NEPA Decision Summary for Permit **#13-210-101r**

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 one year permit (#**13-210-101r**) to plant a five acre site in St. Croix, Virgin Islands, no more than two times with rice (*Oryza sativa*) plants genetically engineered to produce **lactoferrin, lysozyme, serum albumin, transferrin and thirteen other pharmaceutical protein combinations** (claimed as Confidential Business Information - CBI) in rice seeds.

Based on a review of Permit #**13-210-101r**, 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 field trials of rice. Ventria has previously grown genetically engineered (GE) rice containing plant-made pharmaceuticals (PMP) in this location in St. Croix annually since winter 2008-2009 and also in other locations in Kansas annually since 2007, and they have satisfactorily managed those plantings.

   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 of Ventria's engineered genes including their newly developed ones (CBI) are codon optimized and designed with regulatory promoters such that expression occurs only in the rice endosperm, thus limiting the possibility that the engineered proteins could be exuded through the root systems. Furthermore, 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.

   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. **Transferrin** is an iron binding glycoprotein similar in form, function and use to lactoferrin except that it is mainly found in blood plasma instead of milk. Transferrin is found in many organisms and provides a broad range of protective functions through controlling iron uptake. The other thirteen gene product combinations being developed (claimed as CBI) also have expected pharmaceutical uses and most have been previously field tested in VI and KS by Ventria. None of these other thirteen gene product combinations are commonly characterized as allergens, nor do they share any amino acid homology with known toxic peptides in a database search (Swiss-PROT). The target molecules claimed as CBI are antigenic, membrane-binding- and glyco-proteins, a growth factor peptide, an antimicrobial protein, cytokine and plasma glycoproteins and a metabolic enzyme. 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. Selectable marker genes with a safe history of use, either phosphinothricin acetyltransferase, hygromycin phosphotransferase or phosphomannose isomerase, were also used in each of the seventeen constructs. 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.

Environmental assessment (EA) documents have been prepared for some of these gene products produced in rice for locations in North Carolina and Kansas. A review of the current application submitted by Ventria Bioscience, given the small size of this planting (no more than two releases on five acres) raised no new issues, so previous EAs are applicable.

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 seventeen introduced gene combinations originate from rice, corn and/or *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 other than expression of the desired genes of interest and selectable markers. 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. 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 St. Croix. 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 since 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 (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 bermed by natural topography in the St. Croix site and has no outlet for the irrigation water, therefore any seed that is moved by the recirculated irrigation water or rain events 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.

In previous field tests, seed dormancy in rice has not been observed. Following harvest, the fields will be mowed, burned, disked, and/or a no-till cover crop planted, and off-season flushing may be used to accelerate germination of any remaining seed. After harvest the field will be fallowed through the summer season until the following winter cropping season. Scouting will occur every 45 days for one 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. A non-dedicated, off-site seed dryer and temporary storage area may also be used. In this case the seed will be dryed inside envelopes or sacks, so there will be no direct contact of the seed with the dryer. APHIS has reviewed and approved protocols for their use and believes that they are sufficient to prevent commingling of Ventria's rice with other seed. An APHIS inspection will be required before such equipment can be returned to general use. All production personnel are trained in these methods.

Ventria's proposed planting in mid-November will be conducted mostly outside of what is typically considered "hurricane season" (June 1- November 30), so dispersal of plant materials outside the trial site is unlikely. Even if plant material were dispersed beyond the trial site by extreme weather events, given the requirement of highly managed fields for growth and persistence of rice plants, it is unlikely that seed or plants would grow or persist.

5. **ESA Asessment:** There are no threatened and endangered species (TES) in the action area. Federally listed threatened or endangered animal species in the Virgin Islands include three species of sea turtles (green , hawksbill and leatherback), two species of whales (finback and sperm), two species of coral (elkhorn and staghorn),

two species of birds (the roseate Western Hemisphere tern and the piping plover) and two species of reptiles (the St. Croix ground lizard and the Virgin Islands tree boa). Plant species in St. Croix include Vahl's boxwood (*Buxus vahlii*), the evergreen shrub *Calyptranthes thomasiana*, the small spiny shrub *Catesbaea melanocarpa* and the St. Thomas prickly ash (*Zanthoxylum thomasianum*). Individual FWS recovery plans for these species indicate that *C. thomasiana* and *Z. thomasianum* do not occur on St. Croix and that *B. vahlii* is assumed to have been extirpated from St. Croix. The nearest critical habitat to the trial site in St. Croix is over 1 mile away in the ocean. None of the species listed grow in or inhabit agricultural pasture in the planting location or in rice fields in general, or consume rice, so would not be expected to be impacted by this planting. Ventria's primary products have not shown toxicity 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. Therefore, these field trials will not harm or have adverse or other significant effects on threatened or endangered species and no consultation with Fish and Wildlife Service is required prior to issuing this permit.

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 cattle, sheep and goats for the past 30 years, and some of the land has previously been used for Ventria trials of their GE pharma rice. 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 up to two plantings of up to seventeen genotypes planted on up to five acres in St. Croix 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

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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 Service Species Reports
http://ecos.fws.gov/tess_public/countySearch!speciesByCountyReport.action?fips=78010
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http://ecos.fws.gov/tess_public/pub/stateListingIndividual.jsp?state=VI&status=listed
(accessed 09-30-2013)

U.S. Fish and Wildlife Services Critical Habitat Portal
http://criticalhabitat.fws.gov/crithab/ (accessed 09-30-2013)

Signed: _______/s/_______________

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