NEPA Summary for Permit #09-082-102r

SemBioSys, Inc. has requested a permit for a confined field release of genetically engineered safflower plants of up to 50 acres at multiple release sites (see permit for gps coordinates) in the state of Washington within Grant, Lincoln and Douglas counties. APHIS Biotechnology Regulatory Services (BRS) concludes this is a confined release of genetically engineered safflower plants. The tests are not expected to present a significant risk of plant pest introduction or dissemination for the reasons cited below:

1. APHIS has processed 16 safflower notifications and permits to date since 2003. Two Environmental Assessments (EAs) on safflower have been written by APHIS covering the confinement, plant pest and NEPA issues of safflower in Washington State in all counties (Grant, Lincoln and Douglas) where the safflower in this permit is to be planted (EAs are associated with permit numbers 06-250-02r, 06-363-103r and 07-021-101r). In the prior EAs on safflower, APHIS considered 10 acres, 250 acres and 1000 acres of safflower to be planted and determined that the confinement conditions were adequate for all of those size field trials. APHIS and the permit applicant are familiar with safflower biology, confinement and agricultural practices. The applicant has provided APHIS with their Standing Operating Procedures for employee training, equipment cleaning and confinement practices and APHIS has deemed them sufficient to meet all Supplemental Permit Conditions for PMP (plant made pharmaceuticals). The land that this safflower is to be planted on has been in agricultural use for >10 years. Up to 50 acres per site will be planted in this field trial.

2. Safflower is a minor crop of North America and is grown mainly for its seed, which is used as edible oil, meal and birdseed. Safflower is a highly branched herbaceous annual thistle, usually with sharp spines on the leaves. The S-317 safflower cultivar to be planted in this permit is 85-90% self-pollinating with bees being primarily responsible for the remaining percentage of pollination. 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). As stated in the previous EAs on safflower, *C. oxyacantha* has been reported in California (specifically in Monterey County), but overall this sexually compatible species is rare and none have been detected in WA. Furthermore it is considered a noxious weed and so quarantine and eradication efforts would be triggered if it were to be detected. Therefore no out-crossing to wild relatives is reasonably foreseeable.

3. Based on the method of transformation using *Agrobacterium*, only the genetic constructs that are designed to be expressed in the genetically engineered safflower line is expected to be efficiently inserted into the safflower genome. SemBioSys has provided a molecular analysis of the inserted gene and APHIS has determined that no plant pest vectors are associated with the transformed safflower line as a result of the transformation process. None of the genes encoding the desired traits or the selectable

marker, nor the regulatory elements controlling their expression, have any inherent plant pest characteristics, and thus as described in the EA for permit 06-363-103r, p. 24, it is not reasonably foreseeable that this plant will be a plant pest risk based on the gene sequences introduced through the genetic engineering.

4. The field sites (up to 50 acres) will be located on private property in Grant, Lincoln and Douglas Counties, WA. This county contains a mix of agricultural sagebrush-steppe ecosystems with an average rainfall between 7-10 inches per year (www.worldclimate.com). The adjacent agricultural lands will grow barley and wheat. There are no bodies of water (lakes, streams or rivers) within a 5 mile radius of the proposed field test site. Since the bovine prochymosin 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) and described in EA for permit 06-250-02r on page 8 and in EA for permit 06-363-103r on page 8-9. The confinement measures described in the application and analyzed in the previous EAs should be sufficient to prevent any unplanned releases of the transgenic plant material or transgenic seed or the persistence of the transgenic material or its progeny in the environment.

5. The transgenic safflower variety (Centennial) is similar to the S-317 variety described in previous EAs (06-250-02r, p.7 and 06-363-103r, p.22) it that it produces striped seed hulls. It produces more linoleic acid and higher oil as well as being resistant to Alternaria leaf spot and Pseudomonas bacterial blight disease. The transgenic safflower is 85-90% self-pollinating. In addition to the large degree of self-pollination of safflower plants, other mitigating measures such as the perimeter fallow zone and field site isolation from any commercially planted safflower are implemented to prevent gene flow through pollen dispersal to any compatible species or by seed dispersal. The field site will be isolated from 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.

6. Safflower seed produced from the field trials will be retained for the next planting or shipped to other facilities. Waste or plant material generated by sample processing or handling will be returned to the field site for disposal and monitoring for volunteers, or devitalized to render plant propagules non-viable.

7. 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. Seed harvesting may be performed by single or multiple plant threshing by machine. Containment and processing of the safflower seed and viable plant parts will be in a secure limited access facility adequate for USDA

compliance. After final seed harvest, the remaining plant material will destroyed by incorporation into the ground by tillage, hand weeding, grinding, or chemical application.

8. As described in the both prior safflower EAs, (06-250-02r, p.8; 06-363-102r, p. 8-9), cultivated safflower is not observed to be capable of establishing persistent populations in unmanaged environments; it is reliant on continuous human intervention for its survival. Safflower has no seed dormancy and seed heads can even germinate on the plant if there are excessive moisture conditions before harvest. Safflower also has no weedy tendencies. Volunteer monitoring conditions can be found in the supplemental permit conditions.

9. Threatened and Endangered species listed for Washington State include 9 threatened or endangered plant species and 28 animal species. 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*) and gray wolf (*Canis lupus*).

   Of the nine plants listed, only one threatened species potentially resides in Lincoln County and another threatened species in Douglas County; Spalding's Catchfly (*Silene spaldingii*) in Lincoln County and ladies' tresses (*Spiranthes diluvialis*) in Douglas County. Of those terrestrial species none reside in agricultural fields. All proteins produced by the construct in this permit were analyzed for sequence homology to known toxins. No known toxins were identified. Furthermore, bovine chymosin (rennin) is a common enzyme consumed by humans and vertebrates and is the principle enzyme used in cheese production from milk products. Thus in the unlikely event of transgenic safflower consumption by vertebrate or invertebrate animals, no significant negative effect should occur. APHIS has reached a determination that the release of transgenic safflower (09-082-102r) would have no effect on federally listed threatened or endangered species or species proposed for listing, nor is it expected to adversely modify designated critical habitat or habitat proposed for designation, compared to current agricultural practices.

9. Regulated materials in this field trial are not intended for food and/or feed. Any use of these products for food or feed must be in compliance with the guidelines published in the Federal Register by the United States Food and Drug Administration [57 FR 22984, May 29, 1992].

For the above reasons, APHIS has determined that (1) pursuant to 7 CFR 372, the field trials proposed under Permit number 09-082-102r 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 despite the protective and ameliorative measures specified above. Therefore, this field test is deemed confined and is not classified as an exception to a categorical exclusion within the meaning of 7 CFR 372.5 because the permitted action does not raise new or novel issues.

Signed: _____

Mike Watson
Branch Chief, Plant Pest and Protectants Branch
Biotechnology Regulatory Services

Date:_____
PKB_/s/_