



CASE STUDY (CSSD10):

Submitting data to the Biosafety Clearing House (BCH): A Competent National Authority submits a risk assessment and decision to import herbicide-tolerant cotton.

Objective:

Learn how to submit a risk assessment and a decision in the Biosafety Clearing House.

Scenario

You work for a Competent National Authority and recently approved the attached decision to import herbicide-tolerant cotton. Your task is to submit this LMO, risk assessment, and the regulatory decision to the BCH. You are the contact person for the Competent National Authority.

Release Document CNA2007-17:

Safety Assessment of Roundup Ready® Flex Cotton

Determination by Authority of the CNA for the Environment

I. Identification of the novel organism

Designation: Cotton line MON 88913, UID MON-88913-8
Applicant: Monsanto Inc
Parent species: Cotton (*Gossypium hirsutum* L.)
Introduced trait: Herbicide tolerance (glyphosate)
Proposed use: Production of cotton for fibre, cottonseed, cottonseed meal for livestock feed, and cottonseed oil for human consumption. These materials will be grown in the US, and cottonseed meals will be imported for livestock feed use only.

II. Background information

Monsanto Inc. has developed a cotton line, MON 88913, which has enhanced tolerance to glyphosate, the active ingredient in the herbicide Roundup®. The enhanced herbicide-tolerant trait in cotton line MON 88913 will enable the over-the-top application of Roundup® agricultural herbicides at later stages of development than is possible with cotton lines MON 1445 and MON 1698. This is intended to provide greater flexibility and convenience in weed control options during crop production.





Cotton line MON 88913 was developed using *Agrobacterium*-mediated gene transfer technology, introducing two copies of the *epsps* (5-enolpyruvyl shikimate-3-phosphate synthase) gene from *Agrobacterium sp.* strain CP4. The *cp4 epsps* coding region is fused to a chloroplast transit peptide sequence, which directs the translated protein to the chloroplast, the site of amino acid biosynthesis. The CP4 version of the EPSPS protein imparts reduced glyphosate sensitivity to the modified plant. Using two different promoters to control the expression of the *cp4 epsps* genes confers the enhanced tolerance to glyphosate.

The Authority reviewed references to relevant scientific publications. The CP4 EPSPS protein from MON 88913 was shown to be equivalent to the protein produced in an *Escherichia coli* expression system developed to produce the protein. The *E. coli*-produced CP4 EPSPS generated sufficient quantities of pure protein for safety studies.

Phenotypic data for cotton line MON 88913 were collected from fourteen field locations under confined research field trial conditions. Compositional and expression data were collected from four of these locations. Agronomic characteristics of cotton line MON 88913, such as plant morphology, disease susceptibility, agronomic performance, and reproductive fitness, were compared to those of unmodified cotton counterparts. Nutritional components of cotton line MON 88913, such as proximates, amino acids, and fatty acids, were compared with unmodified cotton counterparts.

The following assessment criteria were considered when determining the safety and efficacy as livestock feed and the environmental safety of this novel feed:

- potential impact of cotton line MON 88913 on livestock nutrition,
- potential impact of cotton line MON 88913 on livestock and workers,
- potential of cotton line MON 88913 to become a weed of agriculture or be invasive of natural habitats,
- potential for gene flow from cotton line MON 88913 to wild relatives whose hybrid offspring may become weedier or more invasive,
- potential of cotton line MON 88913 to become a plant pest,
- potential impact of cotton line MON 88913 or their gene products on non-target species, including humans, and
- potential impact of cotton line MON 88913 on biodiversity.

III. Description of the Novel Traits

Development Method:

Cotton line MON 88913 was created through the insertion of a fragment of DNA containing two copies of a gene derived from the *Agrobacterium sp.* strain CP4 (*cp4 epsps*), which imparts field-level tolerance to glyphosate, the active ingredient in Roundup® herbicides. Cotton line Coker 312 was transformed with a plasmid vector carrying the synthetic *cp4 epsps*



genes. Both had been fused with a plant-derived coding sequence expressing an optimized chloroplast transit peptide.

Glyphosate Tolerance:

EPSPS is an enzyme in the shikimic acid metabolic pathway essential for producing aromatic amino acids. The native cotton EPSPS enzyme is sensitive to glyphosate. The herbicide disrupts the shikimic acid pathway, leading to growth suppression or death of the plant. The CP4 EPSPS version of this enzyme is expressed in cotton line MON 88913. It confers glyphosate tolerance since it continues to catalyze the production of aromatic amino acids in the presence of glyphosate due to a reduction in the binding of glyphosate to the CP4 EPSPS in comparison to the native EPSPS.

The expression of the novel enzyme in the plant is driven by constitutively active promoters and was quantified by enzyme-linked immunosorbent assay (ELISA). The CP4 EPSPS protein levels were assessed in young leaf, overseason leaf (OSL), root, seed, and pollen tissues collected from field trials performed in 2002. ELISA assays demonstrated that the mean CP4 EPSPS protein levels across four sites for young leaf, OSL1, OSL2, OSL3, root, and seed tissues were 970, 1400, 690, 630, 99 and 340 $\mu\text{g/g}$ dry weight, respectively. The mean CP4 EPSPS protein level in pollen across the four sites was 4.0 $\mu\text{g/g}$ fresh weight.

Unlike typical allergens, the CP4 EPSPS protein is present at low levels in cotton line MON 88913 (less than 0.12% of total protein in seed), is not glycosylated, and was shown to be labile to digestion. Following incubation in a simulated gastric fluid, it was shown, through western blotting, that more than 95% of the CP4 EPSPS protein was digested within 15 seconds. Also, unlike known allergens, it was shown that EPSPS activity was reduced by more than 90% within 15 seconds of incubation time in simulated gastric fluid.

The amino acid sequence of the CP4 EPSPS protein was compared to several protein sequence databases. It shares no significant structural similarity with known toxic, allergenic, or pharmacologically relevant proteins. Further analysis also showed that the CP4 EPSPS protein lacks immunologically relevant sequences. An acute mouse study reported no deleterious side effects when animals were administered CP4 EPSPS protein by oral gavage at doses up to 475 mg/kg.

Due to the low levels of CP4 EPSPS protein expressed in cotton line MON 88913, it was necessary to produce CP4 EPSPS by bacterial fermentation to obtain sufficient quantities to conduct some of the safety studies (acute oral mouse toxicity study, simulated gastric fluid digestion study). The bacterially-produced protein was compared to the plant-produced protein and shown to have similar molecular weight, immunological reactivity, and functional activity as the plant-produced protein.



Southern blot analysis showed stability of the introduced DNA in the first five generations removed from the original transformant. Data presented also demonstrated that the genes segregated according to Mendelian inheritance in these generations.

The Authority has been provided with a method for detecting and identifying MON 88913.

IV. Criteria for the Environmental Assessment

1. Potential of Cotton line MON 88913 to Become a Weed of Agriculture or be Invasive of Natural Habitats

Cotton (*Gossypium hirsutum*) is a perennial member of the Malvaceae (Mallow family) but is cultivated annually. Neither plants nor seeds of *Gossypium* can survive freezing temperatures. The genus *Gossypium* shows no particular weedy aggressive tendencies. Cotton has been grown for centuries and has never been reported as a serious weed problem.

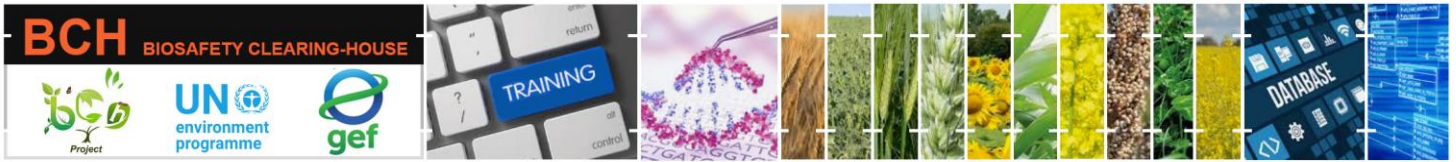
Dormancy and germination characteristics of line MON 88913, the negative segregant control (which shares similar background genetics to MON 88913), and several reference varieties were measured in temperature-controlled growth chambers under seven different temperature regimes. All values for MON 88913 were either within the range of reference values or incrementally outside of the range provided. No significant differences were detected in the percent viable hard seed (a potential measure of dormancy).

Measurements on height, yield, and seed/boll characteristics of line MON 88913 and the control were recorded at harvest. When pooled across all locations, there were no significant differences between height, number of nodes, the total number of bolls, number of vegetative bolls, number of abnormal bolls, whole seed per boll, mature seed per boll, or immature seed per boll. A significant difference of 0.3 grams was recorded for seed index (grams per 100 fuzzy seeds), but this difference likely has a little biological impact on weediness.

Line MON 88913 is therefore considered substantially equivalent to lines 1445 and 1698 regarding invasiveness and potential to become a weed of agriculture. CFIA concludes that cotton line MON 88913 is unlikely to become a weed of agriculture or invasive of natural habitats.

2. Potential for Gene Flow to Wild Relatives and Potential for Hybrid Offspring to Become More Weedier or More Invasive

Cotton pollen remains viable for about 12 hours, but the grains are relatively large and heavy and not easily dispersed by wind. *G. hirsutum* is generally self-pollinating but can exhibit outcrossing in the presence of suitable insect pollinators (such as bumble bees (*Bombus spp.*) and honey bees (*Apis mellifera*)). The outcrossing frequency decreases with increasing



distance from the pollen source. Wild species of *Gossypium* are generally restricted to arid tropical or subtropical regions.

The Authority has concluded that gene flow from glyphosate-tolerant cotton lines to wild *Gossypium* relatives is very unlikely in managed or unmanaged ecosystems in this country.

3. Altered Plant Pest Potential

Cotton is not a plant pest, and the intended effect of the novel trait is unrelated to plant pest potential. The susceptibility of line MON 88913 to insect, disease, and abiotic stressors was evaluated in field experiments at 14 locations. Susceptibilities to aphids, beet armyworm, cotton bollworm, lygus, pink bollworm, stink bugs, tarnished plant bugs, thrips, tobacco budworm, whiteflies, boll rot, Pythium, Rhizoctonia, Verticillium, cold, drought, and heat were similar in line MON 88913 to the susceptibilities of the control plants to these stressors. No differences between line MON 88913 and the control were observed that would contribute to increased plant pest potential.

The Authority has therefore determined that Roundup Ready® Flex cotton line MON 88913 does not display any altered plant pest potential.

4. Potential Impact of Non-Target Organisms

The source of the coding sequence for the CP4 EPSPS protein produced in line MON 88913 is a common soil bacterium that is not a known human or animal pathogen and has not been previously reported as an allergen. The protein is rapidly digested in simulated gastric fluid (more than 95% of the protein was digested within 15 seconds). No immunologically relevant homologous sequences to any known allergenic proteins indicated a lack of allergenic potential. Additionally, no documented cases of allergy or adverse effects from consuming this protein in foods or feeds derived from other Roundup Ready® crops have been reported since their introduction in 1996.

EPSPS proteins naturally occur in plant and microbially-based foods with a history of safe consumption by humans and animals. CP4 EPSPS has been consumed directly or as processed products of Roundup Ready® crops since their commercialization in 1996 with no reported cases of toxicity. A compositional and nutritional assessment compared cottonseed, oil, and meal from line MON 88913 to cottonseed, oil, and meal from a negative control cotton line with similar background genetics. This assessment found no component values in cotton line MON 88913 that were outside the range of those found in the unmodified counterparts.

Cotton is well-known for naturally occurring toxicants and antinutrients (cyclopropenoid fatty acids and gossypol). Aflatoxins are toxic by-products of several varieties of fungi that can grow in cotton. Line MON 88913 was tested for four aflatoxins (B1, B2, G1, and G2),



but because 50% of all the samples taken were below the LOQ, further statistical analyses were not conducted. Cyclopropanoid fatty acids (malvalic acid, dihydrosterculic acid, and sterculic acid) levels in line MON 88913 were assessed and were found to be within the range of values observed in conventional cotton counterparts. Levels of gossypol were assessed and were also found to be within the range of values observed in conventional cotton counterparts. An acute oral toxicity study using E. coli-produced CP4 EPSPS protein was administered to mice. No adverse effects were observed at 475 mg of CP4 EPSPS protein/kg mouse body weight, and there were no differences in body weight, cumulative body weight, or food consumption between mice fed CP4 EPSPS protein and mice fed with a control protein (bovine serum albumin) at that level.

The Authority concludes from the above data that using line MON 88913 will not have a greater impact on non-target organisms (including humans) than conventional cotton.

5. Potential Impact on Biodiversity

No varieties of cotton or wild relatives that can readily interbreed with cotton can grow in the local environment. Roundup Ready® Flex cotton line MON 88913 has no observed or expected modifications that would allow it to survive in the local environment better than unmodified cotton. The Authority, therefore, concludes that line MON 88913 will have no impact on biodiversity in this country.

V. Criteria for the Environmental Assessment

1. Potential Impact on Livestock Nutrition

The composition of cottonseed, cottonseed meal, and cottonseed oil from line MON 88913 was compared with a negative-segregant control line. Acid-delinted whole cottonseed was analyzed in two trials. The analysis included proximates, minerals, amino acids, fatty acids, cyclopropanoid fatty acids, gossypol, and vitamin E. Cottonseed meal and cottonseed oil were analyzed in one of the trials. Cottonseed meal analysis included proximates, minerals, amino acids, cyclopropanoid fatty acids, and gossypol. In contrast, cottonseed oil analysis included fatty acids, cyclopropanoid fatty acids, gossypol, and vitamin E. Oleic acid was significantly altered when expressed as a percentage of total fatty acids. Still, this difference was due to elevated values in the control line. When expressed as a percentage of total amino acids, phenylalanine was significantly higher in line MON 88913 than its control in both trials. However, both line MON 88913 and its control line fell within the range of the commercial reference varieties and the range of literature values for this analyte. There was no difference in phenylalanine when expressed as a percentage of total amino acids in cottonseed meal derived from whole cottonseed used in this trial. Several other analytes (tryptophan, linoleic acid, and manganese) were found to be significantly different, but all of these values fell within the tolerance interval for the commercial reference varieties.



seed overwinter. Therefore the release of the feed into the environment would result in neither intended nor unintended environmental effects.

Livestock feed use of cotton line MON 88913 is therefore authorized as of November 23, 2007. Cotton line MON 88913 and any other cotton lines derived from it may be imported and/or released, provided no inter-specific crosses are performed, provided the intended uses are similar, and provided it is known, based on characterization, that these plants do not display any additional novel traits and are substantially equivalent to currently grown cotton in terms of their specific use and safety for the environment and human and animal health. Cotton line MON 88913 is subject to the same phytosanitary import requirements as its unmodified counterparts.

VIII. Regulatory Contact

Competent National Authority for the Environment
123 Authority Street
National Capital 9999

Note: This release document is also available on the Internet at
www.decisions.com/CNA2007-17.pdf

Important note:

Please ensure you are using the BCH Training Site for this exercise!

Mechanics:

Participants should be divided into four groups of four members each. Each group will be assigned a given country. Participants in each group will take turns assuming the BCH National Focal Point role while the rest are National Authorized Users.

Groups and log-in details are summarized below:



Group	Country	Role	User name	Password
1	Greece	NFP	greece.nfp@gmail.com	Abdef1234\$
		NAU	greece.nau1@gmail.com	Abdef1234\$
		NAU	greece.nau1@gmail.com	Abdef1234\$
		NAU	greece.nau1@gmail.com	Abdef1234\$
2	Angola	NFP	angola.nfp@gmail.com	Abdef1234\$
		NAU	angola.nau1@gmail.com	Abdef1234\$
		NAU	angola.nau2@gmail.com	Abdef1234\$
		NAU	angola.nau3@gmail.com	Abdef1234\$
3	Andorra	NFP	andorra.nfp@hotmail.com	Abdef1234\$
		NAU	andorra.nau1@hotmail.com	Abdef1234\$
		NAU	andorra.nau2@hotmail.com	Abdef1234\$
		NAU	andorra.nau3@hotmail.com	Abdef1234\$
4	Comoros	NFP	comoros.nfp@hotmail.com	Abdef1234\$
		NAU	comoros.nau1@hotmail.com	Abdef1234\$
		NAU	comoros.nau2@hotmail.com	Abdef1234\$
		NAU	comoros.nau3@hotmail.com	Abdef1234\$

Participants should visit the BCH 'Training Site' (<https://bch.cbd.int>> Help> Training Site). Then log in using the assigned country user names and passwords.

Following the assigned role (NAU1, NAU2, etc.), each participant should submit a 'Country Decisions or any other Communications (DEC)' record using the above fictitious scenario information. After that, S/He will see its status as 'Pending BCH-NFP Approval.'

The BCH-NFP can also submit some records (note that, in this case, it will be automatically published with a need to be validated).

When the group's participants have entered at least one record, the BCH-NFP can practice the 'Approve,' 'Edit,' or 'Reject' functionalities. The NAUs could watch the process on BCH-NFP's computer. Practice 'reject' for at least one submission. Participants should exchange roles between them so that each participant in the group can play the role of a BCH-NFP.

During this exercise, participants can also explore the following functions:

- Submitting the record in multiple languages by selecting the languages in the 'Please select in which language(s) you wish to submit this record' drop-down menu and including translation in the newly added fields.
- Use the 'Review' tab to check entered data and if any mandatory data is missing quickly.
- Use the 'Save Draft' functionality to save your data and make changes later.