly with other countries including: Argentina, Australia, Canada, China, Japan, Korea, Philippines, South Africa, Switzerland, and the United Kingdom.

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X. APPENDICES: Summary of permit data and information considered in completing environmental assessment

Appendix I. Biology of Safflower

In this section of the environmental assessment, the biology of safflower and plants related to safflower are considered along with potential routes of gene escape. Because the mechanism by which genes are moved from one flowering plant to another is through cross pollination of sexually compatible plants, the plants with which safflower can cross-pollinate are also described. Below is an analysis of the biology of safflower. This review focuses solely on safflower in the United States.

Systematics of Safflower

Safflower, *Carthamus tinctorius* L., is a member of the family Compositae (Asteraceae) tribe Cardueae, and subtribe Centaureinae (Garcia-Jacas et al., 2002). Safflower is a highly branched herbaceous annual thistle, usually with sharp spines on the leaves. Plants are 30-150 cm tall with globular flower heads (capitula) and, commonly, brilliant yellow, orange or red flowers. Each branch usually has one to five flower heads, and the flower head typically has 15 to 20 (but up to 180) florets, each of which can produce a dry fruit (achene) with a single large seed (Dajue and Mündel, 1996). The taproot can penetrate to 8 to 10 feet if subsoil temperature and moisture permit. As a result, safflower is more tolerant to drought than small grains (Berglund et al., 1998). The florets are self-pollinating, but seed set can be increased by bees or other insects.

Origin and Distribution of Safflower

Safflower originated in southwest Asia (where the genus is native) and moved to India and China. It is a minor crop of North America and is grown mainly for its seed, which is used for edible oil, meal and birdseed. India produces about half of the world's safflower each year (430,000 tons) compared to 89,000 tons in the combined United States Great Plains area and southwestern prairies of Canada (Ekin, 2005). The U.S. safflower acreage varies widely (annual acreage ranges between 100,000 and 200,000 acres) with the majority of production in California (USDA APHIS, 2004). The oilseed crop is also adapted to the cereal grain areas of North and South Dakota as well as Montana.

Outcrossing

Outcrossing between safflower crops has been reported to be anywhere from 0 to 100% (Claassen, 1950) with an average between 15 and 20% (based on dominant flower-color markers) (Knowles, 1980). The S-317 safflower cultivar is 85-90% self-pollinating with bees being primarily responsible for the remaining 10-15% of outcrossing.

Cultivated safflower originated in the Euphrates Basin and from this center of origin expanded to Egypt, Ethiopia, southern Europe and the Far East (McPherson et al., 2004). It can potentially hybridize with at least six species of wild *Carthamus* (McPherson et al.,

2004). Of the four naturalized wild relatives in the New World, only *C. oxyacanthus* (or *C. oxyacanthus*) and *C. creticus* (*C. lanatus* subsp. *creticus*, *C. baeticus*), have produced fertile F1 hybrids when crossed with *C. tinctorius* (McPherson et al., 2004). *Carthamus creticus* and *C. oxyacanthus* have been reported to occur in several U.S. states (Kartesz, 2004) and are listed as noxious weeds, so they must be removed wherever they are found (thus also minimizing the potential for outcrossing).

There are some areas in the United States and Canada where no cultivated or wild *Carthamus* are currently found; hence cultivation in isolation is possible. For example, no wild relatives of safflower occur in Washington State, whereas *C. oxyacanthus* has been reported in California (Kartesz, 2004); (Kiel and Turner, 1993). Overall, the sexually compatible weedy wild *Carthamus* are quite rare and their presence can be verified in each county because they are noxious weeds.

Dispersal/Pollination Mechanisms

Safflower is largely self-pollinated, and bees are occasionally a pollen vector, whereas wind is not known to be a significant dispersal agent (Knowles, 1980);(Langridge and Goodman, 1980). Morphological characteristics (e.g., moderately large pollen grains, with a mean diameter of 53-56 μm) and the behavior of the floret in pollen presentation (Knowles, 1980) help to explain the apparent lack of significant wind dispersal (Carapetian, 1994). Most pollen movement has been thought to occur within 2 meters of the source (USDA APHIS, 2004).

Bees are the main pollinator moving pollen among flowers (florets) and flower heads (Langridge and Goodman, 1980). Most honey bee colonies are managed, as there are very few feral populations left in the United States due to invertebrate pests (e.g., varroa mites and wax moths) and disease (e.g., foul brood and chalk brood). Honey bees are the most important contributors to long-distance pollen movement. The average foraging radius of honey bees from the colony is only a few hundred meters in agricultural areas and they typically do not move beyond 1.6 km (1 mi) (Winston, 1987). However, foragers may fly up to 10 km (6.25 mi) and cover a 100 km^2 (38 sq mi) area around the hive (Seeley, 1995), and there is evidence of honey bees flying several kilometers (2-3 mi) between apiaries and to safflower fields (Gary et al., 1977). Native bees tend to fly shorter distances than honey bees. Although bumble bees typically forage close to their nests, they may travel 5 km (3 mi) from the nest (USDA APHIS, 2004).

Pollen Competition and Viability

Issues related to pollen viability include how far pollen can travel and how long it will stay viable on the pollinator. Bees tend to prefer foraging on viable pollen. Pollen viability is influenced by environmental factors such as relative humidity and temperature, and can vary among cultivars. Safflower is typically grown in dry conditions, where pollen is expected to desiccate rapidly. The viability of pollen is variable between safflower varieties, but pollen viability is very short, lasting less than 24 hours and perhaps into the following day (Knowles, 1980).

Appendix II. Description of the Regulated Safflower Plant

SemBioSys, Inc has engineered safflower to contain the human proinsulin gene fused with an oleosin gene from *Arabidopsis*. The recipient organism, *Carthamus tinctorius*, cv. S-317, is a common commercial cultivar and is grown mainly for its seed, which is used for its oil in both food and industrial processing. The resulting transgenic safflower seed will be used to develop isolation techniques for human proinsulin for future clinical trials.

The Vectors

The genes were transferred into safflower plants via a vector system, disarmed *Agrobacterium tumefaciens*. This process is a well characterized transformation system which integrates the donor genes into the chromosome of the recipient plant cell (Bechtold et al., 1993). The donor DNA sequences are stably and irreversibly integrated into the plant's chromosomal DNA, where they are maintained and inherited as any other genes of the plant cell.

Parsley (*Petroselinum crispum* L.) and common bean (*Phaseolus vulgaris*) are donors for non-coding DNA regulatory sequences that are associated with the introduced genes to facilitate expression in plants. The regulatory sequences from parsley are the ubiquitin (*ubi*) promoter and terminator regions for the selectable marker. The regulatory sequences from bean are the phaseolin promoter and terminator regions for the gene of interest. None of the DNA regulatory sequences can cause plant disease by themselves or in conjunction with the genes that were introduced into the transgenic safflower plant.

The Selectable Marker

To facilitate the selection of transformed plants, the safflower plants were engineered with the [CBI, reviewed by APHIS] gene flanked by the ubiquitin promoter and terminator from parsley (*Petroselinum crispum* L.).

The [CBI, reviewed by APHIS] gene is devoid of inherent plant pest characteristics and is the most commonly used selective marker in plants and has been safely used in many previous field trials (CBI).

The Gene of Interest

Safflower plants were engineered so a modified human proinsulin gene was fused to an *Arabidopsis* oleosin gene to be exclusively expressed in the seeds. SemBioSys has determined that the attachment human proinsulin and other similar experimental proteins to plant oilbodies (e.g. *Arabidopsis* oleosin gene) allows proper folding and stable accumulation of the protein within seeds while preventing unwanted glycosylation.

The modified human proinsulin gene has two basic amino acids removed for added stability in plants plus eleven C-terminal amino acids. The added C-terminal amino acids act as a protein signal that ensures the retention of the fusion protein in the endoplasmic reticulum of the plant seed cell and the removal in downstream processing. SemBioSys

has provided data that demonstrates that proinsulin is only produced in the developing seeds and nowhere else in the plant.

Expression of this gene is controlled by the phaseolin promoter and terminator sequences from *Phaseolus vulgaris* L. (common bean). The phaseolin promoter drives the exclusively seed-specific transcription of human proinsulin. As expected, no detectable fusion protein was found in leaf, root and floral (including pollen) material when analyzed by western blots. See Appendix IV for a more in-depth review of human proinsulin.

Appendix III. Field Test Release and Agricultural Practices

SemBioSys has provided APHIS with detailed SOPs [CBI, reviewed by APHIS] for field release, movement and containment of GE plants. Below is a summary of the permit information provided by SemBioSys.

Plot Design and Location

A field site (<1 acre) will be located on private property in Lincoln County, WA. The experimental plot will be bordered on all sides by a 50 ft fallow strip. The adjacent agricultural lands will be planted with barley and wheat.

Agricultural Practices

Agricultural practices consistent with growing healthy safflower plants will be used; weeds will be controlled by herbicide applications.

There are no serious insect threats to safflower in the United States. If necessary, pesticides such as insecticides and/or fungicides will be used to control insect pests and disease that would diminish the health of the plant and subsequent seed yield. EPA registered chemical pesticides are likely to be used to control insect pests on this crop. Any pesticides or herbicides used will be applied by personnel trained in their use and application. The field will be monitored for noxious weeds and other plant pests during the growing season.

During the growing season the plants will be inspected for traits such as weediness, resistance/susceptibility to insects or disease, or unusual differences in plant growth or morphology.

Termination of the field test and final disposition of the test plants

The harvested seeds will be placed in dedicated storage bags on site and immediately shipped for further processing in a non-food or feed seed grinding facility owned by SemBioSys in Canada.

After harvest, as soon as possible as the weather allows, SemBioSys, Inc. will till the field and apply the herbicide glyphosate to kill all plants remaining in the field. The field site will be monitored quarterly for one year following the termination of the field test and any volunteer safflower plants will be destroyed by application of glyphosate, removed and autoclaved.

Volunteer Monitoring

Volunteer monitoring by the researcher will be done for 1 year (1 growing season) after the end of the field test. Safflower seeds generally lack dormancy and can even germinate in the seed head if too much rain occurs before harvest. Despite limited potential for seed dormancy, the field site will be monitored quarterly for one year (one growing season) following the termination of the field test and any volunteer safflower plants will be destroyed by application of glyphosate, removed, and autoclaved.

Appendix IV. Human Proinsulin

With the growing market demand for insulin, SemBioSys has developed a genetically engineered safflower that produces an oleosin-human proinsulin fusion protein exclusively within its seeds. SemBioSys believes that safflower-produced insulin will help meet the growing market demands while reducing equipment and manufacturing costs compared to current processes.

Proinsulin is produced in the beta cells of the pancreas and is the precursor molecule to the active forms of insulin and C-peptide. Proinsulin itself only has 10% of the activity of insulin and works slower to decrease blood sugar when injected subcutaneously (Rosak et al., 1988). Because of its longer half-life, injected human proinsulin was initially tested as a substitute for faster acting insulin in the late 1980s and early 1990s (Glauber et al., 1987; Tillil et al., 1990; Galloway et al., 1992). More recently, the role of naturally existing proinsulin levels in the blood have been used to distinguish type 1 and type 2 diabetes in patients with newly diagnosed high blood sugar (Bolinder et al., 2005). It has also been suggested that high levels of proinsulin found in the blood may become an autoantigen; a protein that the body unfavorably reacts to causing an allergic response (e.g. inflammation) (You and Chatenoud, 2006).

Proinsulin has no biological activity when ingested. Even the task of developing an oral insulin delivery method has been unsuccessful in the last several decades because both insulin and proinsulin are peptides that are easily and quickly digested. Like most peptides and proteins that animals eat as food sources, the proinsulin within the safflower seed will be broken into amino acids or small peptides within the stomach before they can be absorbed in the intestine. This breakdown of protein begins in the stomach where hydrochloric acid (HCl) denatures the protein and facilitates the action of pepsin, the major gastric enzyme that splits the peptide bonds. Other proteolytic enzymes (enzymes that break down protein bonds) involved in the gastric process are trypsin, chymotrypsin, carboxypeptidase and elastase (Zeitlin et al., 1999). In simulated digestive studies, active insulin is completely digested in 60 minutes whereas the proinsulin seed fusion protein is completely digested in 15 minutes (SemBioSys, permit application data). In the unlikely event of an exclusive diet of transgenic proinsulin safflower seeds, no effects of human proinsulin are expected on animals due to the digestion process.

Appendix V. Threatened and Endangered Species Analysis

The proposed field sites are for the confined releases of the regulated article into the environment in Lincoln County, WA. There are no listed critical habitats for any threatened and endangered animal species in Lincoln County, WA according to the U.S. Fish and Wildlife database (http://criticalhabitat.fws.gov/) and therefore the confined release of the regulated article is not expected to affect any critical habitats.

On the basis of our review of permit 06-363-103r and the analysis of the threatened and endangered species found below, we conclude that controlled field testing of the genetically engineered safflower plants described in this application would not present any risk of new plant pest introduction, would have no significant impact on non-target organisms and on the threatened or endangered species, and therefore constitutes a confined field trial. Furthermore, if the field test is performed with conditions outlined here and in the permit, the risk to the environment would be negligible.

APHIS evaluated plant pest impacts related to the transformation method used in this permit and concluded that the DNA inserted into the plants does not have any inherent plant pest characteristics and is not likely to pose a plant pest risk for the following reasons:

1. The safflower plants were transformed using a disarmed *Agrobacterium tumefaciens* protocol which does not cause plants to exhibit plant disease.
2. The selectable marker gene and all of the non-translated regulatory elements are well characterized.
3. The introduced DNA will not lead to the expression of a toxin. A BLAST search using the amino acid sequence of the human proinsulin-oleosin domains did not reveal any significant homology (> 50%) to the amino acid sequence of proteins other than oleosin and human proinsulin proteins.
4. Although part of the inserted oleosin gene shares a 70% sequence homology within its hydrophobic domain (central part) to a filbert (hazelnut) oleosin that has been implicated as a candidate allergen, this should not be of a concern since the hydrophobic domain is highly conserved among oleosins of many food species that are consumed by humans and animals. The three domains (C-terminal, central core, and the N-terminal) of the *Arabidopsis* oleosin protein share a significant overall homology to the oleosin protein of many food species such as corn, rice, canola, sesame seed and rye) with the highest sequence homology to canola oleosin.

APHIS evaluated potential plant pest impacts related to the quarantine and final disposal of transgenic plants and concluded that the field trial is a confined release and has no significant impact on the environment. The following containment measures should be sufficient to prevent any unplanned release of the transgenic plant material or transgenic seed; or the persistence of the transgenic material or its progeny in the environment:

1. Dedicated equipment will be used for planting and harvesting and will be labeled accordingly. This precaution ensures that the transgenic safflower plants are not inadvertently removed from the field and therefore eliminates dispersal and gene flow of the transgenic safflower plants.
2. A perimeter fallow zone of 50 feet will be maintained around the transgenic test site to ensure that transgenic safflower are not inadvertently commingled with plants to be used for food or feed.
3. In addition to the large degree of self-pollination of safflower plants, other mitigating measures are implemented to prevent gene flow through pollen dispersal to any compatible species or by seed dispersal. The field site will be isolated from sexually compatible wild safflower or any other commercial safflower seed production areas by at least 2 miles. Additionally, the applicant presented a procedure to report to APHIS any unauthorized or accidental release of the transgenic material. These measures would further ensure that the transgenes do not enter the commercial safflower seed supply.
4. The test plots will be monitored weekly for weed, disease, and insect infestation.
5. In addition to lack of seed dormancy of safflower where seed can germinate in the head if rain fall occurs at harvest time (http://www.ipgri.cgiar.org/publications/pdf/498.pdf), the field will be monitored for safflower volunteers for one growing season after harvest. In the growing season following the harvest, the test area will be left fallow.
6. It is unlikely for safflower (*Carthamus tinctorius*) to become a weed under most agricultural situations. Safflower is unable to persist in the environment without continuous human intervention and is not reported to be an agricultural weed. Wild relatives of safflower are not found in Washington and the wild safflower (*Carthamus oxyacanthus*), which is on the Federal Noxious Weed list, is not sexually compatible with *Carthamus tinctorius*. The gene function is known and the phenotype will not confer any traits associated with weediness to safflower.
7. The proposed cultivation practices involved in growing these transgenic safflower plants are similar to growing practices for normal commercial safflower and as a result no "unusual" growing practices should be expected to increase weediness or volunteers.

APHIS evaluated the potential impacts on non-target organisms, including threatened or endangered species (TES).

16 U.S.C., Section 1531, Endangered Species Act (ESA) of 1973, as amended, requires Federal agencies to utilize their authorities to conserve listed species, and to ensure their actions do not jeopardize the continued existence of a listed species. Section 7 (a) (2) of the ESA states that each Federal agency shall, in consultation with and with the assistance of the Secretary (Interior), insure that any action authorized, funded, or carried out by such agency is not likely to jeopardize the continued existence of a listed threatened or endangered species, or result in the destruction or adverse modification of

designated critical habitat. Regulations are found in 50 CFR Part 402, Interagency Cooperation Regulations.

APHIS has analyzed the effects of Alternative B on federally listed threatened and endangered species and proposed species and designated critical habitat and habitat proposed for designation.

An examination of the U.S. Fish and Wildlife threatened and endangered species system (TESS) http://ecos.fws.gov/tess_public/StateListingAndOccurrence.do?state=WA on December 20, 2006 showed that 9 threatened or endangered plant species and 28 animal species exist in Washington. Of the 28 animal species listed, only four animals potentially reside in Lincoln County, WA:

- Bald eagle (*Haliaeetus leucocephalus*)
- Pygmy rabbit (*Brachylagus idahoenis*)
- Columbian white-tailed deer (*Odocoileus virginianus leucurus*)
- Gray wolf (*Canis lupus*)

The bald eagle (*Haliaeetus leucocephalus*) lives near large bodies of open water such as lakes, marshes, seacoasts and rivers, where there are plenty of fish to eat and tall trees for nesting and roosting. The nearest sources of waters with potential roosting places for the bald eagle are the Grand Coulee River, Columbia River and Banks Lake. All three water sources are >25 miles from the field test site, so it is unlikely that this species would be found in the area of the field tests. Therefore, it is unlikely there would be any effect on this threatened bird.

The pygmy rabbit (*Brachylagus idahoenis*) inhabits areas throughout the Great Basin and prefers dense, tall stands of big sagebrush that are usually along intermittent streams or riparian areas in sagebrush grasslands. Ninety nine percent of their diet in the winter consists of sagebrush while grasses and forbs are eaten in mid- to late summer. The pygmy rabbit is not expected to be impacted by the field test site due to its preference for sagebrush and riparian habitats. Therefore, it is unlikely there would be any effect on this endangered mammal.

The Columbian white-tailed deer (*Odocoileus virginianus leucurus*) occurs in farmlands, brush areas, woods, suburbs and gardens. They feed on green plants, acorns, beechnuts, and other nuts and corn in the fall and in winter they feed on woody vegetation, including the twigs and buds of viburnum, birch, maple, and many conifers. The S-317 cultivar is a spiny variety (comparable to many other spiny thistles) that discourages large animals to browse the fields after flowering. S-317 safflower also has a striped hull, and is not as palatable (due to odor and color) as the white hulled variety. Because the Columbia Whitetail Deer would be unlikely to feed on S-317 safflower, it is unlikely there would be any effect on this endangered mammal.

The Gray Wolf (*Canis lupus*) The Northern Rocky Mountain gray wolf is currently endangered in Colorado, Idaho, Michigan, Montana, North Dakota, South Dakota, Washington and Wisconsin and threatened in Minnesota. Wolves are carnivorous but may also feed on earthworms, berries or grasshoppers. It is unlikely that the Gray Wolf would be found on agricultural land under cultivation, or feed on the genetically engineered plants if it were to enter the release site. Therefore, it is unlikely there would be any effect on this endangered mammal.

Of the nine plants listed, only one threatened species potentially resides in Lincoln County (http://ecos.fws.gov/):

- Spalding's Catchfly (*Silene spaldingii*) – Lincoln County

Spalding's catchfly is endemic and is restricted to remnants of the prairie grasslands of eastern Washington, northeastern Oregon, northern Idaho, and western Montana (barely extending into British Columbia, Canada). This species is restricted to Palouse Prairies, sometimes extending into areas where the grasslands are intermingled with ponderosa pine (*Pinus ponderosa*) woodlands. Sites are often near lower treeline, or near scattered ponderosa pine trees. A significant amount of habitat has been lost to conversion to agriculture, restricting most remaining occurrences to small, isolated fragments of native vegetation, where they are vulnerable to degradation. Most remaining populations are small and threatened by weed invasion (including yellow starthistle in places), herbicide treatment (particularly because many populations are small and located near farmlands and roads), and livestock grazing. Activities such as road construction and maintenance, gravel mining, off-road vehicles, and urban developments are additional threats. Recovery of this species requires habitat improvement through site monitoring and restricting public access to areas where they occur. The FWS recovery plan (USFWS, 2005) indicates no occurrences of Spalding's catchfly have been noted in the areas proposed by SemBioSys, Inc., [CBI, reviewed by APHIS] within Lincoln County. In addition, the land is currently used for agriculture. Therefore, it is unlikely there would be any effect on this threatened plant.

Based on the reasons listed below, APHIS is confident that these field trials will not harm or have any direct or indirect effects on threatened or endangered species either by direct or indirect exposure.

1. The introduced DNA will not lead to the expression of a toxin or other product that is known to affect the metabolism, growth, development, or reproduction of animals, plants, or microbes. Sequence alignments and homology searches of the human proinsulin-oleosin protein using the BLAST search of non-redundant GeneBank coding sequence translations plus RefSeq, SwissProt and PDB showed more than 50% similarity to oleosin and human proinsulin proteins. It did not show significant similarity to proteins that are known toxins or allergens. Therefore, the protein expressed in the transgenic

safflower plants should have no known or foreseeable toxic or allergenic effects to humans or animals.

2. Literature searches of the NCBI's PubMed databases did not reveal any evidence for toxic or allergenic effects of proinsulin on human or animals. The publications that were brought up using the search terms allergy, allergic, allergenicity, and immunogenicity were decades old. These publications investigated the common allergic responses to bovine and porcine insulins that were administered to diabetics at that time.
3. The expression of the transgene is driven by a seed specific promoter and the protein does not accumulate in any safflower tissues other than the seed. Because safflower has tightly closed seed heads, predation by birds is minimized.
4. Only trained employees will perform activities related to this permit including planting and harvesting of the transgenic safflower. All activities will be conducted according to the procedures described in the field guide that the applicant submitted for APHIS' approval. This will also minimize any accidental release or possible animal exposure.
5. Several field trials have been performed with transgenic safflower plants under APHIS authority, and APHIS is familiar with safflower biology and methods to manage confined safflower field trials.
6. Safflower does not outcross with any of the plant species that are on the Federal list of threatened or endangered species.

This field release does not involve new species or organisms or novel genes that raise new issues. Many field trials have been performed with transgenic safflower plants under APHIS authority. APHIS is familiar with the biology of safflower and methods to manage confined safflower trials.

For the above reasons, APHIS has determined that (1) pursuant to 7 C.F.R. §372, the field trials proposed under permit #06-250-02r will not significantly affect the physical environment and (2) there are no applicable, extraordinary, or other reasonably foreseeable circumstances under which significant environmental effects could occur given the protective and ameliorative measures specified above.

The proposed field sites for the confined releases of the regulated article into the environment are in Lincoln County, WA. No federally listed threatened or endangered species has designated critical habitat within Lincoln County, WA. (U.S. Fish and Wildlife database http://criticalhabitat.fws.gov/).

In conclusion, APHIS has determined that choosing Alternative B will have no effect on federally listed threatened and endangered species or species proposed for listing, and will have no effect on designated critical habitat or habitat proposed for designation. Likewise, the selection of Alternative A, the no-action alternative, will have no effect on federally listed threatened and endangered species or species proposed for listing, and will have no effect on designated critical habitat or habitat proposed for designation.

Consequently, consultation under Section 7 of the Endangered Species Act with the U.S. Fish and Wildlife Service will not be required.

Appendix VI. Standard Permit Conditions for APHIS Form 2000 (7 CFR 340.4)

Permit conditions: A person who is issued a permit and his/her employees or agents shall comply with the following conditions, and any supplemental conditions which shall be listed on the permit, as deemed by the Deputy Administrator to be necessary to prevent the dissemination and establishment of plant pests:

(1) The regulated article shall be maintained and disposed of (when necessary) in a manner so as to prevent the dissemination and establishment of plant pests.

(2) All packaging material, shipping containers, and any other material accompanying the regulated article shall be treated or disposed of in such a manner so as to prevent the dissemination and establishment of plant pests.

(3) The regulated article shall be kept separate from other organisms, except as specifically allowed in the permit.

(4) The regulated article shall be maintained only in areas and premises specified in the permit.

(5) An inspector shall be allowed access, during regular business hours, to the place where the regulated article is located and to any records relating to the introduction of a regulated article.

(6) The regulated article shall, when possible, be kept identified with a label showing the name of the regulated article, and the date of importation.

(7) The regulated article shall be subject to the application of measures determined by the Administrator to be necessary to prevent the accidental or unauthorized release of the regulated article.

(8) The regulated article shall be subject to the application of remedial measures (including disposal) determined by the administrator to be necessary to prevent the spread of plant pests.

(9) A person who has been issued a permit shall submit to APHIS a field test report within 6 months after the termination of the field test. A field test report shall include the APHIS reference number, methods of observation, resulting data, and analysis regarding all deleterious effects on plants, non-target organisms, or the environment.

(10) APHIS shall be notified within the time periods and manner specified below, in the event of the follower occurrences:

i. Orally notified immediately upon discovery and notify in writing and within 24 hours in the event of any accidental or unauthorized release of the regulated article;

ii. In writing as soon as possible but not later than within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the application for a permit or suffers any unusual occurrence(excessive mortality or morbidity, or unanticipated effect on non-target organisms).

(11) A permittee or his/her agent and any person who seeks to import a regulated article into the United States shall:

i. Import or offer the regulated article for entry only at a port of entry which is designated by an asterisk in 7 CFR 319.37-14 (b);

ii. Notify APHIS promptly upon arrive of any regulated article at a port of entry, or its arrival by such means as a manifest, customs entry document, commercial invoice, waybill, a broker's document, or a notice form provided for such purpose: and

iii. Mark and identify the regulated article in accordance with 7 CFR 340.7.

Rev. 3/2003

Appendix VII. Supplemental Permit Conditions for APHIS Form 2000 (7 CFR 340.4)

SUPPLEMENTAL PERMIT CONDITIONS

For Release of Safflower, *Carthamus tinctorius* L.

USDA-APHIS-BRS Permit 06-363-103r

Compliance with Regulations

1. Any regulated article introduced not in compliance with the requirements of 7 Code of Federal Regulation Part 340 or any standard or supplemental permit conditions, shall be subject to the immediate application of such remedial measures or safeguards as an inspector determines necessary, to prevent the introduction of such plant pests. The responsible party may be subject to fines or penalties as authorized by the Plant Protection Act (7 U.S.C. 7701-7772).

2. This Permit (APHIS form 2000) does not eliminate the permittee's legal responsibility to obtain all necessary Federal and State approvals, including: (A) for the use of any non-genetically engineered plant pest or pathogens as challenge inoculum; (B) plants, plant parts or seeds which are under existing Federal or State quarantine or restricted use; (C) experimental use of unregistered chemicals; and (D) food, feed, pharmacological, biologic, or industrial use of regulated articles or their products and co-mingled plant material. In the latter case, depending on the use, reviews by APHIS, the U.S. Food and Drug Administration, or the U.S. Environmental Protection Agency may be necessary.

3. The procedures, processes, and safeguards used to prevent escape, dissemination, and persistence of the regulated article as described in the permit application, in APHIS-approved Standing Operating Procedures (SOPs) and, in the supplemental permit conditions must be strictly followed. The permittee must maintain records sufficient to verify compliance with these procedures, including information regarding who performed the activity. Persons performing such activities shall have received training as described in a training program submitted to and approved by APHIS. These records are subject to examination by APHIS. APHIS, BRS must be notified of any proposed changes to the protocol referenced in the permit application.

I. Reporting Unauthorized Releases and Unintended Effects

According to the regulation in 7 CFR § 340.4(f)(10)(i), APHIS shall be notified orally immediately upon discovery and notified in writing within 24 hours in the event of any accidental or unauthorized release of the regulated article.

- For immediate oral notification, contact APHIS/BRS Compliance Staff at (301) 734-5690 and ask to speak to a Compliance and Inspection staff member.

- In the event of an emergency and you are unable to reach the BRS Compliance Staff at the above number, you may call:

  The APHIS/BRS Regional Biotechnology Coordinator assigned to the state, where the field test occurs

  For Western Region, contact Ralph Stoaks by phone at (970) 494-7573 or e-mail Ralph.D.Stoaks@aphis.usda.gov

  For Eastern Region, contact Ashima SenGupta by phone at (919) 855-7622 or e-mail Ashima.SenGupta@aphis.usda.gov

  Or

  The APHIS/PPQ Regional Biotechnology Coordinator assigned to the state where the field test occurs

  For Western Region, contact Stacy Scott by phone at 970-494-7577 or e-mail Stacy.E.Scott@aphis.usda.gov

  For Eastern Region, contact Susan Dublinski by phone at (919) 855-7324 or e-mail Susan.G.Dublinski@aphis.usda.gov

  Or

  The APHIS State Plant Health Director for the state where the field test occurs. The list of APHIS State Plant Health Director is available at http://ceris.purdue.edu/napis/names/sphdXstate.html

  For Washington State:

  Barbara Chambers, Seattle
  Phone: (206) 592-9057
  Fax: (206) 592-9043
  Email: barbara.a.chambers@aphis.usda.gov

1. According to the regulation in 7 CFR § 340.4(f)(10)(ii), APHIS shall be notified in writing as soon as possible but within 5 working days if the regulated article or associated host organism is found to have characteristics substantially different from those listed in the permit application or suffers any unusual occurrence (excessive mortality or morbidity, or unanticipated effect on non-target organisms).

2. Written notification should be sent by one of the following means:

3.

By e-mail:
BRSCompliance@aphis.usda.gov
By mail:
Biotechnology Regulatory Services (BRS)
Compliance and Inspection Branch
USDA/APHIS
4700 River Rd. Unit 147
Riverdale, MD 20737

II. Perimeter Fallow Zone

1. To ensure that transgenic plants are not inadvertently commingled with plants to be used for food or feed, a perimeter fallow zone of at least 50 ft. must be maintained around the transgenic test site in which no crops are grown to be harvested or used for food or feed.

2. The permitted border rows of non-transgenic plants that are the same as, or sexually-compatible with, the regulated article are considered part of the field test. The perimeter fallow zone shall start outside the border rows.

3. The perimeter fallow zone shall be managed in a way that allows detection and destruction of volunteer plants that are the same as, or sexually compatible with, the transgenic plants.

III. Dedicated Planting and Harvesting

1. To ensure that the regulated article is not inadvertently removed from the site, planting and harvesting equipment must be dedicated for use in the permitted test site(s) from the time of planting through the end of harvesting.

2. After harvest, you will not be required to obtain APHIS authorization to use this equipment on APHIS -permitted sites (same sites or different sites) planted with same transgenic crop, with the target protein(s) authorized under this permit, in

subsequent growing seasons under an extension of this permit or a different permit.

3. Authorization is required from APHIS before this planting and harvesting equipment can be used on sites planted to crops not included under this permit. The permittee must notify APHIS/BRS and the State Regulatory Official at least 21 calendar days in advance of cleaning this equipment for this purpose so that APHIS may schedule an inspection to ensure that the equipment has been cleaned appropriately.

IV. Cleaning of Equipment

1. To minimize the risk of seed movement and commingling, equipment used for planting and harvesting, as well as other field equipment (e.g. tractors and tillage attachments, such as disks, plows, harrows, and subsoilers) used at any time from the time of planting through the post-harvest monitoring period must be cleaned in accordance with procedures submitted to and approved by APHIS before they are moved off of the test site.

2. Equipment used to transport seeds or harvested material must be cleaned prior to loading and after transportation to the authorized site in accordance with procedures submitted to and approved by APHIS.

3. Seed cleaning and drying must be performed in accordance with the procedures submitted to and approved by APHIS to confine the plant material and minimize the risk of seed loss, spillage, or commingling.

V. Use of Dedicated Storage Facilities

1. Dedicated facilities (locked or secured buildings, bins, or areas, posted as restricted to authorized personnel only) must be used for storage of equipment and regulated articles for the duration of the field test.

2. Before returning these facilities to general use, they must be cleaned in accordance with procedures submitted to and approved by APHIS. **The permittee must notify** APHIS/BRS and the State Regulatory Official at least 21 calendar days in advance to allow for APHIS to schedule an inspection to ensure that the facilities have been cleaned appropriately. APHIS authorization should be received before facilities are returned to general use.

VI. Post Harvest Monitoring

The field test site including the perimeter fallow zone must be monitored for the presence of volunteer safflower plants for one (1) year after termination of the field test. Viable plant material should not remain at the test site following termination.

VII. Post Harvest Land Use Restrictions

1. Production of food and feed crops at the field test site and the perimeter fallow zone is restricted during the growing season that follows harvest or termination of the field test.

2. Permission must be obtained from APHIS/BRS prior to planting any food or feed crop at the field test site and perimeter fallow zone during the post-harvest monitoring period. Requests for such permission are not encouraged and will not be granted in cases where there is a reasonable potential for plant material derived from, or originating from, the regulated articles to become mixed with the proposed food or feed crop during harvesting.

VIII. Inspections

1. APHIS Biotechnology Regulatory Services (BRS) and/or an APHIS/PPQ Regional Biotechnologist, APHIS/BRS Regional Biotechnology Coordinator or APHIS State Plant Health Director may conduct inspections of the test site, facilities, and/or records at any time.

2. APHIS may invite the FDA or State Regulatory Officials to participate in these inspections.

3. Inspections will likely correspond to the beginning of the field test, mid-season or during flowering, at and/or following harvest, and during the post-harvest monitoring period.

4. Inspections will include examination of records that verify compliance with regulations and SOPs.

IX. Reports and Notices

Send notices and all reports (CBI and CBI-deleted or non-CBI copies) to BRS by e-mail, mail, or fax.

BRS E-mail:
BRSCompliance@aphis.usda.gov

and please cc the review biotechnologist:
Patricia.K.Beetham@aphis.usda.gov

BRS Mail:
Biotechnology Regulatory Services (BRS)
Compliance and Inspection Branch
USDA/APHIS
4700 River Rd. Unit 147
Riverdale, MD 20737

BRS Fax:
Compliance and Inspection Branch
(301) 734-8669

In addition, fax the CBI deleted or non CBI version of the pre-planting and pre-harvest (termination) notices to the State Regulatory Official(s):

Brad White, Ph.D., Program Manager
Plant Services Division
Washington State Dept. of Agriculture
P. O. Box 42560
Olympia, WA 98504-2560
Phone: 360-902-2071
Fax: 360-902-2094
Email: bwhite@agr.wa.gov

A. Pre-Planting Notice

At least 7 calendar days before planting, submit a Pre-Planting notice that includes the following information for each field test site:

i. Provide APHIS with the contact information for each field test site.
ii. Indicate if planting and harvesting equipment will be moved between authorized field test sites.
iii. A map that clearly identifies the site location to facilitate any inspections by USDA personnel.
iv. The planned numbers of acres for each gene construct.
v. The planned planting date

B. Planting Report

Within 28 calendar days after planting, submit a planting report that includes the following information for each field test site:

i. A map of the site, with sufficient information to locate it, that includes: the state, county, address, GPS coordinates for each corner of the plot (inclusive of the border rows of any sexually compatible plants);
ii. The location and the approximate number and/or acres of transgenic plants which were actually planted at the test site for each of the target proteins;
iii. The total acreage of the test plot (exclude border rows, if any);
iv. The distance from the genetically engineered plants to the nearest plants of the same crop which will be used for food, feed, or seed production. A survey should be done within the distance specified in the permit. APHIS requires a distance of two (2) miles from any commercially planted safflower fields.
v. The actual planting date.

C. Pre-Harvest/ Termination Notice

At least 21 calendar days prior to the anticipated harvest or termination, submit a Notice indicating the planned date of harvest **or** termination and the contact information for each field test site. For multiple harvests, submit the notice prior to the initial harvest.

D. Field Test Report

Within 6 months after the end of the field test (final harvest or crop destruct), the permittee is required to submit a field test report. Field test reports shall include:

i. APHIS reference number
ii. Methods of observation.
iii. Resulting data.
iv. Analysis of all deleterious effects on plants, non-target organisms, or the environment.
v. A list of the lines planted at each site
vi. Disposition table
The disposition table should contain the following information: site name (or GPS), crop, gene, harvest date, and disposition of harvested material. The disposition table is a formal record of how the regulated material was removed from the environment. An accounting of the harvested material should be provided with regards to what material is harvested, how much material is harvested per site, what is done to devitalize residual and harvested material at the site, where the harvested material is transported, stored and further processed up to the time it is taken to a contained facility.

E. Monitoring Report

Within 3 months after the end of the monitoring period, submit a volunteer monitoring report. The report must include:

i. Dates when the field site and perimeter fallow zone were inspected for volunteers.
ii. Number of volunteers observed.
iii. Any actions taken to remove or destroy volunteers.