NEPA Decision Summary for Permit #20-080-102r

Under the APHIS National Environmental Policy Act Implementing Procedures at 7 CFR § 372.5(c)(3)(iii) "Permitting, or acknowledgement of notifications for, confined field releases of genetically engineered (GE) 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."

iBio CDMO has requested a one-year permit for confined field release of GE tobacco (*Nicotiana tabacum*) on one release site (5 acres) in Hidalgo County, Texas with the number of planting up to 14 times. The introduced genetic material is designed to produce pharmaceutical proteins. The application involves five genes of interest and one well characterized selectable marker (*nptII*).

Genetically engineered tobacco has been approved in more than 260 (290 if all *Nicotiana* species) applications for field releases under APHIS authority. APHIS is familiar with tobacco biology and up-to-date methods to manage confined field trials. APHIS has also issued at least 6 permits for pharmaceutical tobacco permits. Although those previous permits are for plants on a hard surface rather than in tillable fields, the transplanting and no seed setting conditions for current permit indicate that current field trial does not pose new type of risks that BRS is unfamiliar with.

The location of the confined field release, the familiarity with the tobacco plant, gene donors, and well-tested functionality of most of the genes do not raise any new issues about the field release of GE tobacco plants. A review of this permit application #20-080-102r raised no new issues with respect to potential impacts resulting from these genotypes; therefore an environmental assessment is not necessary.

1. **Purpose and Design of the Field Trial**
   iBio will use Texas A&M AgriLife Research to grow and harvest vegetative tissue exclusively of a transgenic tobacco plant producing recombinant human procollagen type I. The collagen will be processed to commercial products for clinical use.

   The seedlings will be produced in an iBio indoor growth room under controlled access to authorized and trained personnel only. The seedlings (21 to 45 days old) will then be transplanted to a Texas A&M field site with controlled access. Any flowers will be removed to prevent any pollen escape or seed setting. After

about 70 days of growth, the leaves will be harvested and transported back to the iBio facility for the extraction and purification of recombinant collagen.

2. **Familiarity with the Crop and the Adequacy of Confinement**
Both the applicant and APHIS BRS are familiar with tobacco biology and ecology. Over 260 (290 if all Nicotiana species) field trials, including 6 for pharmaceutical products, have been performed with GE tobacco plants under APHIS BRS authority.

   Tobacco is primarily considered a self-pollinating with a 95% frequency of self-pollination (Litton and Stokes, 1964), any outcrossing relies on pollinators that feed on flowers and pollen, such as birds and insects with a 0.5-4% frequency of pollination (Free, J.B, 1970). There is no commercial tobacco being grown in South Texas. Environment release risk for this pharmaceutical GE plant is greatly reduced by the fact that only seedlings produced from the greenhouse will be transplanted by hand in the field, all flowers will be topped to prevent pollen escape and seed setting, and only the leaves will be harvested. Flowers are expected to appear about 35 day post-transplant. Plants will be monitored 5 times a week and topped to remove all inflorescence, eliminating any outcrossing start not later than 25 days post-transplant. Any non-GE tobacco grown for any other reason within the regulated field trial will be treated as regulated material. Additionally, the applicant will monitor 5 times a week within a ½ mile radius isolation area around the regulated plot for other tobacco or sexually compatible plants.

   After the termination of the field trial, the field will not be planted with food/feed crop for 12 months. Scouting for volunteers at least every month for 1 year from the date of harvest and removal of any volunteer tobacco before it flowers within the field plot and the 50 foot perimeter zone, will ensure no volunteer tobacco plants occur. All production personnel at iBio CDMO and Texas A&M are trained to implement these confinement procedures.

3. **Familiarity with the Traits and Genetic Component.**
Under this permit, iBio CDMO intends to conduct a confined field release of tobacco plants that have been genetically engineered (GE). The introduced genetic material is derived from plant (*Chrysanthemum morifolium*, florist's daisy; *Hordeum vulgare*, barley), bacteria (*Agrobacterium tumefaciens*, *Escherichia coli*), human (*Homo sapiens*), and plant virus (non-coding DNA). The non-coding regulatory regions of the constructs come from organisms that are well-tested for their safety and have been in use for over 10 years in genetically engineered crop plants. The majority of the genetic components and associated gene donors of the GE tobacco in this application have been previously approved for confined field release by APHIS BRS. Texas A&M contractor for iBio CDMO has previous experience in growing tobacco, including flower topping stage operations. Neither the intended product nor the biochemical process to

produce such a product is expected to cause toxicity, allergenicity, or malnutrition.

4. Safety of the Transformation Process

Based on the methods of transformation, the genetic material is stably integrated into the tobacco genome and no plant pest vector sequences that can cause plant disease will be associated with the transformed tobacco line 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 code toxic or allergenic compounds, neither will they lead to the expression of other new toxin or other substance, or alter any existing toxin or other substance in tobacco plant to harm non-target organisms. No mutations were introduced to any recombinant DNA sequences.

5. Safety of the Gene Products

The safety of iBio CDMO pharmaceutical proteins is summarized in their CBI documents, including the product description and associated references submitted with the permit application.

Because collagen is part of the meal of most of the carnivores, it is well digested and tolerated. Studies have demonstrated for over 40 years that collagen is being processed and breaks down in the stomach and intestine in animals. Type I collagen is the product of two genes (*COL1α1* and *COL1α2*) and constitute extracellular matrix and connective tissue of the human body. Recombinant collagen is used in numerous medical applications including regenerative medicine. Analytical studies demonstrated the breakdown of collagen into small peptide during digestion. Therefore, the ingestion of recombinant collagen in small quantities (less than 100μg/g of plant tissue) by animals is not expected to trigger any toxicity. iBio will monitor and document the expression levels of procollagen expressed in tobacco plants grown in the field.

Additional genes coding for three proteins (human propyl 4-hydroxylase subunit α and β (P4H), and human lysyl hydroxylase 3 (LH3)) were introduced to ensure proper post-translational modification and maturation of recombinant procollagen type I. Posttranslational modifications involving prolyl hydroxylase and lysyl hydroxylase are conserved between plants and animal. Plant source prolyl hydroxylase exhibits relatively loose substrate sequence specificity. Lysyl hydroxylase is unable to sufficiently hydroxylate collagen lysines. Plant leaves, which account for at least 75% of the plant biomass, will be harvested and removed from the field. With only roots and stalk stay in the field after termination, the amount of P4H and LH3 protein remaining in the field will be limited, especially because the tobacco stalk is constituted of up to 80% of lignin and cellulose combined.

The genes and non-coding regions regulating their expression are not likely to pose a plant pest risk. The selectable marker *nptII* (neomycin phosphotransferase II) is widely used and known to be safe. It has been part of at least two dozen EAs/EISs, prepared by APHIS on petitions seeking deregulation of genetically engineered crop events. There have been no reports of adverse effects such as toxicity and allergenicity of either NPTII protein or the *nptII* gene on humans, animals or the environment (Miki and McHugh 2004). There are no new or novel modifications that raise new issues.

None of the tobacco from the confined field releases will be used as food or feed. No toxic effects on birds, reptiles, insects or mammals are expected from the confined field tests. There are no known beneficial invertebrates of tobacco production. After the termination of the field trial, 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 tobacco plants.

6. **ESA Assessment:** In Hidalgo County, Texas, eight species are listed as threatened or endangered species, including two bird species, three flowering plant species (non-sexually compatible), and two mammal species (https://ecos.fws.gov/ecp0/reports/species-by-current-range-county?fips=48215, accessed 01/30/2020). Northern Aplomado Falcon (*Falco femoralis septentrionalis*) does not feed plants. The bird Red knot (*Calidris canutus rufa*) eat shoots, buds, leaves, and seeds early in the breeding season (when insects may be scarce) (https://www.nwf.org/Educational-Resources/Wildlife-Guide/Birds/Red-Knot). The birds arrive in the Hidalgo County, TX after they arrive in Houston around May (https://houstonaudubon.org/birding/gallery/red-knot.html). It is unlikely they will feed on tobacco due to a lack of food sources. Tobacco is not the food source for carnivals like Gulf Coast jaguarundi (*Herpailurus (=Felis) yagouaroundi cacomitli*). The trial site is a part of a university research station that involves no critical habitat for any endangered species. In addition, the facility where the trial is conducted is all fenced and the surrounding areas are just agricultural lands and city development. Thus, 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.

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. The genetic constructs do not result in the production or increase the production, of a toxin, natural toxicant, allelochemical, pheromone, hormone, etc. in tobacco 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.

7. **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) are not expected to have a potential for significant environmental impacts. With only roots and stalk stay in the field after termination, the amount of protein remaining in the field is expected to be small as the tobacco stalk is constituted of up to 80% of lignin and cellulose combined. Bees or other pollinating insects are not likely to be impacted because of the removal of floret. The only past, present, and reasonably foreseeable actions specifically associated with the locations for the proposed release are those related to agricultural production. The proposed release site has been under use for agriculture cropping system in a variety of crops including vegetables and row crops. The proposed release will not result in a change in the agricultural status of this land. The size of the present environmental release comprises 14 plantings of one genotype planted on a five acre sites in Hidalgo County, TX for a period not to exceed one year without the issuance of a new permit or deregulation, both of which would involve a separate NEPA assessment. The applicant will be required to report any unusual occurrence in the final report. The introduced trait, with its 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 site and areas being monitored, should prevent its persistence in the environment, and should prevent any gene flow. 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)(iii), 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

AOSCA (2012) Seed Certification Handbook. pp. 166. Association of Official Seed Certifying Agencies, Moline, Illinois.

Free, J. B. 1970. Insect Pollination of Crops. Academic Press, New York, New York. Pp. 355-356

Litton, C. C. and Stokes, G.W. 1964. Outcrossing in Burley Tobacco. Tobacco Science 8: 113-115.

Miki B and McHugh S. 2004. Selectable marker genes in transgenic plants: applications, alternatives and biosafety. Journal of Biotechnology 107, pp. 193-232.

Stein H., Wilensky M., Tsafrir Y., Rosenthal, M., Amir R., Avraham T., Ofir K, Dgany, O.,Yayon, A., and Shoseyov,O. 2009. Production of Bioactive, Post-Translationally Modified, Heterotrimeric, Human Recombinant Type-I Collagen in Transgenic Tobacco. Biomacromolecules 10, 2640–2645

U.S. Fish and Wildlife Services - ECOS Environmental Conservation Online System https://ecos.fws.gov/ecp0/reports/species-by-current-range-county?fips=48215 accessed 05/01/2020.

U.S. Fish and Wildlife Services Critical Habitat Portal http://ecos.fws.gov/crithab/ accessed 05/01/2020

See also the supporting documents in the ePermits folder entitled “Product Description” and other references.

Signed: _____/s/_____

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