CBI INDICATED IN BRACKETS [ ]

NEPA Decision Summary for Permit #10-355-104rm-a1

Genetically Modified Crambe

Crambe or Abyssinian mustard (*Crambe abyssinica*) is an annual herbaceous plant belonging to the family Brassicaceae (Cruciferae) and originated in the Mediterranean region (Leppik and White 1975). Crambe has been evaluated and grown as an industrial oilseed crop due to its high levels of erucic acid in the seed. It was introduced to the United States by the Connecticut Agricultural Experiment Station in the 1940s. Evaluations for strains of the crop began in Texas in 1958. Crambe has since been successfully grown in several areas of the United States (Oplinger et al. 1991). In 2002, crambe was grown in 54 farms in the United States and was harvested on 12,320 acres, with a total production of 13.3 million pounds. North Dakota was the leading producer with 34 farms and harvested 7.5 million pounds from 7,481 acres. Montana had 20 farms and harvested 5.8 million pounds from 4,839 acres (Hansen 2010). Crambe has been commercially grown in the northern plains since the early 1990s. Commercial interest in crambe as an alternative crop developed in part because it can be grown with conventional small-grain equipment. It also required less pest management inputs and demonstrated good tolerance of variable growing conditions (Hansen 2010).

Crambe oil is high in non-edible erucic acid (22:1; 50 to 60 percent), is incredibly stable at high temperatures, and is used for industrial non-food uses as a lubricant or lubricant additive (Sell et al. 1992). The unacceptably high levels of glucosinolates and erucic acid in the crambe seed limit its use in animal feed. The meal contains 25 to 35% protein when the pod is included and 46 to 58% protein when the pod is removed (ARS 1962). It has a well-balanced amino acid content (Miller et al. 1962) and solvent extracted meal (which contains not more than 4% of oil or 4% of glucosinolate) has been approved by the FDA for use in feedlot cattle rations in an amount not to exceed 4.2% of the total ration (21 CFR 573.310 at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=573.310). Oilseed meals containing glucosinolates reduce feed intake and animal performance when fed to non-ruminant animals, but ruminants can tolerate glucosinolates at higher levels without negative effects (Carlson et al. 1996). Crambe is considered as an attractive alternative for production of waxy esters because it contains high erucic acid oil, which is a precursor of wax biosynthesis.

MacIntosh & Associates, Inc. has requested a permit for a confined field release of 0.51 acre of genetically engineered crambe in Codington and Hyde Counties, South Dakota. **This permit is for interstate movement and for field release of regulated crambe lines engineered for altered fatty acid profile for 1 year.** Based on a review of the permit, the following determinations were made:

1. Under this permit, MacIntosh & Associates, Inc. intends to conduct a field test of genetically engineered crambe to express genes that alter fatty acid profile or wax esters in crambe seed tissues. The transgenes expected to be expressed in the genetically engineered crambe **[ ] and [ ]** were obtained from jojoba (*Simmondsia*

chinensis) and silver dollars (*Lunarua annua*) plant species, respectively. The application also contains a selectable marker gene, *npt* II (neomycin phosphotransferase II). The marker gene has routinely in use to select transgenic plants. Moreover, the *npt* II gene was the subject of 28 EAs involving several crop species that have already been deregulated by APHIS BRS. The protein products of all above-mentioned transgenes have been well-characterized. The non-coding regulatory regions of the construct come from organisms that are well-tested for their safety and have been in use for several years to genetically modify crop plants. The genes and non-coding regions regulating their expression are not likely to pose a plant pest risk.

2. Based on the method of transformation (disarmed *Agrobacterium tumefaciens*), only the genetic construct is expected to be efficiently inserted into the crambe genome. The disarmed *A. tumefaciens* technique integrates into the crambe genome those portions of the genetic construct that are designed to be expressed in the genetically engineered crambe lines. No plant pest vector is associated with the transformed plant lines as a result of the transformation process. None of the gene sequences inserted into the plants have any inherent plant pest characteristics and are not likely to pose a plant pest risk. In addition, the introduced DNA will not lead to the expression of a toxin or other product that is known to affect the metabolism, growth, development, and reproduction of animals, plants, or microbes.

3. Although the present permit application is the first transgenic crambe submitted for APHIS BRS approval, crambe crop has been in cultivation in the U.S. since the late 20th century. The literature on crambe cultivation suggests that the agroecology of crambe is very similar to other Brassica crops such as canola and mustard. The following measures will be used to prevent mechanical co-mixing or to confine the field trial to prevent pollen-mediated gene flow or seed flow outside of the authorized field trial.

   (a) The transgenic material will be surrounded by a 10 ft border of conventional nontrangenic crambe that is further surrounded by a 50 ft fallow zone. Crambe is primarily a self-pollinated crop (Beck et al. 1975; Campbell et al. 1986), yet has the potential for some outcrossing (Vollmann and Ruckenbauer 1991). Crambe is not known to outcross with other species (or genera) in the family Brassicaceae (Youping and Peng 1998). Long distance gene flow mediated by insect pollination is not expected from this field trial due to its small size (0.01 acre for each of two sites), the use of a pollen sink border and isolation from sexually compatible plants. The nontransgenic border rows are expected to work as pollen sink and will be treated as regulated article. These field plots will be located at least 660 ft away from any other crambe crops. According to the applicant, there are no other crambe crops being grown in the counties where the proposed field release experiments are going to take place.

(b) All agronomic activities in the field will be done by hand except for standard farm equipment that may be used to apply pesticides. The only other equipment that will be used is at harvest when a Mechanical Roller and an Air Blast Seed Cleaner will be used to remove the seed from the straw and chaff. A clean-out process is included in Annex 3 in the permit.

(c) All plant materials collected for laboratory analysis will be frozen or devitalized. Unused plant materials will be disposed by autoclave, composting or buried at the planting site. The harvested seed material will be stored in a locked box or locked freezer within a secured location for additional testing purposes, including future field trials.

(d) Crambe does not survive the harsh South Dakota winter; therefore volunteers in the subsequent season are unlikely to occur. However, a few volunteers may show up in subsequent growing seasons due to postharvest seed dormancy. Although crambe seeds show some dormancy, it is not a major problem as seeds are capable of germinating in a variety of environments (Ellis et al. 1985). The regulated field will be monitored every two weeks after harvest until the first frost and any volunteers will be destroyed. Monitoring will continue on a monthly basis for two years after trial termination to remove all plants coming from spring germination of over wintering seed. Volunteers will be destroyed by hand, by mowing, or with herbicide treatment.

(e) A training session on the latest information on field trial will be conducted for field trial personnel. The training course will include all the most recent information from BRS for ensuring regulatory compliance. The training outline is described in Annex 4 in the epermit.

4. According to the U.S. FWS' Critical habitat Portal (http://criticalhabitat.fws.gov/) none of the threatened and endangered species (TES) has a designated critical habitat in Codington and Hyde Counties of South Dakota. The proposed field release locations are situated in agriculture lands that have been under managed agriculture activities for several years. The field release locations are several miles away from the nearest designated critical habitat. Therefore, the proposed release is not going to impact, in any way, the critical habitats of TES.

5. APHIS' analysis of the location of the proposed field trial indicates that it is occurring on an agricultural land, so there is no change in land usage. Furthermore, crambe is not sexually compatible with any listed or proposed threatened or endangered species. The other TES that occur in the state are either aquatic species that do not inhabit agricultural land (e.g. the mussels, Topika shiner, and pallid sturgeon), or other species that do not typically occur in agricultural fields. A search of the species listed or proposed at the county level (http://www.fws.gov/southdakotafieldoffice/endangered_species_newVersionP2.htm accessed May 23, 2011) shows that the only two species reported or known to occur in the counties of release are Topeka shiner and the whooping crane,

neither of which would occupy agricultural fields or feed on crambe. Crambe is not generally eaten by birds (Carlson et al. 1996), 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 TE species or species proposed for listing. The genes and gene products (waxy esters) are common to several edible plant species **[ ]**, and the waxy esters are found on most plant leaves. APHIS has reached a determination that this field release would have no effect on listed (or proposed) species. There is no designated or proposed critical habitat within or near the action area. Therefore a written concurrence or formal consultation with either Fish and Wildlife Service, or National Marine Fisheries Service is not required.

6. The proposed field trial plots will not be used for food or feed purpose. Any use of these products for food or feed must be in compliance with the guidelines published in the Federal Register by the United States Food and Drug Administration (57 FR 22984, May 29, 1992).

7. The distribution of the regulated article will occur only between personnel mentioned in the permit application and approved by APHIS. All regulated crambe materials mentioned in the application are only for experimental purposes and no sale of the materials will occur.

8. The only impacts from the proposed release are related to typical agricultural production practices. The proposed environmental release of 0.51 acre of transgenic crambe at two locations is for one year. The proposed field sites have been managed for row crops for several years. The small experimental plot size and the short duration of the proposed trial are not expected to significantly alter the agroecosystem of the release area. The only past, present, and reasonably foreseeable actions associated with the locations for the proposed release are those related to agricultural production. APHIS does not expect there to be a change in the baseline in the type or magnitude of effects related to agricultural production as a result of the proposed field release. APHIS has determined that the incremental impact of the proposed action will not aggregate with effects from past, present, or reasonably foreseeable actions 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 site or the ecosystem in which it is 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 document, APHIS has determined that this permit involves 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 state is required.

Signed: _____/s/_____
Susan Koehler
Branch Chief, Plants
Biotechnology Regulatory Services

Date:_____6/20/2011
SH_/s/_

References

Agricultural Research Service. 1962. Crambe, A potential new crop for industrial and feed uses. A.R.S.34-42. U.S.D.A., Washington, D. C.

Beck L.C., Lessman, K. J., and Buker, R. J. 1975. Inheritance of pubescence and its use in out crossing measurements between *Crambe hispanica* and *C. abyssinica* Hochst. ex. R.E.Fries. Crop Science 15: 221-224.

Campbell, I.A., Crock, J., Williams, J. H., Hang, A. N., Sigafus, R. E. Schneiter, A. A. McClain, E. F., Graves, C. R., Woolley, D. G., Kleiman, R., and Adamson, W. C. 1986. Registration of "BelAnn" and "BelEnzian" crambe. Crop Science 26: 1082-1083.

Carlson, K.D., Gardner, J.C., Anderson, V.L. and Hanzel, J.J. 1996. Crambe: New Crop Success, *in* Progress in New Crops, J. Janick (ed.), ASHS Press, Alexandria, VA. Proceedings of the 3rd National Symposium on New Crops: New Opportunities, New Technologies, Indianapolis, IN October 22-25, 1996. (Accessed on May 23, 2011 at http://www2.bioversityinternational.org/publications/Web_version/52/ch17.htm).

Ellis, R.H., Hong, T.D., and Roberts, E.H. 1985. Handbook of Seed Technology for Genebanks – Volume II. Compendium of Specific Germination Information and Test Recommendations. Chapter 32. Cruciferae. International Board for Plant Genetic Resources, Rome. Accessed May 23, 2011 at http://www2.bioversityinternational.org/publications/Web_version/52/ch17.htm.

Hansen, R. 2010. Crambe Profile. Agricultural Marketing Resource Center, Iowa State University, Iowa. (Updated August 2010 by D. Huntrods, Iowa State University).

Leppik, E.E., and White, G.A. 1975. Preliminary assessment of *Crambe* germplasm resources. Euphytica 44: 681-689.

Miller, R. W., VanEtten, C. H., McGrew, C., Wolff, I. A., and Jones. Q. 1962. Amino acid composition of seed meals from forty-one species of Cruciferae. Journal of Agricultural and Food Chemistry 10: 426.

Oplinger, E. S., Oelke, E. A., Kaminski, A. R., Putnam, D. H., Teynor, T. M., Doll, J. D., Kelling, K. A., Durgan, B. R., and Noetzel, D. M. 1991. Crambe *in* Alternative Field Crops Manual. University of Wisconsin Cooperative or Extension Service, University of Minnesota Center for Alternative Plant & Animal Products, and University of Minnesota Extension Service.
Weblink: http://www.hort.purdue.edu/newcrop/afcm/crambe.html

[ ]

Sell, R.S.; Watt, D. L.; and Johnson, R. G. 1992. Crambe as a Specialty Crop in North Dakota, Agricultural Economics Report No. 268. Department of Agricultural Economics, North Dakota State University, Fargo, ND.

Vollmann, J., and Ruckenbauer, P. 1991. Estimation of outcrossing rates in crambe **(***Crambe abyssinica* Hochst. Ex. R. E. Fries**)** using a dominant morphological marker gene. Weblink: www.boku.ac.at/diebodenkultur/volltexte/band-42/heft-4/vollmann.pdf

Youping, W., and Peng, L. 1998. Intergeneric hybridization between *Brassica* species and *Crambe abyssinica*. Euphytica 101: 1-7.