NEPA Decision Summary for Permit **#12-274-101r**

Under the APHIS National Environmental Policy Act Implementing Procedures at 7 CFR § 372.5(c)(2)(ii) "Permitting, or acknowledgement of notifications for, confined field releases of genetically engineered organisms and products" are APHIS actions that are categorically excluded from the requirement to prepare an environmental assessment (EA) or environmental impact statement (EIS) under the National Environmental Policy Act of 1969 (NEPA), provided that none of the exceptions to categorically excluded actions at 7 CFR § 372.5(d) apply. These exceptions include actions that individually or cumulatively "may have the potential to affect 'significantly' the quality of the human environment", for example 7 CFR § 372.5(d)(4): "When a confined release of genetically engineered organisms or products involves new species or organisms or novel modifications that raise new issues."

Ventria Bioscience (Nathan Fortner) has requested a permit (#12-274-101r) for a confined field release to plant four sites (acreage is Confidential Business Information - CBI) in Geary and Riley Counties, KS, with rice (*Oryza sativa*) plants genetically engineered (GE) to produce one or two of thirteen (13) pharmaceutical proteins for a total of seventeen (17) unique genotypes (phenotypic designations). 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 ePermits file associated with this application. These conditions further insure that the field release will be confined and any impacts from the release are expected to be insignificant. APHIS BRS has concluded based on the analysis below that issuing Permit number 12-274-101r is a categorically excluded action under section 7 CFR § 372.5(c)(ii) because it is a confined field release of genetically engineered organisms, and that none of the exceptions at 7 CFR § 372.5(d) apply.

1. **Familiarity of the Crop and the Traits**: Hundreds of field trials have been performed with GE rice plants under APHIS authority, and APHIS is familiar with rice biology and methods to manage confined rice field trials. Ventria previously grew GE rice in Kansas from 2007 through 2012 and satisfactorily managed those plantings. The proposed plantings in 2013 include the same previous field locations in Geary County approved since 2008 plus a new location in Riley County never previously approved but within the same ecoregion of both previously approved sites in Geary and Riley Counties. An Environmental Assessment (EA) has been prepared previously for field tests totaling 3,200 acres in Geary County, KS for Ventria's rice expressing three proteins that also occur in the current permit application, e.g. lysozyme, lactoferrin and serum albumin. A review of this permit application 12-274-101r raised no new issues with respect to potential impacts resulting from the additional genotypes or from the additional location in Riley County, so the previous EA is applicable to this application.

   The safety of Ventria's pharmaceutical proteins is summarized in their CBI documents "2013 KS TES Evaluation" and "2013 KS Supporting Docs" and associated references submitted with the permit application. No toxic effects on

birds, reptiles, insects, or mammals are expected from these confined field tests. Because of the tissue specific expression system employed, all of Ventria's pharmaceutical proteins are expressed only in the rice seed endosperm, not in other plant tissues. 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. All viable transgenic plant material will be removed from the test site or devitalized in the field and/or destroyed in the processing facility, so there should 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 newer products (transferrin and nine other pharmaceutical proteins claimed as CBI) and it is likely that they would also not be found in soils. A selectable marker gene with a safe history of use, either phosphinothricin acetyltransferase or hygromycin phosphotransferase, was also used in each of the 17 genotypes.

- Lactoferrin is an iron-binding protein found in breast milk and cow's milk. 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. The results of a toxicity study commissioned by the applicant indicated that there was no negative effect seen at any of the dose treatments given to rats and dogs, with the highest treatment dose equivalent to the amount of lactoferrin that would be ingested if they consumed 56% and 28%, respectively, of their body weight of whole grain Ventria GE lactoferrin rice.
- Lysozyme is a protein found in most mammalian breast milk, and several other secretions. Similar homologous proteins are found in a variety of diverse species. 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 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. Ventria compared the stability and sensitivity to gastric digestive conditions of native serum albumin and rice-derived serum albumin, and found that both are rapidly degraded at 60^{o}C and neither survives pepsin digestion beyond ½ minute. Based on the similarity of mammalian and bird gastric fluids, digestive enzymes and pancreatic systems, Ventria concludes that avian species would not be affected by consumption of rice-derived serum albumin.
- Transferrin is an iron binding protein similar to lactoferrin, although found predominantly in blood serum as opposed to milk. Due to the shared sequence homology between transferrin and lactoferrin (50 to 70%) and their overall similar structural characteristics, transferrin and its homologs share important functional properties such as their strong yet reversible capability to bind iron to

control iron levels in body fluids, and their strong bacteriostatic activity related to the tight control of iron in the body which limits the availability of iron for the growth of invading bacteria. Transferrin is extensively used in the cell culture industry for pharmaceutical manufacturing.

The other nine gene products (claimed as CBI) and their combinations also have expected pharmaceutical uses and several have been previously field tested in KS by Ventria. None of these nine gene products are commonly characterized as allergens, nor do they share any amino acid homology with known toxic peptides. The pharmaceutical gene products claimed as CBI include: a membrane protein which is an effective antigen for Lyme disease; a growth factor peptide important for fetal cell growth and development; an antimicrobial protein; a glycoprotein member of the cytokine family; a nontoxic antigenic membrane-binding protein subunit; an antigenic protein; a plasma glycoprotein; and a metabolic enzyme important for the treatment of a disease. A gene silencing construct under the control of a constitutive promoter (Ubi1) from maize is included with one of the constructs to improve the quality of the final protein product with respect to its therapeutic use. Although some of the CBI genes are derived from pathogens, they do not encode infectious agents. The purified gene products are intended to immunize against, treat or prevent disease or otherwise be used for *in vitro* cell culture or other indirect therapeutic purposes. The potential for exposure to the expressed proteins by consumption of rice seed from these GE plants will be mitigated by the confinement protocols. Most of the proteins are not orally active, as they completely degrade in the intestinal tract and/or are denatured by cooking, and never reach systemic circulation; therefore they require injection for therapeutic delivery. In addition to these barriers to exposure to the GE proteins in a biologically active state from whole grain rice, if an animal or human were to somehow have the opportunity to consume this GE rice, the quantity of rice required in order to receive a modest therapeutic or biologically meaningful dose of at least some of the proteins would far exceed the amount likely to be consumed (on a per body weight basis) under normal scenarios of consumption.

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 thirteen 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 of this introduction is for grain production and germplasm evaluation and selection of Ventria's genetically engineered pharmaceutical rice. The following activities will be conducted:
   - Production of rice grain for Ventria's commercial needs.
   - Production of rice seed for future release permits.
   - Assessment of gene stability.
   - Replicated trials comparing yield and agronomic traits of untransformed parental lines and transgenic lines.
   - Evaluation and selection of breeding lines.
   - Development of breeder seed from new breeding lines.
   - Production of breeder seed.
   - Production of seed for laboratory analysis of the proteins of interest.
   - Characterization of the plants (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 GE rice lines to anyone. Most of Ventria's GE rice is dehusked, debranned, ground into flour and processed in their dedicated facilities for protein extraction and purification, in preparation for transfer to third parties to be used as raw materials for the manufacture of cell culture media and drug formulations. Remaining seed is used for expression analysis in the lab, or propagation and/or crossing in their greenhouse for eventual planting in future confined field trials.

4. **Crop Biology and Adequacy of Confinement:** Rice is highly self-pollinated due to flower morphology and limited pollen viability. The 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 of these field sites since rice has not been grown in the area in the past. Ventria scouted for weedy rice in this area from 2007 through 2012 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.

   Ventria maintains a closed cultural system for its rice by using a variety of methods. 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 (Powers et. al, 1978; Smith and Sullivan, 1980). A more extensive bird digestion study was conducted by Cummings et al. (2008) that concluded that rice seeds, whether free-fed or force-fed, were digested and did not pass viable through the digestive tract of mallard ducks, ring-necked pheasants, red-winged blackbirds and rock pigeons.

The rice seed will be ground seeded (never 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 (2013 KS Project Guide and Procedures) which have been reviewed by APHIS. An APHIS inspection will be required before such equipment can be returned to general use.

In previous field tests, seed dormancy in rice has not been observed. At harvest, combine design and prevailing wind speed and direction are taken into account to ensure light seed and straw do not travel into adjacent fields. Following harvest, as soon as possible and as weather conditions allow, all the fields will be mowed and usually also burned and either disked or no-till seeded with a cover crop. In the event that Ventria does not incorporate plant residue and remaining seed into the soil after harvest (for instance, during drought), they must verify that adjacent fields are planned to be managed in a way that would destroy any rice seedlings (e.g., spring cultivation, glyphosate tolerant crops). Off-season flushing will be used to accelerate germination of any remaining rice seed. After harvest, the field will be fallowed for one full cropping season, about 18 months. Scouting for volunteers at least every 45 days 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 foot isolation zone, particularly the 50 foot fallow zone, will ensure that there are no issues related to volunteer rice plants. All production personnel at Ventria are trained in these confinement methods.

5. **ESA Assessment:** APHIS has reached a determination that this action would have no effect on federally listed threatened or endangered species and designated critical habitat, and is unlikely to jeopardize the continued existence of a proposed species or adversely modify proposed critical habitat; therefore, conference, written concurrence, or formal consultation with either the United States Fish and Wildlife Service, or National Marine Fisheries Service is not required. There are no threatened and endangered species (TES) in the action areas and no critical habitat. There are two TES birds known to occur in Riley County (the Interior Least Tern and Piping Plover) and a TES fish (Topeka shiner) known to occur 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 along shorelines primarily on small fish and insects, so exposure to Ventria's planting sites would also 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. **Cumulative 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 will not result in a change in agricultural practices. The size of the present environmental release comprises no more than three plantings of up to seventeen genotypes planted on the four sites (two in Geary County and two in Riley County) (acreage CBI) 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 Geary County sites have been planted with Ventria's rice since 2008, while the Riley County sites have never been planted with Ventria's rice, but all the proposed release sites have been used for crop production for over 50 years. 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.

References

Powers, K. D., Noble, R. E., & Chabreck, R. H. 1978. Seed distribution by waterfowl in Southwestern Louisiana. *J. Wildlife Management* 42:598-605.

Smith, R. J., & Sullivan, J. D. 1980. Reduction of red rice grain in rice fields by winter feeding of ducks. *Arkansas Farm Research* 29, 4:3.

Cummings, J.L., Handley, L.W., MacBryde, B., Tupper, S.K., Werner, S.J., & Byram, Z.J. 2008. Dispersal of viable row-crop seeds of commercial agriculture by farmland birds: implication for genetically modified crops. *Environ. Biosafety Res.*7:241-252.

See also the supporting documents in the ePermits folder entitled 2013 KS TES Evaluation, 2013 KS Supporting Docs and 2013 KS References.

Signed: _____

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