

NEPA Decision Summary for Permit #14-030-102r

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.”

Applied Biotechnology Institute (Ismeralda Resella) has requested a one year permit (#14-030-102r) to plant a site (acreage claimed as Confidential Business Information - CBI) in California (County claimed as CBI) one time with corn plants (*Zea mays*) genetically engineered to produce 22 pharmaceutical and/or industrial molecules predominantly expressed in corn seeds, including Hepatitis B virus surface antigens, Brazzein sweet proteins, plus proteases, hydrolases, endo- and exo-cellulases and protease inhibitors which have all been claimed CBI, and the *pat* selectable marker gene.

Based on a review of Permit #14-030-102r, the following determinations were made:

- 1. Familiarity of the Crop and the Traits:** Thousands of field trials have been performed with genetically engineered corn plants under APHIS authority, and APHIS is familiar with corn biology and methods to manage confined field trials of corn. Applied Biotechnology Institute has previously received permits to grow genetically engineered (GE) corn containing plant-made pharmaceuticals (PMP) and industrial molecules in this location in California annually since 2009, and they have satisfactorily managed those plantings.

Applied Biotechnology Institute has proposed gene constructs for environmental release that are expected to result in the production of products intended for various purposes:

- pharmaceutical (Hepatitis B virus surface antigen for use in vaccine production, hydrolases for medical use),
- industrial (proteases and cellulases for use as reagents claimed as CBI),
- industrial with food applications (Brazzein for use as a nutritive sweetener),
- industrials combined with pharmaceuticals (protease inhibitors for use as reagents as well as medical treatments, claimed as CBI), and
- the selectable marker phosphinothricin N-acetyltransferase (PAT).

All 22 of Applied Biotechnology Institute’s engineered genes are designed with regulatory promoters that are expected to direct expression to occur predominantly in

the embryo of maize (Belanger and Kriz. 1989), thus limiting the possibility that the engineered proteins could be present in the foliage or stems, or exuded through the root systems. Furthermore, because virtually all GE corn seed is intended to be removed from the test site, there should be no foreseeable cumulative impacts resulting from field trials of these GE lines.

Hepatitis B virus surface antigen is the product of five gene constructs in the application. The antigen is a structural protein and has no enzymatic or toxic activity. Although this antigenic protein was derived from the pathogen Hepatitis B virus, the antigen does not encode infectious agents. The purified antigenic proteins are intended to immunize against, treat or prevent disease. This antigen is currently used to make the commercial vaccine for hepatitis B (e.g. Recombivax HB by Merck) and millions of people have been injected with this protein. Two gene constructs produce **Brazzein**, a protein derived from the African plant *Pentadiplandra brazzeana* Baillon that has an intrinsic sweetness 500-2,000 times that of sucrose. Brazzein-containing fruit is consumed locally in parts of tropical Africa with a safe history of use. The other fifteen gene products being developed (hydrolases, proteases, cellulases and protease inhibitors claimed as CBI) also have expected pharmaceutical and industrial uses such as for *in vitro* cell culture or indirect and direct therapeutic purposes. Most (nine of these 15) have been previously field tested in CA by Applied Biotechnology Institute. None of these other fifteen products are commonly characterized as allergens, nor do they share amino acid homology with known toxic peptides in a database search such as Swiss-PROT. A selectable marker gene with a safe history of use, phosphinothricin acetyl-transferase, was also used in each of the 22 constructs.

The potential for exposure to the expressed proteins by consumption of corn seed from these GE plants will be mitigated by the confinement protocols. A barrier to exposure for most of these GE proteins is that they would degrade in the intestinal tract and/or are denatured by cooking, and would never reach systemic circulation if they were accidentally consumed. Furthermore, if an animal or human were to somehow have the opportunity to consume this GE corn, the quantity of corn 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.

A review of the current application submitted by Applied Biotechnology Institute, given the small size of this planting (no more than one releases on a small acreage (CBI) raised no new issues. None of the genes encoding the desired traits or the selectable marker, nor the regulatory elements controlling their expression, have any inherent plant pest characteristics, have any known toxicity, and they are not likely to pose a plant pest risk.

2. **Method of Transformation:** All transformations were performed with disarmed *Agrobacterium tumefaciens*. Based on the method of transformation, only the genetic constructs that are designed to be expressed in the genetically engineered corn lines are expected to be efficiently inserted into the plant genome. No plant pest vectors or

vector agents are expected to be associated with the transformed corn lines as a result of the transformation process. The genetic insertion in each plant is expected to remain stable for generations. The target molecules are expected to undergo post-translational transport to the corn endosperm and are free of potential contaminants from either human or GE animal or plant systems.

3. **Purpose and Design of the Field Trial:** The purpose for this introduction is to test various constructs for expression under the control of different promoters. Seeds from all of these constructs will be analyzed for expression level of the protein at the ABI laboratory in California. Additional breeding experiments to optimize agronomic characteristics will occur for the most promising candidates. Some of the material may be later processed and used in application tests. None of this corn is intended to be used as food or feed.
4. **Crop Biology and Adequacy of Confinement:** Corn is an annual, wind-pollinated crop which lacks sexually compatible wild relatives (including threatened or endangered plant species) in the U.S. or its Territories, and exhibits very limited, if any, seed dormancy or weedy behavior. The Association of Official Seed Certifying Agencies (AOSCA) certified seed regulations for foundation corn seed require a minimum isolation distance from other corn varieties of at least 660 feet. However, regulated pharmaceutical and industrial GE corn is held to a stricter isolation standard based on APHIS regulations (68 FR 11337-11340):

There will be a 50 foot fallow zone surrounding the field trial. The isolation distance from the edge of the field trial site to reproductively compatible plants (corn, *Zea mays*) will be by one of two following methods:

- No corn grown within 1.0 mile (5280 feet) of the open-pollinated GE corn field or
- No corn grown within 0.5 mile (2640 feet) of tassel-bagged or de-tasseled GE corn and
 - the GE corn must be planted no less than 28 days before or 28 days after any corn growing in a zone extending from 0.5 mile to 1 mile from the field trial and
 - GE corn plants must be checked weekly for intact bags and tassels.

The entire isolation distance to sexually compatible plants will be monitored for corn plants at 2-4 week intervals beginning in May until flowering is complete in August-September.

Dedicated equipment (seed jabber, seed sheller, any hand tools (knives, pruners, hoes) and backpack sprayer when not in use will be stored at a locked dedicated sites separately from equipment used for food or feed. Before removal from the field trial site, all field equipment (tractor and attachment –disk, ring roller and harrow) will be thoroughly cleaned to remove seed and plant parts that may adhere to the equipment according ABI submitted and APHIS approved SOPs. All equipment will be cleaned to remove plant parts. If cleaning occurs off-site, then the equipment will be placed in

a secure plastic bag so that no regulated material will be released during transportation to the dedicated storage facility.

Ears will be hand harvested at maturity and transferred into containers and stored at locked dedicated storage facilities. A chain of custody document will accompany the seed harvested from the field trial site. Seed, ears, and ear fragments not bagged and transported to the storage location will be buried at the site to a depth of at least 36 inches with markings to identify the location. No recovered plant material (including seeds) will be used for food or feed.

The field trial site and the perimeter fallow zone will be monitored for the presence of volunteers for 12 months after completion of the harvest. Monitoring will occur after the first rain (approximately Nov 1st) until the end of the rainy season (approximately May 1), every month during the time when conditions are conducive to germination. Any growth from germinated seeds from the previous year's field trial will be destroyed before flowering. Volunteers will be destroyed by one or more of the following methods:

- Thorough mowing of the entire site
- Herbicide application
- Plowing under the entire site and incorporation of any plant material into the soil
- Hand removal of the volunteer and incorporating it into the soil.

In the following growing season, the field trial site and the perimeter fallow zone will not be used for crops that are used for food or feed. Planting of cover crops that will not be harvested is allowed. No plants will be grown on the field trial site that will interfere with the identification and destruction of volunteers.

All field operations personnel are trained by the Field Test supervisor according to APHIS approved training related to their specific job responsibilities. The field trial size will be inspected by an internal auditor throughout the duration of the field trial to verify compliance with site security. An internal auditor will inspect records to verify compliance.

Movement of seed off-field by waterfowl and establishment in other fields is unlikely because studies have shown that viable corn 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 corn 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.

5. **ESA Assessment:** Federally listed threatened or endangered animal and plant species that are known to or are believed to occur in the County include several dozen species including mammals, birds, reptiles, snails, insects, fish, crustaceans and amphibians, as well as flowering plants (http://ecos.fws.gov/tess_public/) A detailed analysis

(saved in a separate document) has lead APHIS to the conclusion that none of these animals or plants would be expected to be present in the field site. None of the species listed grow in or inhabit agricultural pasture in the planting location or in corn fields in general, or consume corn, so would not be expected to be impacted by this planting.

Of the TES with critical habitat in the County, according to the USFWS website at <http://ecos.fws.gov/crithab/>, the release site is located within designated critical habitat for the California red-legged frog and approximately 100 feet from critical habitat for the steelhead. The other closest critical habitat is over one mile away from the release site. The endangered **steelhead (southern California evolutionary significant unit) (*Oncorhynchus mykiss irideus*)** typically spends two years in fresh water, migrates to marine waters where they spend 2-3 years, and then returns to natal stream to spawn. Main threats to the species are habitat loss through de-watering and dam construction and also introduction of rainbow trout. (NatureServe 05/27/2014). The stream adjacent to the release site (100 feet) is designated critical habitat for this species but the extent of the habitat is limited to the high-water line or bankfull elevation (70 Federal Register 52487-52627).

Because the field test site is approximately 100 feet from the Critical Habitat of the Steelhead (*Oncorhynchus mykiss irideus*), there is a potential for a small amount of the GE pollen to drift into the stream and expose the steelhead to the engineered products. This is not likely to occur, because most of the pollen would land on the ground surrounding this field test site and on the shrubs and trees that border the stream, and the genes are under the control of embryo/seed specific promoters so only minute amounts of the engineered product would be present in the pollen. Considering the exceedingly low level of exposure and the lack of known toxicity, it is determined that there will be no effect on the steelhead or its critical habitat.

The threatened **California red-legged frog (*Rana draytonii*)** is the largest native frog in the western United States (USFWS 2002 Recovery Plan for the California Red-legged Frog (*Rana aurora draytonii*)). The release site is in designated critical habitat for the CRLF. Almost the entire landscape, not just breeding ponds and streams, may become suitable habitat for the adults during periods of above average rainfall. The California red-legged frog uses a variety of areas, including aquatic, riparian, and upland habitats. They may complete their entire life cycle in a particular habitat (e.g. pond is suitable for all life stages), or they may seek multiple habitat types depending on climatic conditions or distance between and availability of wetland and other suitably moist environments. Following the breeding season, which typically runs from November through April, tadpoles emerge and feed on algae. Metamorphosis typically occurs from July to September. In summer, frogs remain in small mammal burrows, leaf litter, or other moist sites in or near (within a mile of) riparian areas. During periods of wet weather starting with the first rains of fall, some individuals may make overland excursions through upland habitats. Most of these overland movements occur at night. Individuals may range far from water along riparian corridors and in damp thickets and forests. Diet includes various terrestrial and

aquatic invertebrates, mainly invertebrates of shoreline or water surface. Adult males range from 3 to 4.6 inches in body length, while females are larger and range from 3.4 to 5.4 inches.

There are three small ponds within 3,124 feet (0.6 mile) from the release site, the closest being approximately 790 feet. It is unknown if the ponds are used as breeding habitat. The site is also within 100 feet of a semi-permanent stream. The release site itself has been used for planting agricultural crops for over 50 years. The release will occur during the summer months when the frogs remain in moist areas in small mammal burrows or plant debris. A planted corn field would be unlikely to be used as summer habitat. Considering the species' habitat requirements, life history, and food habits; and the timing of the release; it is determined that the planned release under this permit will have no effect on the California red legged frog.

The field test site occurs in the critical habitat of the California red-legged frog¹ and has been reported to occur in the Creek² that is located 100 feet from the field site (personal communication from John Howard, 4-8-10). Critical habitat for the species has four primary constituent elements as described in the Federal Register notice (aquatic breeding habitat, aquatic non-breeding habitat, upland habitat, and dispersal habitat). Dispersal habitat is described as: "upland and riparian habitat contiguous with breeding and nonbreeding aquatic habitat that is free of barriers, and connects two or more patches of aquatic habitat within 1 mi (1.6 km) of one another. Dispersal barriers include heavily traveled roads that possess no bridges or culverts, moderate- to high-density urban or industrial developments with large expanses of asphalt or concrete that do not contain the PCEs or features essential to conservation of the species, and large lakes or reservoirs over 50 ac (20 ha). Agricultural lands such as row crops, orchards, vineyards, and pastures do not constitute barriers to California red-legged frog dispersal." (75 FR 12816-12959). It is possible that the California red legged frog could enter the release site when moving between the small stream and the ponds near the release site. The proposed fence around the field will have a three inch clearance below which will allow passage of the frogs. However, such movement is not likely to take place during the release because although dispersal may occur at any time of year, movement beyond 98 feet occurs mostly during winter rain events. The release site has a history of agricultural production as does the area surrounding the site, and the activities associated with the release will be consistent with that use.

The applicant will comply with the October 20, 2006, U.S. District Court for the Northern District of California imposed no-use buffer zones around California red-legged frog and the aquatic habitats for certain pesticides (<http://www.epa.gov/espp/litstatus/redleg-frog/rlf.htm>). Glufosinate will be used as an herbicide in weed control. This herbicide is not listed in the list of prohibited chemicals. An information sheet was provided for use by the applicant to educate all

¹ 76 FR 12816-12959 Final rule. Revised designation of critical habitat for the California Red-Legged Frog. Published March 17, 2010. Rule is effective April 16, 2010

² USFWS 2002 Recovery Plan for the California Red-legged Frog (*Rana aurora draytonii*).

workers entering the sites within the range of the California red-legged frog. The information sheet covers the life history and description of the frog, and action that can be followed to reduce pesticide exposure in accordance with EPA guidance. It also provides instructions and information necessary to notify the USFWS if the species is encountered.

Because the field release site is within the critical habitat for the California red-legged frog, an informal consultation was carried out with US Fish and Wildlife Service (USFWS). USFWS concurred with APHIS's determination of may affect but is not likely to adversely affect the federally threatened California red-legged frog (*Rana draytonii*) based on the following:

1. Although the project is located in proximity to potential habitat for the California red-legged frog (*Rana draytonii*), the site is surrounded by existing agricultural fields, making it unlikely that a frog would enter the project site.
2. Numerous measures as provided in the supplemental permit conditions "USFWS Measures to Avoid Harm to the CRLF, dated 10-1-2012" and "APHIS CRLF Information sheet" will be taken to avoid potential adverse effects.
3. Restrictions on work activities will be implemented during and after measurable precipitation and on pesticide use in the project area.

On 5/27/14, Michael Blanchette reviewed the 10-10-12 email from Douglas Cooper (FWS) and the proposed actions in previous and this permit, and made the determination that the 10-10-12 FWS consultation made for permit 12-021-103r-a1 and 13-030-104r would apply to this permit due to the following:

- It is unlikely that the CRLF would enter the field test site.
- The field site is surrounded by land that is in agricultural production.
- The measures and activities to avoid potential adverse effects to the CRLF that were in place for the most recent permit 13-030-104r will be put into place for the current permit.

Based on these factors, no further consultation under section 7 of the Endangered Species Act is required.

TES Analysis Conclusion: No federally listed threatened or endangered species or species proposed for listing are likely to found at the release site. If they were to enter the sites, their presence would be fleeting as the habitat is either not suitable or does not contain constituent elements required by the species. Field activities will result in no changes to the habitat used by any listed species or species proposed for listing. With the exception of the California red-legged frog and Steelhead, the sites are not within or near designated critical habitat or habitat proposed for designation. Therefore, except for the California red-legged frog, the action will have no effect on listed species or species proposed for listing and would not affect designated critical habitat or habitat proposed for designation; and the action may effect but is not likely to adversely affect the California red-legged frog. USFWS concurred with APHIS's

determination of may affect but is not likely to adversely affect the federally threatened California red-legged frog (*Rana draytonii*).

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 managed agricultural production for forage crops, cover crops and grapes for over 50 years, and some of the land has previously been used for Applied Biotechnology Institute's trials of their GE pharma corn. The proposed release will not result in a change in agricultural status of this land. Although the specific agricultural practices used in corn cultivation are different than grape management, the surrounding land is already used for agricultural production. The size of the present environmental release comprises a single planting of up to twenty-two genotypes planted on less than 10 acres (actual acreage CBI) in California 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

68 Federal Register 11337 (March 10, 2003). Field Testing of Plants Engineered To Produce Pharmaceutical and Industrial. Retrieved on March 27, 2014 from <http://www.gpo.gov/fdsys/pkg/FR-2003-03-10/pdf/03-5427.pdf>

70 Federal Register 52487-52627 (September 2, 2005). Endangered and Threatened Species; Designation of Critical Habitat for Seven Evolutionarily Significant Units of Pacific Salmon and Steelhead in California. Retrieved on March 27, 2014 from <http://www.gpo.gov/fdsys/pkg/FR-2005-09-02/pdf/05-16389.pdf>

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U.S. Fish and Wildlife Services Critical Habitat Portal
<http://criticalhabitat.fws.gov/crithab/> (accessed 05-27-2014)

U.S. Fish and Wildlife Service Species Reports
<http://ecos.fws.gov/speciesProfile/profile/speciesProfile.action?sPCODE=D02D>
and associated recovery plans
<http://ecos.fws.gov/speciesProfile/profile/speciesProfile.action?sPCODE=D02D#recovery>
(accessed 05-27-2014)

Signed: _____/s/_____

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