NEPA Summary for Permit #10-334-104r

The National Environmental Policy Act of 1969 (NEPA) is the mandate of any federal agency or department for the protection of the environment. NEPA requires all federal agencies to consider the values of environmental preservation for all significant actions and prescribes procedural measures to ensure that those values are in fact fully respected.

The Council on Environmental Quality (CEQ) developed the categorical exclusion process to reduce the amount of unnecessary paperwork and delay associated with NEPA compliance.

The categorically excluded actions for APHIS Biotechnology Regulatory Service (BRS) processes are listed in 7 CFR 372.5(c)(2)(ii):

"Permitting, or acknowledgment of notifications for, confined field releases of genetically engineered organisms and products"

However, the CEQ acknowledged that, from time to time, exceptions to a categorical exclusion may arise. As a result, the CEQ requires all agencies to develop procedures to determine whether a normally excluded action may have a significant environmental effect. Exceptions to categorically excluded actions for APHIS BRS are determined by the following criteria found in 7 CFR 372.5(d)(4):

"When a confined field release of genetically engineered organisms or products involves new species or organisms or novel modifications that raise new issues."

SemBioSys, Inc. has requested a permit for a confined field release of genetically engineered safflower plants of up to 20 acres at two release sites (see application) in the state of Washington within Lincoln and Grant counties. Safflower plants were engineered so a modified human proinsulin gene was fused to an *Arabidopsis* oleosin single chain antibody to be exclusively expressed in the seeds. The GE safflower also contains a selectable marker, the PAT gene, which confers resistance for the herbicide glufosinate . Expression of this gene is controlled by the phaseolin promoter and terminator sequences from *Phaseolus vulgaris* L. (common bean). The phaseolin promoter drives the exclusively seed-specific transcription of human proinsulin.

APHIS BRS has reviewed the permit application and has set permit conditions for the activities to be authorized under this permit. These conditions can be found in the e-permits file associated with this application. APHIS BRS has concluded that issuing Permit number 10-334-103r is categorically excluded action under section 7 CFR 372.5(c)(ii) because it is a confined field release of genetically engineered organisms.

The field release is confined for the following reasons:

1) The proposed field release site is for up to 10 acres, in two locations (total of 20 acres in the permit). There will be one planting per location. The small experimental plots with limited plant numbers decrease the pollination potential (pollen pool) of the field site to other plants not involved in the study. Studies

have suggested large field sites and therefore, large pollen pools increase the potential for pollination and gene escape.[1]

2) Experimental plots will be conducted in Grant and Lincoln counties. There will be no other GE plants within two miles of the experimental plots and sexually compatible wild relatives do not exist in the area. The closest commercial safflower field is over 10 miles away and the safflower is morphologically distinct from what is being grown. No organic safflower is grown in these counties. This prevents escape of the genetic traits via pollination.
3) Dormancy is reported to be very short. Short dormancy ensures that rogue volunteer plants will not appear after the experiment has terminated and the volunteer monitoring period has ended.
4) Safflower seed will be imported to the field sites and stored in locked, labeled facilities.
5) Waste or plant material generated by sample processing or handling will be destroyed by tilling or herbicide application. Destruction prevents inadvertent plant material escape.
6) The regulated area will be monitored monthly for a year after termination of the trial. If regrowth is found, it will be destroyed immediately. Should regrowth be found during the last monitoring month (month 12) of the year, monitoring will continue and APHIS BRS Compliance will be notified.

APHIS has determined that the exception for categorically excluded actions (7 CFR 372.5(d)(4)) Do not apply to this action for the following reasons:

1) This GE safflower is not a new species to APHIS. APHIS has processed over 25 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 both counties (Grant and Lincoln) 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, 08-205-101r). In the prior EAs on safflower, APHIS considered 10, 100, 250 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.
2) The introduced traits do not raise new issues. Human proinsulin biologically inactive and is destroyed readily by digestive acidity and enzymes when ingested. Thus in the unlikely event of transgenic safflower consumption by vertebrate or invertebrate animals, no significant negative effect should occur.
3) APHIS BRS has issued prior permits for GE safflower with proinsulin, carp growth hormone, human apolipoprotein and rennin and have found no significant impacts to the human environment. All proteins produced by the construct in this permit were analyzed for sequence homology to known toxins. No known toxins were identified.

[1] USDA APHIS (2004). Workshop on the Confinement of Genetically Engineered Crops during Field Testing, September 13-15, 2004. Washington, D.C.

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

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

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

Proinsulin has no biological activity when ingested. Even the task of developing an oral insulin delivery method has been unsuccessful in the last several decades because both insulin and proinsulin are peptides that are easily and quickly digested. Like most peptides and proteins that animals eat as food sources, the proinsulin within the safflower seed will be broken into amino acids or small peptides within the stomach before they can be absorbed in the intestine. This breakdown of protein begins in the stomach where hydrochloric acid (HCl) denatures the protein and facilitates the action of pepsin, the major gastric enzyme that splits the peptide bonds. Other proteolytic enzymes (enzymes that break down protein bonds) involved in the gastric process are trypsin, chymotrypsin, carboxypeptidase and elastase (Zeitlin, Cone et al. 1999). In simulated digestive studies, active insulin is completely digested in 60 minutes whereas the proinsulin seed fusion protein is completely digested in 15 minutes (SemBioSys, 08-205-101r permit application data). In the unlikely event of an exclusive diet of transgenic proinsulin safflower seeds, no effects of human proinsulin are expected on animals due to the digestion process.

In addition to determining that the field trial is confined and that the exclusions do not apply to this action, APHIS has also concluded that there are unlikely to be any significant impacts from the authorization of this field trial because:

1) The field release is limited in time and space. The plants will be in the field for less than one year in an area equal to or less than 10 acres per site.
2) The genes do not code for toxins or any other substance that is likely to harm any animals or humans that may encounter the plants.

3) The GE plants do not encode any substances that will persist in the soil, water or air. Most of the genes alter the expression levels of naturally occurring compounds. The marker gene codes for a protein that will degrade in the environment like other proteins native to the plant.
4) The genetically engineered safflower will not be used for food or feed.

APHIS analyzed the potential for effects to federally-listed threatened or endangered species and their critical habitat. Based on the analysis below APHIS has determined that there is no effect to any of these species or that the activities authorized in this permit would result in the alteration of any designated critical habitat.

Threatened and Endangered species listed for Washington State include 9 threatened or endangered plant species and 19 animal species. Of the 19 listed, only one animal potentially resides in Grant and Lincoln Counties, WA (http://ecos.fws.gov/tess_public/StateListingAndOccurrence.do?state=WA; accessed on December 20, 2010); pygmy rabbit, *Brachylagus idahoenis*. This animal is not traditionally found in or around safflower or use safflower as a primary food source.

Of the nine plants listed, only one threatened species potentially resides in Lincoln County and another threatened species in Grant County (http://ecos.fws.gov/); Spalding's Catchfly (*Silene spaldingii*) in Lincoln County and Ute ladies' tresses (*Spiranthes diluvialis*) in Grant County. Of those terrestrial species none reside in agricultural fields. APHIS has reached a determination that the release of transgenic safflower (10-334-103r) 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.

This GE safflower field trial will occur on land that has been cultivated for agricultural purposes since for over 10 years and therefore will not eliminate habitat that may contain a threatened, endangered species.

The experimental plot will also not affect any TES species listed for Grant or Lincoln counties as safflower is not a primary food or habitat for any TES species in these two counties.

Signed: ____/s/_____
John M. Cordts, M.S.
Chief, Plant Pests and Protectants Branch
Environmental Risk Analysis Division
Biotechnology Regulatory Services

Date: ____12/29/2010___

References

Bolinder, J., P. Fernlund, et al. (2005). "Hyperproinsulinemia segregates young adult patients with newly diagnosed autoimmune (type 1) and non-autoimmune (type 2) diabetes." Scand J Clin Lab Invest **65**(7): 585-594.

Galloway, J. A., S. A. Hooper, et al. (1992). "Biosynthetic human proinsulin. Review of chemistry, *in vitro* and *in vivo* receptor binding, animal and human pharmacology studies, and clinical trial experience." Diabetes Care **15**: 666-692.

Glauber, H. S., R. R. Henry, et al. (1987). "The effects of biosynthetic human proinsulin on carbohydrate metabolism in non-insulin-dependent diabetes mellitus." New England Journal of Medicine **316**: 443-449.

Rosak, C., B. O. Boehm, et al. (1988). "Biosynthetic human proinsulin, a new therapeutic compound for diabetics? A comparative study of biosynthetic human proinsulin with biosynthetic human insulin." Horm Metab Res Suppl **18**: 16-21.

Tillil, H., B. H. Frank, et al. (1990). "Hypoglycemic potency and metabolic clearance rate of intravenously administered human proinsulin and metabolites." Endocrinology **127**: 2418-2422.

You, S. and L. Chatenoud (2006). "Proinsulin: a unique autoantigen triggering autoimmune diabetes." The Journal of Clinical Investigation **116**: 3108-3110.

Zeitlin, L., R. A. Cone, et al. (1999). "Using monoclonal antibodies to prevent mucosal transmission of epidemic infectious diseases." Emerging Infectious Diseases **5**(1): 54-64.