NEPA Decision Summary for Permit #14-224-103r

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 has requested a one year permit for confined field release of genetically engineered (GE) rice (*Oryza sativa*) to plant one release site (acreage is CBI) on St. Croix, Virgin Islands, no more than three times. The introduced genetic material is designed to confer production of pharmaceutical proteins and the expression of a selectable marker. Hundreds of GE rice field releases have been previously authorized by APHIS BRS including over 20 GE rice field release permits approved for Ventria Bioscience. Based on a review of the permit, the following determinations were made:

1. **Familiarity of the Crop and the Traits:** Under this permit, Ventria Bioscience intends to conduct a confined field release of 31 rice lines that have been genetically engineered (GE) to confer production of one or two of eighteen distinct (18) pharmaceutical proteins and the expression of a selectable marker gene. The introduced genetic material is derived from plant, bacterial, animal, viral and non-coding, regulatory plant viral DNA sequences. The non-coding regulatory regions of the constructs come from organisms that are well-tested for their safety and have been in use for several years to genetically engineer crop plants. GE rice containing all of the same genetic components and associated gene donors has been approved for confined field release previously by APHIS BRS. Ventria Bioscience has grown similar GE rice in Kansas since 2007 and on St. Croix, Virgin Islands (USVI) since winter 2008-2009 and has satisfactorily managed these plantings. The proposed USVI planting in 2014 includes the same field location approved for the USVI planting in 2013. An Environmental Assessment (EA) has been prepared for confined field releases totaling over 3000 acres in Geary County, KS for Ventria's GE rice expressing three pharmaceutical proteins that are also included in this current permit application, e.g. lactoferrin (LF), lysozyme (LZ) and serum albumin (HSA). The location of the confined field release, familiarity of gene donors, and well-tested functionality of most of the genes do not raise any new issues about the field release of GE rice plants. A review of this permit application #14-224-103r raised no new issues with respect to potential impacts resulting from these genotypes; therefore the previous EA is applicable to this application.

The safety of Ventria Bioscience's pharmaceutical proteins is summarized in their CBI documents "2014 USVI TES Evaluation", "2014 USVI Supporting Docs" and associated references submitted with the permit application. No toxic effects on birds, reptiles, insects or mammals are expected from the confined field tests. Ventria Bioscience employs a tissue specific expression system resulting in the expression of the pharmaceutical proteins in the rice seed endosperm only. The pharmaceutical proteins are not expressed in any other plant tissues. Ventria Bioscience has monitored for the presence of three of the engineered proteins (lactoferrin, lysozyme and serum albumin) in soils for several growing seasons and none has been detected. All viable transgenic plant material will be removed from the field test site or devitalized in the field and/or destroyed in the processing facility. There should be no foreseeable cumulative impacts resulting from confined field tests of these GE lines. The genes and non-coding regions regulating their expression are not likely to pose a plant pest risk. A selectable marker gene with a safe history of use (phosphinothricin acetyltransferase, hygromycin phosphotransferase or phosphomannose isomerase) has been inserted into each of the 31 genotypes.

- Lactoferrin (LF) is an iron-binding glycoprotein found in breast milk and cow's milk. Lactoferrin is ubiquitous in humans and provides a broad range of protective functions. It helps the body to modulate inflammatory and immune responses, in particular in infants. The U.S. Food & Drug Administration has recognized that some bovine-milk derived lactoferrins are GRAS (Generally Recognized As Safe) under the intended conditions of use.
- 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 Bioscience 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 Bioscience concludes that avian species would not be affected by consumption of rice-derived serum albumin.

- Transferrin is an iron binding protein similar to lactoferrin; however it is predominantly present 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 fourteen gene products (claimed as CBI) and their combinations also have expected pharmaceutical uses and most of them have been previously field tested by Ventria Bioscience. None of these fourteen 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; an essential peptide for normal fetal growth and development; an antimicrobial protein; a glycoprotein member of the cytokine family; a non-toxic antigenic membrane-binding protein subunit; an antigenic protein; a plasma glycoprotein; a metabolic enzyme important for the treatment of a disease; a prohormone precursor; a lipid-binding protein; transmembrane proteins; a surface protein; and a surface glycoprotein. 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 no encode infectious agents. The purified gene products are intended to immunize against, treat or prevent disease. Otherwise, they will be used for *in vitro* cell culture or other indirect therapeutic purposes. The potential for exposure to the expressed pharmaceutical 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. They never reach systemic circulation requiring injection for therapeutic delivery.

Rice seed harvested under this permit will be:

- Transported to the processing facility in Junction City, Kansas for target protein extraction, experimental analysis, propagation and crossing in the greenhouse, or for propagation under a future release permit.
- Shipped to Ventria Bioscience labs in Ft. Collins, Colorado for experimental analysis; or
- Documentation of the ultimate destination of the material (and quantities) will be maintained by Ventria Bioscience

All seed movement will be carried out under a separate movement permit with approved shipping variances # 05-035 and #10-012.

2. **Method of Transformation**: Based on the methods of transformation, the genetic material is stably integrated into the plant genome and no plant pest vector sequences that can cause plant disease will be associated with the transformed rice lines as a result of this process. The method of transformation is commonly used and is familiar to APHIS BRS. The gene sequences inserted into the plants do not have any inherent plant pest characteristics and are not likely to pose a plant pest risk. The introduced DNA will not lead to the expression of a toxin or other substance that is known or likely to be toxic to or cause disease in non-target organisms.

3. **Purpose and Design of the Field Trial:** The purpose of Ventria's Breeding Nursery introduction is germplasm evaluation and selection of genetically engineered pharmaceutical rice. The following activities may 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 the rice from the confined field releases will be used as food or feed. 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:** Both the applicant and APHIS BRS are familiar with rice biology and ecology. Over 300 field tests have been performed with GE rice plants under APHIS BRS authority. 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 requires a minimum isolation distance from other rice varieties of at least ten feet when hand or machine planted.

   There are no commercial rice fields on St. Croix. There is no weedy red rice in the immediate area of the confined field release site since rice has never been grown in this area. Ventria Bioscience scouted for weedy rice in this area from 2008 and none was found. Any non-GE rice lines used as controls or grown for any other reason within the regulated field trial will be treated as regulated

material.

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 (over 100 times the AOSCA standard) proposed by the applicant should be more than adequate to prevent gene flow.

Ventria Bioscience 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 Bioscience manages its rice fields to discourage waterfowl from landing during seed set and maturation and (2) studies 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 viably pass 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 to prevent encroachment on the 50 foot fallow zone. Ventria Bioscience 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 (2014 USVI 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. As soon as possible following harvest and as weather conditions allow, all the fields will be mowed and usually burned and either disked or no-till seeded with a cover crop. In the event that Ventria Bioscience 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 left fallow 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

no issues related to volunteer rice plants occur. All production personnel at Ventria Bioscience 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. On St. Croix, two coral species, Coral, Elkhorn (*Acropora palmata*) and Coral, Staghorn (*Acropora cervicornis*), one reptile species, Lizard, St. Croix Ground (*Ameiva polops*), one plant species, No common name (*Catesbaea melanocarpa*), and one turtle species, Sea turtle, Leatherback (*Dermochelys coriacea*) are listed as an endangered species. The nearest critical habitat to the trial site on St. Croix is over 1 mile away in the marine environment. None of the species listed grow in or inhabit agricultural pasture in the planting location or in rice fields. They do not eat rice. APHIS BRS's analysis of the location of the proposed field trial indicates that it is occurring on agricultural land, indicating that there is no change in land usage.
   Rice is not sexually compatible with any other listed or proposed threatened or endangered species, and the genetic constructs do not result in the production, or increase the production, of a toxin, natural toxicant, allelochemical, pheromone, hormone, etc. that could directly or indirectly result in killing or interfering with the normal growth, development, or behavior of a federally listed threatened or endangered species or species proposed for listing. Therefore, release under this permit will have no effect on federally listed threatened or endangered species, species proposed for listing, designated critical habitat, or habitat proposed for designation.

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 site has been used for pasture for the past 30 years. The proposed release will not result in a change in agricultural status of this land. Although the specific agricultural practices used in rice cultivation are different than pasture management, the surrounding land is already used for agricultural production. The size of the present environmental release comprises no more than three plantings of up to thirty-one genotypes planted on one site on St. Croix, Virgin Islands (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 same release site has been planted with Ventria's rice since winter 2008-2009. 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

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.

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.

U.S. Fish and Wildlife Services Caribbean Endangered Species Map http://www.fws.gov/caribbean/es/PDF/Map.pdf (accessed 09/09/2014)

U.S. Fish and Wildlife Services Critical Habitat Portal http://ecos.fws.gov/crithab/ (accessed 09/19/2014)

See also the supporting documents in the ePermits folder entitled 2014 USVI TES Evaluation, 2014 USVI Supporting Docs and 2014 USVI References.

Signed: _____/s/_____
Susan M. Koehler
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Date: _____09/24/2014
KLR_/s/_