NEPA Decision Worksheet

Permit #	05-320-01r
Institution	SemBioSys
Organism	Safflower
Category	OO

Gene	Oleosin from Arabidopsis and Carp Growth Hormone
1. Confinement	
Confinement and mitigation conditions have been reviewed and determined to be adequate	X
2. Threatened or Endangered Species or its habitat	
Resident or migratory in counties and harm to threatened or endangered species or habitat is likely	
Resident or migratory in counties and harm to threatened or endangered species is unlikely	
None observed in area (no harm to threatened and endangered species)	X
New or Novel	
3. New or Novel Crop	
Never used in a field trial	
Not new but no prior EA	X
Not new and prior EA	
4. New or Novel Trait (gene product)	
Never used in a field trial	
Not new but no prior EA	X
Not new and prior EA	
Raises new issues	
5. Cumulative Effects	
Cumulative effects likely	
Cumulative effects possible	
Cumulative effects unlikely	X
6. Plant Pollination	
Primarily bee or insect pollinated crop	
Primarily wind pollinated food or feed crop	
Primarily self fertilized food or feed crop	X
Non-food or feed crop	
7. Effects on Food/Feed Supply	
Known allergen, antinutritive, oral toxicant	X
Food safety not established	
GRAS status or approved food additive for native protein	
GRAS status or approved food additive for plant produced protein	
8. Isolation Distance	
AOSCA standard for crop	
Proposed isolation distance	2 MILES
9. Scale	
>100 acres/trait/crop/institution/year	
50-99 acres/trait/crop/institution/year	
10-49 acres/trait/crop/institution/year	Two sites of 10 acres each
<10 acres/trait/crop/institution/year	
10. Effects (positive or negative) on other species	
Significant effects expected/observed	
Minimal, non-cumulative effects expected/observed	
No effects expected/observed	X
11. Sexually Compatible Relatives	
Relatives within dispersal distance	
Relatives not within dispersal distance	X
12. Seed Dormancy	
>3 years	
3 years	
2 years	
<2 years	X[1]
13. Persistence in environment	
Crop can naturalize	
Crop can persist 3-5 years without human intervention	
Crop does not persist without intervention	X

14. Comments

1.Safflwer generally lacks seed dormancy and can germinate in the head if rain fall occurs at harvest time (http://www.ipgri.cgiar.org/publications/pdf/498.pdf)

Additional supporting documentation is found in the summary risk assessment completed on March 13, 2006

/s/

Rudaina Alrefai
Biotechnologist
APHIS/BRS/PPP

NEPA Decision Summary of Permit 05-320-01r

Pursuant to the regulations found in 7 CFR Part 340, SemBioSys Genetics, Inc submitted a permit application (APHIS # 05-320-01r, dated November 10, 2005) to conduct field tests of genetically engineered Safflower expressing a fusion protein, called ImmunoSphere, with industrial applications. The fusion gene encodes three domains: 1) the oleosin protein from *Arabidopsis thaliana*; 2) Synthetic linker sequence of six amino acids; and 3) the somatotropin growth hormone protein from carp (*Cyprinus carpo*). The purpose of the field test is to increase seed production of transgenic safflower (*Carthamus tinctorius*) plants that are harvested at Peru, Chile. The proposed field testing of these plants will begin in April, 2006 in Douglas County and Lincoln County, WA with a total acreage of up to 20 acre (10 acres per site).

On the basis of our review of permit 05-320-01r, 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:

- The safflower plants were transformed using a disarmed *Agrobacterium tumefaciens* protocol which does not cause plants to exhibit plant pathogenic properties.
- The selectable marker gene and all of the non-translated regulatory elements are well characterized.
- 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 ImmunoSphere domains did not reveal any significant homology (> 50%) to the amino acid sequence of proteins other than oleosin and carp growth hormone proteins.
- 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.

- Literature search of the NCBI's PubMed 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 *Gen Comp Endocrinol* **89**: 51-61.)

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:

- 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.
- 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.
- In addition to the large degree of self-pollination of safflower plants, redundant 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. SemBioSys will also maintain at least 5 miles isolation distance from safflower seed production for food use. 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.
- The test plots will be monitored weekly for weed, disease, and insect infestation.
- 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 two growing seasons after harvest. In the growing season following the harvest, the test area will be left fallow.
- 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.
- 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 also 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 March 2006 showed that 9 threatened or endangered plant species and 33 animal species exist or once existed in Washington. 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.

- 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 search of the ImmunoSphere 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.
- Literature search of the NCBI's PubMed 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.
- 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.
- 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.
- 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.
- Safflower does not outcross 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 #05-320-01r 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.

Signed: _____/s/_____
Michael Watson, Ph.D.
Director
Plant Pest Protectants Branch
USDA/APHIS/BRS

Date: _____03/30/2006_____

Initial: RA
cc:
Ingrid Berlanger