Finding of No Significant Impact and Decision Notice

Animal and Plant Health Inspection Service

Issuance of a permit to grow GE Safflower containing carp growth hormone

The Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA) has received a permit application (APHIS number 06-250-02r) from SemBioSys Genetics, Inc. to conduct field tests with GE safflower line GHB618 containing carp growth hormone found exclusively within its seeds. A description of the field test may be found in the attached Environmental Assessment (EA) which was prepared pursuant to APHIS regulations at 7 CFR 372, promulgated under the National Environmental Policy Act. The field tests are scheduled to begin in April 2007 in Lincoln, Grant and Douglas Counties, WA.

APHIS proposed two different actions to take in response to the permit application: the denial of the permit (Alternative A) and the granting of the permit with Supplemental Permit Conditions containing additional environmental safety requirements and a requirement for the filing of field test reports with APHIS (Alternative B).

A draft EA was prepared and submitted for public comment for 30 days. Twenty-three individual and eight public interest groups' comments were received and addressed, where appropriate, in the preparation of the final EA, which is attached to this document.

Based on the analysis documented in its EA, APHIS has selected the action proposed in Alternative B. APHIS has determined that the proposed action will not have a significant impact, either individually or cumulatively, on the quality of the human environment and that no Environmental Impact Statement will be prepared regarding this decision. Pursuant to its regulations (7 CFR 340) promulgated under the Plant Protection Act of 2000, APHIS has determined that this field trial will not pose a risk of the introduction or dissemination of a plant pest for the following reasons:

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. The one sexually-compatible, wild relative of safflower that grows in the U.S.A., *Carthamus oxyacanthus*, is not found in Washington State. 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.

For the reasons enumerated above, which are consistent with regulations implementing the Plant Protection Act, the field trial of GE safflower containing carp growth hormone is hereby authorized.

for Rebecca Bech
Deputy Administrator
Biotechnology Regulatory Services
Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: 6-7-07

Attachment
Finding of no significant impact
Response to comments
APHIS No. 06-250-02r

In response to a notice published in the Federal Register (Docket No.APHIS-2006-190) on February 5, 2007, APHIS received 33 comments on permit 60-250-02r and environmental assessment during the 30-day comment period closing on March 7, 2007. There were 2 individuals that supported the planting of GE crops in general, but did not raise any specific points from the EA. There were 23 individuals that were opposed to the use of biotechnology in food crops in general but did not cite specific plant pest risk issues associated with this particular EA. One public interest group uploaded 20,360 nearly identical letters from individuals opposing pharmacological proteins produced in food crops in general without addressing specific issues within the EA. Another public interest group had 25 signatures to their letter representing various organizations that oppose pharmacological proteins in food crops and addressed specific issues within the EA. In total, eight public interest groups wrote letters in opposition to allowing the planting of GE safflower, citing issues that will be discussed below.

The majority of general comments and a comment from one public interest group opposed to this field release expressed the opinion that all genetically engineered plants are unwanted and are dangerous, causing harm to humans (especially those with "pharma" proteins expressed within them). APHIS disagrees with the statement that all genetically engineered plants are dangerous and unwanted. First it must be noted that genetically engineered crops have a history of safe use. Over a billion acres have been planted with genetically engineered crops and there are no instances of any physical harm to humans and the environment (ISAAA Briefs 30, by Clive James; http://www.europabio.org). The fact that the adoption of genetically engineered crops keeps increasing is inconsistent with the argument that genetically engineered plants are unwanted. The SemBioSys field trial includes the following numerous redundant safeguards that should minimize the likelihood of harm to humans or impacts to the environment:

1. Surrounding the field with a 50 foot fallow zone;
2. Restrictions on the production of food and feed crops at the field test site and perimeter fallow zone in the following season;
3. Mandatory use of dedicated planters and harvesters in the permitted test sites;
4. Equipment cleaning in accordance with APHIS approved protocols;
5. Mandatory use of dedicated facilities for the storage of equipment and regulated articles for the duration of the field test. Facilities must be cleaned according to APHIS approved protocols prior to general use of the facilities;
6. Mandatory cleaning procedures to minimize the risk of seed movement by field operations or equipment (such as movement of seed on tires of tractors, clothing, etc.);

7. Mandatory training programs approved by APHIS to ensure that personnel are prepared to successfully implement and comply with permit conditions;
8. Multiple field and permit inspections to ensure permit compliance;
9. Use of low production geography. Only 1500 acres of safflower are grown in WA and the crop is grown for birdseed and not crushed for oil for human consumption. WA does not have any seed crushing facilities for oil production;
10. The variety of seed used by SemBioSys is visibly distinct from the locally grown safflower and is a variety that is unpalatable to birds;
11. The mandatory isolation distance to the nearest safflower is 2 miles. Given the low production geography, no safflower is expected to be planted within 10 miles, well outside the foraging range of bees and more than sufficient to reduce out-crossing to *de minimis* levels. Out-crossing refers to the extent to which pollen from an unwanted donor crosses to the receptor plant;
12. Strict chain of custody and closed loop identity preservation systems.

These numerous safeguards are designed to exclude any commingling of pharma crops with food crops. In the unlikely event that all these redundant safeguards fail, very little of the trait is expected to get out because there is little safflower production in WA and gene flow would be detected because the seeds are visibly distinguishable from the bird seed grown in the area. Even if some gene flow did occur, the minute amount of bird seed containing the gene would be unlikely to be consumed by birds because the unpalatable few seeds would be ignored among the vast excess of palatable seeds. As the nearest crushing plants are hundreds of miles away in California, it is not foreseeable that the pharma safflower will be mixed in with material for human consumption. Based on these considerations, APHIS has concluded that this field trial will not have any significant impacts on the human environment and therefore is by extension neither dangerous nor harmful to humans.

Three public interest groups were concerned about equipment movement to and from multiple field sites that might lead to the dissemination of seed. In these field tests, there are harvesters, planters and storage facilities dedicated to each of the pharma field sites. Furthermore, such equipment can not be used on conventional crops unless they undergo specialized cleaning and servicing as specified by SemBioSys' SOPs (reviewed by APHIS) and are inspected by USDA to ensure adequacy of the cleaning.

One public interest group disagreed with the one year fallow period for GE safflower believing that seed can germinate years after harvest. APHIS disagrees with this comment. As described in the EA on p.11, safflower lacks dormancy.

One public interest group was concerned about the use of the GE safflower in shrimp and the impacts of the supplement in oceans. A review of the safflower product outside its intended use is outside the scope of this EA. At this time, SemBioSys is not planning to commercialize this product in the United States; however, SemBioSys is responsible for

complying with all Federal, State and local regulations in the final use of this product. The processed safflower seed will be used as a shrimp feed supplement in confined experimental studies (performed in a university laboratory facility). The shrimp used in this experiment will not be used for food or feed and will not be released into the environment. At the end of the experiments, the shrimp will be disposed of according to university biosafety regulations. All experimentation will be done according to the laboratory biosafety regulations and in compliance with all NIH guidelines. This group also wanted APHIS to review the potential impacts of the supplement on all endangered species in the USA, not just Washington State. APHIS disagrees with this comment. The Endangered Species Act requires an analysis on threatened and endangered species in the affected area (7 U.S.C. § 136, 16 U.S.C. § 1531 et seq). As there is no foreseeable exposure to threatened and endangered species outside the affected area, there is no need to analyze potential impacts on all endangered species in the USA.

Two public interest groups were concerned that studies have not been done to demonstrate the safety of CGH to birds and mammals. They take issue with the justification that CGH is found in fish, and many animals eat fish without ill effects. They raise the point that it is necessary to compare the levels of CGH in fish with the levels of CGH in safflower seed and estimate the exposure of animals to the safflower seed. While the CGH amounts in the GE safflower seed is classified as CBI, APHIS has reviewed the concentrations and made a comparison to the plasma levels found in young Atlantic salmon (0.7-11 ng/mL depending on day length; Bjornsson et al., 2000) and found the CGH concentration per 10 seeds to be considerably less than what is found in a yearling Atlantic salmon.

They also take issue with the comparison between bovine somatotropin (BST) and CGH regarding the lack of effect through oral exposure. They argue that bovine somatotropin is only about 40% similar to CGH so studies demonstrating safety through oral exposure of the former are not applicable to the latter. Another group stated that it is necessary to do further research because the animals that may safely eat CGH in fish are not the same as the animals that may eat CGH in safflower. APHIS disagrees with both arguments. The point is that hormones rarely are biologically active when taken orally because they are digested into inactive peptides. This is a property of proteins and is not based on structure. A similar case could be made for insulin which also has little biological activity when taken orally and has no similarity in structure to the growth hormones. Furthermore, as digestion of proteins is a phenomenon characteristic of all birds and mammals there is no scientific basis to think that some birds or mammals will be more susceptible to orally ingested protein than others.

Commenters further cite a study that reports an immunological response to recombinant BST in rats fed high doses of the protein although the effect is of no known significance. In order to reproduce this effect with GE safflower, animals would need to consume 2500-6500 seeds on a daily basis for 90 days, a dose difficult, if not impossible to reproduce in the laboratory, and even less likely in the field because safflower seed remains tightly associated with the capsule which cannot be opened by most birds and mammals. During harvest, some capsules are cracked (called shattering) and seed may lie

on the ground for about a week before it is tilled into the ground. APHIS does not agree that scientific studies are needed to evaluate the effect of the protein on mammals and birds because exposure of these animals to the seeds will be limited by cultivation practices and mammals and birds that do eat the seeds will digest the protein into inactive peptides.

Two commenters mentioned that the permit application and the company SOPs were both CBI. The company's specific SOPs are CBI, but there exists a non-CBI permit application that can be requested via The Freedom of Information Act (FOIA, 5 U.S.C. 552, as amended). There is a process where the public can request the non-CBI permit application in writing and this information can be readily found on http://www.aphis.usda.gov/footer_items/how_to_submit_a_foia_request.shtml.

Three groups were concerned over the confidential business information (CBI) contained in the document and, because of CBI information, could not individually assess the scientific merit of the permit for themselves. APHIS is required by law to protect CBI data. The APHIS policy on CBI was published in 50 F.R. 38561. That policy requires information that is: 1) asserted to be a trade secret by the applicant; or 2) established by review to potentially cause substantial competitive harm, will be released only if required by statute or court order or otherwise required by law. Information of this nature is protected under the Freedom of Information Act (FOIA) (5 U.S.C. 552). Section (b)(4) of FOIA exempts from disclosure "trade secrets and commercial or financial information obtained from a person and privileged or confidential." 5 U.S.C. 552 (b)(4). Releasing this information is a violation of the Trade Secrets Act (18 U.S.C. 1905). APHIS has reviewed the CBI justification provided by the applicant and is maintaining the information in a way that is consistent with our policy. While CBI information is not available to the public, APHIS has thoroughly reviewed all of the information provided by the petitioner before making a decision.

Two public interest groups were concerned over the potential negative impacts on wild birds consuming GE safflower seeds as well as the dissemination of viable seed away from the field test sites. Like mammals, the avian digestive processes break down all seed proteins to small usable peptides so exposure to active CGH through oral exposure would be limited. The small peptides are biologically inactive and consumption of GE safflower seeds is not expected to have any residual carp growth hormone effect on birds. Viable, intact safflower seed is not expected to pass through a bird's digestive tract. Bird digestion is complex: first the crop softens the food, next the proventriculus continues to soften and breakdown the food, then the muscular gizzard (containing grit and tiny pebbles/stones) grinds and shreds even the hardest of foods, the food then goes into the small intestine where acidic digestive juices continue digestion. Finally the large intestine and cloacoa contain the waste to be voided. Therefore, given the nature of the digestive system of birds, it is unlikely that safflower seed will pass intact through the gut. Birds also have been shown to prefer the white safflower seed over the yellow (used for the background in GE safflower) due to odor and palatability as explained in the EA.

Four special interest groups were concerned about volunteers contaminating neighboring wheat and barley fields. Due to the size of the safflower seeds and how tightly the maturing safflower seed is held within the seed head, dissemination of the seed into neighboring fields past the 50 ft fallow zone is not expected; however, farmers will be monitoring adjacent fields for any safflower volunteers on a weekly basis during the growth period and quarterly for one year after harvest. Safflower is a poor competitor with weeds. It is unlikely to establish in a wheat or barley field which are competitive crops. Furthermore, wheat and barley are short season crops. The safflower variety currently used for this GE safflower requires significantly more growing degree days to mature. Safflower is visibly different than wheat and barley and will be easily detected and controlled by herbicide management programs used in conventional barley and wheat crops. In the unlikely event that any volunteer safflower seed was disseminated, it would be immature at crop harvest and not be viable. Harvest timing, herbicide regimes, the 50 ft fallow zone around the field test sites and volunteer monitoring are some of the confinement measures used in the field tests to prevent GE safflower seeds from mixing with neighboring food or feed crops.

One commenter was concerned about the effect of CGH on native fish populations. First it is unlikely that any safflower seed will find its way into waterways given the distance of the field test sites from any waterways and the numerous confinement measures described above. Second, even if seed did get into waterways, CGH consumed orally is not active in fish. APHIS therefore concludes that there are no foreseeable impacts of the GE safflower field trial on native fish populations.

One group was concerned over the inhalation of GE safflower pollen. Similarly another group was concerned about the wild and commercial honey bee populations. As stated in the EA, the only tissue where the carp growth hormone is found within the GE safflower plant is within the seed endosperm. Pollen contains no detectable CGH and therefore there is no chance of human exposure to CGH through inhalation of pollen. Since the CGH gene is only found in the seeds of GE safflower and not found in the pollen, neither bees nor honey will be affected by the planting of GE safflower.

One public interest group stated there were six wild relatives that could hybridize with safflower. As stated in the EA, these relatives (except for one noxious weed listing in one CA county) are not found in the U.S.A. Therefore there is no chance that safflower could hybridize with any wild relatives.

One public interest group was worried about the endangered pygmy rabbit. Pygmy rabbits are closely associated with tall, dense stands of big sagebrush (*Artemisia tridentata*) growing on deep, loose soil. Pygmy rabbits eat sagebrush leaves and the shrubs provide refuge from predators. They are not known to be consumers of safflower seed. Unsuitable habitats include essentially any habitat lacking sagebrush, especially those such as closed-canopy forest, pasture, cultivated fields, and grassland (http://www.natureserve.org). Since the GE safflower is being grown on fields cultivated for several generations in the absence of sagebrush, the pygmy rabbit will not be affected by these field trials. Even in the unlikely event that pygmy rabbit did consume some GE

safflower, active protein is not likely to get into the animals bloodstream as the protein will be broken down into small inactive peptides during digestion.

One group was concerned the FDA had not been consulted to do a food and feed safety and nutritional assessment. This product is not intended for commercial distribution or for food and feed and as described above, numerous redundant mitigation measures have been implemented to keep the GE safflower out of the food and feed supply. As this product is not entering the food or feed supply and will not be processed at any food or feed seed grinding facility, there is no basis to do a food and feed safety and nutritional assessment. SemBioSys did confer with FDA and was told that FDA has no regulatory authority to review this product.

Two public interest groups believed the EA was inadequate because it failed to address two routes of seed dissemination; the growth and import from Chile of the seed to be planted from Chile and the movement of the harvested seed to SemBioSys storage facility in Washington for further processing. While the EA discusses the impacts from the planting (release) of the GE safflower seed into specified areas, APHIS recognizes the concern about the growth, import and movement of the seed. Chile is a low-production geography for safflower. According to FAO, safflower seed is not listed in the top 20 commodities produced by Chile nor is it listed in the top 20 producers of safflower seed in the world; the lowest producer being Palestine with a production of less than 5 metric tons in the year 2005 (http://www.fao.org). Assuming an average yield of 1 metric ton/ha http://www.fao.org/ag/agl/aglw/cropwater/safflower.stm#yield, 5 metric tons is the equivalent of 5ha which is roughly 10 acres. Therefore there is essentially no safflower production in Chile. There are two weedy relatives to safflower that have been collected in Chile, *C. lanatus* (n=22) and *C. leucocaulos* (n=10). The chromosome number differs from *C. tinctorius* (n=12) and neither are sexually compatible with cultivated safflower. Therefore there is little opportunity for GE safflower to hybridize with any commercial or wild safflower plants or commingle with any commercial safflower. While the GE safflower grown in Chile did not come under APHIS authority, SemBioSys has followed all permitting requirements by Chilean officials without incident. The actual import and movement of GE safflower seeds were done in compliance with triple containment regulations as specified in APHIS regulations 7CFR340.8 which effectively exclude seeds from the environment. APHIS concluded there was an unlikely risk of seed dissemination via seed production in Chile or by its import. APHIS has determined there should be no risk by the subsequent movement of the seeds post-harvest.

Two public interest groups cited the December 2005 OIG report on significant deficiencies in oversight of GE crop trials to request a ban on the outdoor production of pharma food crop trials. OIG began the review of APHIS on April 25, 2003 and this audit focused on GE field testing between 2001 through 2003. Therefore significant changes made within BRS since that time are not captured in the final report. BRS was not formed until August 2002 and the organization has made tremendous progress in improving on areas highlighted in the audit. For instance, BRS has enhanced compliance and inspection, hired a documents control officer and has moved to an ePermitting system to enhance tracking. Long before the report was released, BRS had implemented

most of the recommendations which have significantly enhanced APHIS' internal management controls and regulatory oversight of genetically engineered (GE) crops. BRS has instituted a wide range of controls to provide maximum visibility, accountability, and transparency including a strengthening of requirements for field testing of pharmaceutical plants published in the FR on March 10, 2003 (the response to the OIG report is found on http://www.aphis.usda.gov/brs/brs_oig.html).

One public interest group called for an Environmental Impact Statement to be done before the deregulation of GE safflower. The federal action to allow a permitted field trial is not for deregulation of GE safflower. The GE safflower is being grown under strictly controlled conditions to confine the safflower to the field test site. APHIS' stringent confinement conditions and mitigation measures **control not only the probability of exposure, but the level**. Because of these measures, the probability of a single individual getting repeatedly exposed to biologically significant amounts of the challenged proteins is so low that it is infinitesimal. The proposed feeding studies will occur within a controlled experimental environment as stated in the EA. CEQ regulations state that an Environmental Assessment is adequate when the proposed action will not significantly affect the quality of the human environment. This group also wanted the positive economic impacts to be assessed for aquaculture producers (including shrimp producers). These impacts are not related to any environmental effects relating to this federal action and therefore the aforementioned economic impacts are outside the scope of the EA.

One public interest group was concerned over severe rain and wind events (>50 mph) that could disperse seed beyond the field test sites. Heavy rains, storms and tornado activity are most common in the spring months when the crop is being planted or is immature. The crop does not survive standing water for even a few hours above 20C and heavy rains during flowering leads to crop loss. The seedhead holds tightly onto the seeds and they do not shatter in high winds, although the safflower stalks of maturing plants tend to break due to the weight of the seedhead (as the taproot of safflower can penetrate the soil 2-3 ft). In the event some seed is dispersed by being washed or blown away from the field site, they are unlikely to establish and produce seeds in unmanaged environments. In managed environments, they are unlikely to compete with heavily seeded cereal crops like barley and wheat due to the herbicide management practices for these crops and growth competition. In row crops, they are unlikely to survive weed control programs.

One group was concerned that the seeds harvested could potentially be sold to commercial markets. SemBioSys has a strict chain of custody procedure for handling seed and uses a closed loop identity preservation system. This seed is being specifically grown for non food or feed and is immediately being exported to a private facility that has no connection with food or feed seed processing. Furthermore as the only safflower seed produced in the area is used for bird seed and is visibly different from the seed produced by SemBioSys, there is essentially no chance that the seed will be mixed in with the bird seed produced on other farms. There are also no safflower oil production facilities in the state of WA.

One public interest group compared the long distance pollination of pine trees (conifers) to that of safflower. The study that was cited (Duke University, Spatial modeling of transgenic conifer pollen, http://www.nicholas.duke.edu/people/faculty/katul/book_chapter_williams.pdf) focused on trees that are primarily wind pollinated and whose seeds are designed to disperse maximally with the wind. According to Dajue and Mündel. (1996) wind-pollination does not contribute to
safflower seedset. As such, the comparison made by the commenter between a self-pollinating/insect-pollinating agricultural crop and a wind-pollinated tree is not scientifically valid, despite the similarity in pollen size of the two different species.

One group charged that APHIS fails to assess the containment measures for growing GE safflower at different sites. APHIS disagrees with this comment. Information on confinement measures to be submitted in permits is stipulated in 7 CFR 340.4 and in the APHIS User's Guide. APHIS also provides additional guidance for pharma permits in Federal Register Notices, letters to the applicants, and on the BRS website (http://www.aphis.usda.gov/brs/pharmaceutical.html). APHIS requires the following information in permit applications: 1. the final and intermediate destinations of the plant and its products; 2. the location and specific design of the field test site and conditions of the release; 3. a description of the biological factors and measures that will be taken for physical and reproductive isolation of the plant and its progeny from planting through harvest; 4. how the site will be secured, monitored, and inspected; 5. plans for the termination, destruction, and disposal; and 6. post-harvesting monitoring and subsequent land use to ensure the plants, their progeny, or their active products do not persist or pose a risk in the environment. Up to five inspections per site and two inspections the following season are conducted to ensure compliance with the redundant containment measures.

One public interest group was concerned about the potential theft of the GE safflower seed after harvest. Each test site has a locked storage facility where the seed will be stored until export. SemBioSys is required to report any theft of GE safflower seed immediately to APHIS.

One group believes APHIS should assess the increasing GE crop acreage in general and the effects on growers as well as the effects on threatened and endangered species in Washington State and neighboring states should any contamination from GE crops occur. This EA is solely focused on the environmental effects of GE safflower as specified in the EA. The roles of other GE crops on threatened and endangered species, WA growers and growers in neighboring states are beyond the scope of this EA; however, due to the small size of the proposed release, APHIS has determined that it will not result in a significant increase of total GE crop acreage, nor will it have any effects on TES in Washington or in neighboring states.

One public interest group was concerned with the protocols APHIS has in place to monitor planting and harvesting at the different field site locations and believes APHIS should have a broader monitoring system and detection protocols to see if GE safflower

has escaped. APHIS believes that the current containment measures specified in the EA and permit application, along with the applicant's SOPs are sufficient to detect and destroy safflower volunteers.

One group was concerned that APHIS did not assess the potential for native bee populations and other insects to disseminate pollen from the fields to ornamental safflower growers or people who grow safflower for food in their backyards. Ornamental safflower is not a common annual that is grown in backyards. Processing safflower oil requires specialized equipment and is normally beyond the backyard food grower. APHIS believes the potential for pollen spread by bees or insects to within a 2 mile zone around the field test sites is remote, and even more remote that a backyard safflower enthusiast exists within that zone.

Several groups were concerned over the cumulative impacts of growing GE safflower. The only past, present, and reasonably foreseeable actions associated with the location for the proposed release are those related to agricultural production. The land used for the growth of GE safflower has been in agricultural production for greater than ten years and will continue in agricultural production for the foreseeable future. APHIS has determined that there are no past, present, or reasonably foreseeable actions that would aggregate with effects of the proposed action to create cumulative impacts or reduce the long-term productivity or sustainability of any of the resources (soil, water, ecosystem quality, biodiversity, etc.) associated with the release site or the ecosystem in which it is situated. No resources will be significantly impacted due to cumulative impacts resulting from the proposed action.

One commenter was concerned that the EA failed to address negative economic impacts and potential health impacts to neighboring safflower producers should contamination of conventional or organic safflower crops occur. APHIS has thoroughly considered this possibility and concluded that the likelihood of foreseeable economic impact and health impact is negligible because 1) there is very little safflower production in the state of WA (total of 1500 acres not counting SemBioSys) 2) the limited acreage is separated from the SemBioSys plots by distances that exceed the foraging range of potential pollinators, 3) multiple redundant confinement procedures are in place such as:

- The use of dedicated equipment
- Fallow zones
- Pre-selected contract distributors for shipping of seeds and harvested materials
- Secure distribution of seeds by developer to pre-selected contract growers
- Sealed containers shipped under permit in triple containment with security
- Continuous tracking of inventory
- Direct transport to the processing facility
- Point to point pre-determined chain of custody with sign off and release
- Strictly controlled processing of product in dedicated facilities outside of conventional food and feed channels

Based on these multiple redundant measures, APHIS has concluded that the likelihood of commingling and gene flow to conventional safflower are negligible. Therefore APHIS cannot foresee any economic or health impacts from the field trial.

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USDA-APHIS
Environmental Assessment

In response to permit application (06-250-02r) received from the SemBioSys, Inc. for a field-test to produce carp growth hormone in genetically engineered safflower (*Carthamus tinctorius*) seeds

U.S. Department of Agriculture

Animal and Plant Health Inspection Service

Biotechnology Regulatory Services

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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 request for a permit (APHIS Number 06-250-02r) 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-carp growth hormone (somatotropin from *Cyprinus carpio*) exclusively within its seed to be ground and incorporated into experimental aquaculture feed. This transformed safflower is currently a regulated article under USDA regulations at 7 CFR Part 340, and as such, field tests of transformed safflower have been conducted under a permit issued by APHIS (Permit #05-320-01r). SemBioSys has submitted a permit application to plant GE safflower to increase seed production.

II. INTRODUCTION

Demand for seafood has increased dramatically in recent years (Worm et al., 2006). The aquaculture industry seeks to raise seafood in managed environments often at higher densities than are supported in the wild. High density production methods may lead to detrimental health consequences including more rapid spread of disease and decreased growth rates. The aquaculture industry has been trying to find ways to improve their managed environments without the detrimental health consequences of over-crowding. One current practice that promotes health in farm raised seafood is to include carp growth somatotropin in aquaculture feed. SemBioSys has genetically engineered the safflower cultivar S-317 (moderate oleic acid oil producer) to produce carp growth somatotropin as a fusion protein to oleosin, a naturally occurring seed storage protein. The fusion protein is expressed exclusively within its seed, which will then be ground and incorporated into aquaculture feed. The ground seed supplement in aquaculture feed has been shown to increase experimental farmed fish growth rates and health as well as increasing the immuno-resistance of farmed shrimp to common shrimp diseases such as white-spot virus (unpublished SemBioSys, Inc. internal data). Currently, there are no U.S. Food and Drug Administration (FDA) approved hormones for growth promotion of aquatic animals. The transgenic safflower seed meal is to be used in experimental fish feeding studies by SemBioSys and is not for commercial production. A technical review of carp growth hormone can be found in Appendix IV.

A. USDA Regulatory Authority

The authorities for regulation of genetically engineered safflower is 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 the recombinant DNA technique using the vector, 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. Since this planting is not for commercial distribution, SemBioSys has not submitted a food and feed safety and nutritional assessment summary to FDA for the transformed safflower.

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 Douglas, Grant and Lincoln Counties, WA. The safflower is genetically engineered to express, within its seeds, the carp growth hormone fused to an *Arabidopsis* oleosin. The purpose of this introduction is to obtain a seed increase of material for future use as a supplement in aquaculture meal. The permit application was received by APHIS, BRS on September 5, 2006. It was submitted by SemBioSys Genetics, Inc., W. Sacramento, CA. The application number is 06-250-02r.

APHIS has prepared this EA before making a determination on whether to allow the planting of transformed safflower under APHIS regulations. The developer of this transformed safflower, SemBioSys, submitted a permit application to USDA-APHIS requesting that APHIS make a determination whether to allow planting for seed increase under 7 CFR Part 340. 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 USC 4321 *et seq.*) and the pursuant 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 to increase seed production

For the purposes of this Environmental Assessment, the No Action alternative would be the denial of permit application 06-250-02r. APHIS might choose this alternative if there were evidence to demonstrate a plant pest risk from the cultivation of safflower engineered to express carp growth hormone within its seeds.

The U.S. Department of Agriculture's Animal and Plant Health Inspection Service, Biotechnology Regulatory Services has previously allowed the SemBioSys Genetics, Inc. to plant transgenic safflower plants containing the carp growth hormone-oleosin fusion gene under permit 05-320-01r for a total of 20 acres which is set to expire in April 2007. Under the No Action Alternative, if submitted permit 06-250-02r is denied, the transgenic safflower plants will not be planted. Any safflower plants currently planted under permit 05-320-01r will be removed from the field or devitalized before the expiration of that permit, as required by APHIS regulations in 7 CFR 340.3(c).

B. Issue the Permit: Allow planting with supplemental conditions

In this alternative, issuing this permit would allow the following research to proceed at field sites in Douglas, Grant and Lincoln Counties, WA (see Appendix III for additional details). In this alternative, Supplemental Permit Conditions (Appendix VII), input from the State of Washington and public comment from this environmental assessment would be required. If APHIS chooses this alternative, additional mitigating measures would be required to prevent spread of the organism outside the field production area based on environmental risk of escape of the engineered organism.

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 Standing 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 sites will be geographically isolated from any commercial safflower production fields, ensuring no out-crossing of the GE safflower to food or feed safflower crops. The locations of the field sites are considered CBI. Crops surrounding the field sites will be barley and wheat with the closest commercial safflower production field at least ten miles away; however, APHIS permit conditions only require a 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 seed. The ten mile distance between the SemBioSys field test sites and any commercial safflower fields far exceeds these requirements.

b. The field test sites will be geographically isolated from any sexually compatible wild safflower species to ensure no out-crossing of the GE safflower with 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* with an n=12 chromosome number) is only sexually compatible with the federally listed noxious weed known as wild safflower or jeweled distaff thistle (*C. oxyacanthus* with an n=12 chromosome number). *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 fields will be located.

c. There are no apiaries within a ten mile radius of the field test sites ensuring no pollen flow of safflower by bees and preventing out-crossing of GE safflower in the unlikely event that any safflower (wild or cultivated) is present in the 2 mile isolation zone around the field sites. 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 Douglas, Lincoln and Grant Counties. No apiaries are found within a 10 mile radius of the test plot sites. Wild bees and pollinating insects are not expected to be a problem due to insecticides used on adjacent wheat and barley fields to prevent thrips, wireworms, cutworms and other insect pests (http://www.ipmcenters.org/CropProfiles/docs/wabarley.html, http://www.ipmcenters.org/cropprofiles/docs/IDwheat.html).

d. Each test plot will be surrounded by a 50 ft fallow (bare ground) border to detect any potential vegetative reproduction by the transgenic safflower plants or volunteers that may occur as dedicated equipment is moved through out 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 and to ensure no significant harm to the environment:

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 out-crossing of the GE safflower with any food or feed safflower crop.

C. Preferred Alternative

APHIS has chosen Alternative B as the preferred alternative. This is based on APHIS' scientific analysis of the permit application.

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. The 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 carp growth hormone-oleosin fusion gene is a moderate oleic oil producer (~39% oleic oil production).

Out-crossing between safflower crops 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 of out-crossing.

Wild relatives of cultivated safflower, *Carthamus creticus* and *C. oxyacantha,* 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. oxyacantha* has been reported in California (specifically in Monterey County), overall this sexually compatible species is rare and none have 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.

Up to ten field sites (50-100 ac each site) will be located on private property in Douglas, Grant and Lincoln Counties, WA. These counties contain a mix of agricultural sagebrush-steppe ecosystems with an average rainfall between 7-10 inches per year (www.worldclimate.com). The adjacent agricultural lands are expected to grow barley and wheat. There are no bodies of water (lakes, streams or rivers) within a 3 mile radius of any of the proposed field test sites. Since the carp growth hormone 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).

The proposed field test sites will not be artificially irrigated and will rely on natural precipitation for water. 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^{o}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 head (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 Grant, Douglas or Lincoln Counties in WA (The closest the Pacific Flyway comes to the listed counties 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 near-by 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 sites, 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 WA following SemBioSys' standard operating procedure [CBI, reviewed by APHIS] 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.

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.*, the 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 10 miles from any commercially produced safflower fields, far exceeding the 2 mile requirement set up by APHIS.

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 carp growth hormone 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).

Carp growth hormone is not expressed in pollen or any of the vegetative material except seeds. Because it is not expressed in the pollen, no cumulative effects of carp growth hormone are expected if APHIS chooses to allow planting and the pollen falls outside the field test site, by-passing the redundant mitigation measures in place.

Seed Movement

Movement of whole seed by animals is the major way 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 test sites 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 which would carry the seeds away from the field site. 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 most likely be ground up and digested and are not expected to pass through the avian digestive tract while remaining viable. No cumulative effects of carp growth hormone are expected if any small animals ingest GE safflower seed.

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.

The seeds are the only place where the gene of interest (carp growth hormone) 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 three mile radius of any of the proposed field test sites, it is unlikely that aquatic animals will be exposed to any carp growth hormone should GE safflower seeds be transported outside the field test site despite strict confinement measures. Should APHIS choose Alternative B and allow planting, APHIS is confident 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 carp growth hormone 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 at best because transgene promoters and coding sequences are optimized for plant expression, not prokaryotic bacterial expression.

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. No cumulative effects of carp growth hormone would be expected with either alternative.

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 the detailed description safflower biology).

Because cultivated safflower is not described as a weedy species and none of its sexually compatible weedy species are 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

APHIS has reached a determination that the proposed environmental release will have no effect on federally listed threatened or endangered species or species proposed for listing, and no effect on designated critical habitat or habitat proposed for designation in the action area. Consequently, consultation under Section 7 of the Endangered Species Act with the United States Fish and Wildlife Service is not required for the action described

in the preferred alternative of this EA. Appendix V includes the BRS analysis of threatened and endangered species in the area of the field release.

If APHIS chooses the no action alternative (Alternative A) there would be no impact on non-target organisms or Federally-listed threatened or endangered species.

Safflower has minimal insect pests and none that feed directly on the seed; therefore, APHIS has determined there will be no impact of carp growth hormone on insects should Alternative B be chosen. APHIS has determined there will be no impact of carp growth hormone on birds or scavenging animals that could possibly ingest seed (refer to Seed Movement section). Based on the lack of toxicity of the proteins that will be produced, APHIS concludes that if it chooses Alternative B, there will be no significant effect on any native floral or faunal species for Douglas, Grant and Lincoln Counties.

4. Potential impacts on biodiversity

Analysis of available information indicates that SemBioSys' GE safflower containing the carp growth hormone-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 oleosin-carp growth hormone 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.

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 10 miles away from the closest test plot site and can 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.

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 presence 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 the 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 the permit 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 and 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# 06-250-02r. 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, Brazil, Japan, China, and Korea. Many countries, e.g. Argentina, Australia, Canada, China, Japan, Korea, Philippines, South Africa, Switzerland, 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 ft 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 seedset 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 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 (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.

Out-crossing

Out-crossing 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 out-crossing.

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. oxyacantha* (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. oxyacantha* 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 out-crossing).

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. oxyacantha* 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., pollen grain's moderately large size, 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 m 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 and probably 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 carp growth hormone 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 as a feed additive in aquaculture.

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 has been safely used in many previous field trials (CBI).

The Gene of Interest

Safflower plants were engineered so the carp growth hormone (carp somatotropin from *Cyprinus carpio*) fused to an *Arabidopsis* oleosin gene is exclusively expressed in the seeds. SemBioSys has determined that the attachment of fish growth hormone and similar proteins to plant oilbodies (e.g. *Arabidopsis* oleosin gene) allows proper folding and stable accumulation of the protein within seeds while preventing unwanted glycosylation. Expression of this gene is controlled by the phaseolin promoter and terminator sequences from *Phaseolus vulgaris* L. (common bean). The phaseolin promoter drives the tight, seed-specific transcription of carp growth hormone. 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 carp growth hormone.

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

Up to 10 field sites (50-100 ac each site) are located on private property in Douglas, Grant and Lincoln Counties, WA. The experimental plots 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. 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 aquaculture feed.

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 site. 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. Carp Growth Hormone

Growth hormone (GH) is a single chain peptide produced in the anterior pituitary of all vertebrates. The primary function of GH is to stimulate growth and development either directly or indirectly through the production of insulin-like growth factors (IGFs). GH also influences reproduction and food conversion efficiency as well as osmoregulation and sea water adaptation in fish (Mahmoud et al., 1998).

Carp growth hormone (somatotropin protein; 21.5 kDa) is found naturally within common carp as a product of the pituitary gland regulation of the growth of these fish (Mahmoud et al., 1998). The growth hormones of cyprinids (carp-like fish) belong to a family possessing similar primary structure but are not closely related to those of other animals and share only 36% identity with human and about 40% with bovine somatotropins (Mahmoud et al., 1998).

While the safety of carp growth hormone to humans and animals other than fish has never been investigated, the long history of fish ingestion by numerous mammalian and avian species would indicate that it is not toxic at its natural biological levels. A comparison was made of the safety of the transgenic protein as it relates to recombinant bovine somatotropin, which is very well characterized. As mentioned above, carp growth hormone shares some homology with its bovine counterpart and performs the same basic function within its host. The ingestion safety of bovine somatotropin to mammals such as mice, cattle and humans has been described and it is deemed a safe protein even at high levels of exposure due to the breakdown of proteins in the mammalian and avian digestive process (USFDA, 1993). Part of the difference between the oral efficacy of these hormones on fish versus mammals and birds is that the fish gut can absorb intact proteins into circulation before digestion occurs (McLean and Donaldson, 1990). Another interesting feature of somatotropins is that those belonging to higher organisms such as mammals have a definite biological effect on lower animals such as fish while the fish counterpart does not appear to affect mammals (Hertz et al., 1991; Peter and Marchant, 1995; Pérez-Sánchez et al., 2002).

Appendix V. Threatened and Endangered Species Analysis

The proposed field sites are for the confined releases of the regulated article into the environment in Grant, Douglas and Lincoln Counties, WA. There are no listed critical habitats for any threatened and endangered animal species in Grant, Douglas and Lincoln Counties, 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 06-250-02r, 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 exceedingly low.

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 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 or other product that is known to affect the metabolism, growth, development, or reproduction of animals, plants, or microbes. A BLAST search using the amino acid sequence of the carp growth hormone-oleosin domains did not reveal any significant homology (> 50%) to the amino acid sequence of proteins other than oleosin and carp growth hormone 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.
5. Literature searches of the NCBI's PubMed databases did not reveal any evidence for toxic or allergenic effects of the carp somatotropin growth hormone on human or animals. Fish somatotropin does not appear to have a biological effect on mammals (Fine et al., 1993).

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). 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 2006 showed that 9 threatened or endangered plant species and 28 animal species exist in Washington. Of the 28 listed, only four animals potentially reside in Douglas, Grant and Lincoln Counties, 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 not near the field test sites, so it is unlikely that this species would be impacted by the field tests.

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. The pygmy rabbit is not expected to be impacted by the field test sites due to its preference for sagebrush and riparian habitats.

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. Columbian White-tailed Deer are not expected to be impacted by the field test sites.

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. Since the Gray Wolf habitat does not include maintained agricultural land, the field test site is not expected to impact this endangered species.

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

- Spalding's Catchfly (*Silene spaldingii*) – Lincoln County
- Ladies' Tresses (*Spiranthes diluvialis*) – Douglas 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 and thus, the field trial sites, all agricultural areas, are not expected to impact this threatened species.

Ladies' Tresses (*Spiranthes diluvialis*) is a threatened species listed as potentially occurring in Douglas County, WA; however, Natureserve distribution and the U.S. FWS recovery plan only indicate potential growth sites in Chelan and Okanogan Counties (www.natureserver.org; (USFWS, 1995). Its preferred habitat is moist to very wet meadows along streams or in abandoned stream meanders that still retain ample ground water. It is also found near springs, seeps, and lakeshores. Because of the preferred habitat locations, Ladies' Tresses are not expected to be impacted by the field trial sites which are all areas previously used for agricultural production.

Based on the reasons listed below, APHIS is confident that these field trials will not harm or have any significant adverse 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 carp growth hormone-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 carp growth hormone 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 the carp growth hormone on human or animals. The long history of fish ingestion by numerous mammalian and avian species also confirms that carp growth hormone protein is not toxic at its natural biological levels.
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. Therefore, this field test is deemed confined within the meaning of 7 C.F.R. §372.5.

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 06-250-02r

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

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.

II. 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.

III. 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.

IV. 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.

V. 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.

VI. 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.

VII. 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.

VIII. 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.