NEPA Decision Summary for Permit **#10-343-105r**

Ventria Bioscience (Nathan Fortner) has requested a permit (#10-343-105r) to plant two sites of up to 15 acres each in Geary and Riley Counties, KS, with rice (*Oryza sativa*) plants genetically engineered to produce one of nine pharmaceutical proteins.

Based on a review of Permit #10-343-105r, the following determinations were made:

1. **Familiarity of the Crop and the Traits**: Hundreds of field trials have been performed with transgenic rice plants under APHIS authority, and APHIS is familiar with rice biology and methods to manage confined rice field trials. Ventria previously grew rice in Kansas in 2007, 2008, 2009 and 2010 and satisfactorily managed those plantings. The proposed plantings in 2011 are smaller than those grown in previous years, and include the same previous field location in Geary County plus a new location 15 miles away which shares the same ecoregion, yet is in adjacent Riley County. An Environmental Assessment has been prepared previously for pharmaceutical-producing rice in the original location.

   Ventria has monitored for the presence of three of its engineered proteins (lactoferrin, lysozyme and serum albumin) in soils for several growing seasons and none has been found. Because all viable transgenic plant material will be removed from the test site and/or destroyed, there will be no foreseeable cumulative impacts resulting from field trials of these transgenic lines. Ventria is using the same regulatory sequences in gene constructs for their newly developed products (CBI) and it is likely that they would also not be found in soils.

   Lactoferrin from cow's milk and related products have been granted GRAS status by the FDA. Lactoferrin is used as a food additive and is sold as a nutritional supplement. Egg white lysozyme and related gene products have been granted GRAS status by the FDA. Lysozyme is used as a food additive and is sold as a nutritional supplement. Serum albumin (HSA) is a soluble, monomeric protein which comprises about one-half of the blood serum protein. The protein is encoded by the *alb* gene and is produced in the liver. It functions primarily as a carrier protein for steroids, fatty acids, and thyroid hormones and plays a role in stabilizing extracellular fluid volume. It is used in medical practice to replace blood volume in burn victims, patients suffering acute traumatic shock, and those undergoing certain types of surgery. It has no reported oral or dermal activities. The other gene products being developed (claimed as CBI) also have expected pharmaceutical uses and several have been previously field tested in KS by Ventria for three season since 2008. None of these nine gene products are commonly characterized as allergens, nor do they share any amino acid homology with known toxic peptides. The target molecules claimed as CBI are an iron-binding protein, a growth factor peptide, an antimicrobial protein, a glycoprotein cytokine, and cell-membrane binding proteins. A selectable marker gene with a safe history of use, either phosphinothricin acetyltransferase or hygromycin phosphotransferase, was also used in each of the nine constructs.

An EA was prepared in 2007 for rice producing several of these gene products in this ecoregion. A review of the application submitted by Ventria Bioscience raised no new issues, so the previous EA is applicable to this application.

2. **Method of Transformation**: All transformations were performed with the biolisitc method except for one genotype which was transformed with disarmed *Agrobacterium tumefaciens*. The regulatory elements controlling expression of the nine introduced genes originate from rice and *A. tumefaciens*. The target molecules undergo post-translational transport to the rice endosperm and are free of potential contaminants from either human or transgenic animal or plant systems. No other plant tissue/part expresses any of the target molecules at detectable levels. The constructs and their transgenic lines have been grown for several years under at least greenhouse conditions, if not also in the field, and have demonstrated gene expression and yield stability. There has been no observable phenotypic difference between these transgenic lines and either their untransformed antecedent rice lines or conventional cultivars. Also, Southern analysis has shown stable chromosomal integration/inheritance of the codon optimized synthetic target genes within the rice genome.

3. **Purpose and Design of the Field Trial:** The purpose for this introduction is for germplasm evaluation and selection in Ventria's Breeding Nursery. The following activities will be conducted:
   - Replicated trials comparing yield and agronomic traits of untransformed parental line and transgenic line.
   - Evaluation and selection of breeding lines.
   - Production of breeders seed.
   - Development of breeder seed from new breeding lines.
   - Development of pure line seed stocks.
   - Production of seed for laboratory analysis of the proteins of interest.
   - Assessment of gene stability.
   - Characterization of the plant (e.g. tissue specific expression, Southern analysis etc.).

   None of this rice is used as food or feed. Ventria does not distribute or offer any of its proprietary rice lines to anyone.

4. **Crop Biology and Adequacy of Confinement:** Rice is highly self-pollinated (the pollen is heavy) and is not generally pollinated by insects. Association of Official Seed Certifying Agencies (AOSCA) certified seed regulations for foundation rice seed require a minimum isolation distance from other rice varieties of at least ten feet when hand- or machine-planted.

   There are no commercial rice fields in Kansas. There is no weedy red rice in the immediate area since rice has not been grown in the area in the past. Ventria scouted for weedy rice in this area in 2007, 2008 and 2009 and none was found.

The proposed confinement protocols are adequate to ensure that the field test is confined. A 50 foot fallow zone (maintained with a mowed ground cover) surrounding each release site and a separation distance of 1320 feet from any other rice (one hundred thirty two times the AOSCA standard) as proposed by the applicant should be more than adequate to prevent gene flow.

The 50 foot fallow zone is levied and has no outlet for the irrigation water, therefore any seed that is moved by the recirculated irrigation water will settle within this zone. Measures are in place to keep water from leaving the fields during managed flood periods. Movement of seed off-field by waterfowl and establishment in other fields is unlikely because: (1) Ventria manages its rice fields to discourage waterfowl from landing during seed set and maturation; and (2) studies as summarized in the permit have shown that viable rice does not pass through the gut of waterfowl. In previous field tests, seed dormancy in rice has not been observed. Following harvest, the fields will be mowed, burned, and disked, and off-season flushing will be used to accelerate germination of any remaining seed. After harvest the field will be fallowed for one full cropping season, about 18 months. Monthly scouting for 1 year from the date of harvest and removal of any weedy or volunteer rice before it flowers within the field plot and the 1320 isolation zone, particularly the 50 ft. fallow zone, will ensure that there are no issues related to volunteer rice plants.

The rice seed will be ground seeded (not aerially seeded) with a drill seeder or a ground-scale spreader so as not to encroach on the 50 foot fallow zone. Ventria has a closed-loop growing system using dedicated equipment for all planting, harvesting, seed cleaning, seed handling, drying and storage exclusively for its proprietary rice lines. Cleanout and storage of this equipment is described in their Standard Operating Procedures which have been reviewed by APHIS. An APHIS inspection will be required before such equipment can be returned to general use. All production personnel are trained in these methods.

5. **ESA Asessment:** There are no threatened and endangered species (TES) in the action areas. There are two TES birds noted in Riley County (the Interior Least Tern and Piping Plover) and a TES fish (Topeka shiner) in both Counties. Given the location of the trial sites, the fish would not be expected to be exposed to Ventria's rice. The Tern and Plover feed primarily on small fish and insects and exposure to Ventria's planting sites would not be expected. Regardless, Ventria's primary products have not shown toxicity to birds in their testing work. Some of the new products in development have not been assessed for food safety. Ventria assessed all for similarity to known toxins and found no protein sequence similarity that would indicate toxicity of any of these proteins. The lack of exposure of these TES to Ventria's rice provides further assurance that there should be no effect on TES from growing these rice lines.

6. **Cummulative Impacts Assessment:** The incremental impact of the proposed release when added to other past, present, and reasonably foreseeable future actions (regardless of which agency or person undertakes such actions) is not expected to have a potential for significant environmental impacts. The only past, present, and reasonably foreseeable actions specifically associated with the locations for the proposed releases are those related to agricultural production. The proposed release sites have been used for crop production for over 50 years, and the proposed release will not result in a change in agricultural practices. The size of the present environmental release comprises one-time plantings of up to nine genotypes planted on two sites (one in Geary County and one in Riley County) totaling no more than 15 acres per site for a period not to exceed one year without issuance of a new permit or deregulation, both of which would involve a separate NEPA assessment. The introduced traits, with their lack of toxicity, should not impact biological or physical resources. The location of the trial, the confinement methods and methods for termination of the trial should be adequate to confine the regulated article to the release sites and areas being monitored, should prevent its persistence in the environment, and should prevent gene flow that could impact sensitive markets. 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 sites or the ecosystem in which they are situated. No resources will be significantly impacted due to cumulative impacts resulting from the proposed action.

For the above reasons, and those documented on the NEPA/ESA decision worksheet, APHIS has determined that this permit involves a confined field trial of genetically engineered organisms or products that do not involve a new species or organism or novel modification that raises new issues. Issuance of this permit qualifies for categorical exclusion status under 7 CFR § 372.5(c)(3)(ii), and none of the exceptions for categorically excluded actions under 7 CFR § 372.5(d) apply to this action because APHIS has determined that all environmental impacts resulting from the issuance of this permit will be insignificant. APHIS has determined that this action does not have the potential to significantly affect the quality of the human environment, and neither an environmental assessment nor an environmental impact statement is required.

References

Signed: _____/s/_____

Susan M. Koehler, Ph.D.
Chief, Plant Branch
Environmental Risk Analysis Division
Biotechnology Regulatory Services

Date:_____04/06/2011_____

Prepared by CMCV