CASE STUDY 10:

A CNA provides information to the BCH about a decision.

Scenario:

Upon approval of the importation of DAS-21023-5 x DAS-24236-5 (trade name Widestrike™; see **Case Study 9**) for intentional release into the environment, what information must the South African competent authority provide to the BCH about his decision? As a resource, please refer to the attached document "Decision on Insect Protected Cotton Event, DAS-21023-5 X DAS-24236-5 (Trade Name Widestrike™)." Provide the answer indicating how information was submitted to the Biosafety Clearing House and which article(s) of the Cartagena Protocol are pertinent to this activity.

DECISION ON INSECT PROTECTED COTTON EVENT, DAS-21023-5 X DAS-24236-5 (TRADE NAME WIDESTRIKE™)

DAS-21Ø23-5 x DAS-24236-5

Host Organism / Variety	*Gossypium hirsutum* L. (Cotton) WideStrike™
Trait	Resistance to lepidopteran pests.
Trait Introduction Method	Traditional plant breeding and selection
Proposed Use	Production of cotton for fibre, cottonseed and cottonseed meal for livestock feed, and cottonseed oil for human consumption.
Company Information	DOW AgroSciences LLC

General Description

WideStrike™ cotton (OECD identifier: DAS-21Ø23-5 x DAS-24236-5) was produced by cross-breeding two insect-resistant cotton lines: 281-24-236 (OECD identifier: DAS-24236-5) and 3006-210-23 (OECD identifier: DAS-21Ø23-5). Each of these lines expresses an insecticidal protein. This stacked cotton line is a product of traditional plant breeding.

DAS-24236-5

The parental line 281-24-236 was produced by *Agrobacterium*-mediated transformation of plant cells from the cotton variety 'Germain's Acala GC510.' The pAGM281 plasmid was used for the transformation. It contained the cry1F gene, coding for a full length chimeric Cry1F protein (delta-endotoxin) which confers Lepidopteran insect resistance; a mannopine synthase promoter containing four copies of the octopine synthase enhancer ((4OCS)delta-mas2') from *A. tumefaciens* strain LBA 4404 pTi15955; and a bi-directional

terminator (ORF25polyA) from the same *A. tumefaciens* strain as the promoter. The pAGM281 plasmid also contained a synthetic version of the pat gene, coding for glufosinate ammonium tolerance, and used as a selectable marker. The expression of the pat gene was under the control of a *Zea mays* ubiquitin promoter (UbiAm1). The plasmid backbone, derived from plasmid Rk2, contained an erythromycin resistance gene to allow the selection of bacteria containing pAGM281.

Successful transformants were detected as those tolerant to glufosinate ammonium. Resistance to lepidopteran insects was tested by conducting a bioassay using leaf discs from the successful transformants. Leaf discs were fed to the larvae of cotton bollworm, a target lepidopteran pest. The successful event was designated 281-24-236 and was subsequently introgressed into the elite genotype 'PSC355,' a cultivar with a broad adaptation to the southern United States.

DAS-21Ø23-5

The cotton line 3006-210-23 was produced by *Agrobacterium*-mediated transformation of plant cells from the cotton variety 'Germain's Acala GC510' using the the binary plasmid vector pMYC3006. The plasmid contained the cry1Ac gene, coding for a full length chimeric Cry1Ac protein (delta-endotoxin) which confers lepidopteran insect resistance; a *Zea mays* promoter system (UbiZm1); and a bi-directional terminator (ORF25polyA) from *A. tumefaciens* strain LBA 4404 pTi15955. The pMYC3006 plasmid also contained a synthetic version of the pat gene, coding for glufosinate ammonium tolerance, and used as a selectable marker. The expression of the pat gene was controlled by a mannopine synthase promoter from pTi15955, and four copies of the octopine syntase (4OCS) enhancer from pTiAch5. Polyadenylation sequences were derived from the bi-directional ORF25 terminator from pTi15955. The plasmid backbone, derived from plasmid Rk2, contained an erythromycin resistance gene to allow the selection of bacteria containing pMYC3006.

Successful transformants were detected as those tolerant to glufosinate ammonium. Resistance to lepidopteran insects was tested by conducting bioassays using leaf discs from the successful transformants. These were fed to cotton bollworm, a target lepidopteran pest. The successful event was designated 3006-210-23 and was subsequently introgressed into the elite genotype 'PSC355,' a cultivar with a broad adaptation to the southern United States.

DAS-21Ø23-5 x DAS-24236-5

WideStrike™ expresses two novel proteins: Cry1F and Cry1Ac, delta-endotoxins which confers resistance to lepidopteran pests of cotton, such as the cotton bollworm, pink bollworm and tobacco budworm. The insecticidal protein Cry1F is produced by the *cry*1F gene from cotton line 281-24-236, and Cry1Ac is produced by the *cry*1Ac gene from the cotton line 3006-210-23. The *pat* gene is also expressed in WideStrike™. This gene produces the PAT protein (phosphinothricin acetyltransferate) which confers resistance to the herbicide glufosinate ammonium, and inserted solely to be used as a selectable marker during the transformation that led to the production of 281-24-23 and 3006-210-23.

The inserted genes and their gene products in WideStrike™ cotton have a history of safe use, and have undergone prior review and approval by several regulatory agencies. No interactions among the gene products or negative synergistic effects are expected in

the stacked line. Since neither Cry1F, nor Cry1Ac, have enzymatic activity, these proteins have no effect on plant metabolism. The PAT protein has a very high affinity for L-phosphinothricin, the active ingredient in the herbicide glufosinate ammonium. Cry1F, Cry1Ac and PAT are therefore not expected to interact within, nor affect the metabolism of the stacked hybrid.

The South African competent authority has conducted an environmental hazard assessment of WideStrike™ cotton. Data on the effects of the Cry1F and Cry1Ac were assessed separately, and in combination to detect possible synergistic effects. No synergistic effects were observed, nor any increase in the host range of non-target organisms, from the stacking of both Cry proteins. No harmful effects to aquatic and terrestrial wildlife were observed, and it was concluded that the cultivation of the stacked line would not be hazardous to non-target terrestrial, aquatic and soil organisms. Additionally, this approval permits WideStrike™ to only be used in small scale, experimental field trials grown under conditions of reproductive isolation coupled with additional restrictions and mandatory monitoring of the trial site during the trial and for a period of one year after termination of the trial.

Summary of Introduced Genetic Elements

Code	Name	Type	Promoter, other	Terminator	Copies	Form
cry1Ac	Cry1Ac delta-endotoxin (*Bacillus thuringiensis* subsp. *kurstaki* (Btk))	IR	ubiquitin 1 (*Zea mays*)	3' polyadenylation signal from ORF25 (*Agrobacterium tumefaciens*)	1 functional;	Cry1Ac active insecticidal core and non-toxic portions of the Cry1Ab1 and Cry1Ca3 proteins. Sequence modified for optimal in planta expression.
cry1F	cry1F delta-endotoxin (*Bacillus thuringiensis* var. *aizawai*)	IR	mannopine synthase (d mas 2') promoter from pTi15955 four copies of the octopine synthase (4OCS) enhancer	3' polyadenylation signal from ORF25 (*Agrobacterium tumefaciens*)	1 functional;	Cry1F active insecticidal core and non-toxic portions of the Cry1Ab1 and Cry1Ca3 proteins. Sequence

			from pTiAch5			modified for optimal in planta expression.
pat	phosphinothricin N-acetyltransferase (*S. viridochromogenes*)	SM	mannopine synthase (d mas 2') promoter from pTi15955 four copies of the octopine synthase (4OCS) enhancer from pTiAch5	3' polyadenylation signal from ORF25 (*Agrobacterium tumefaciens*)	1 functional;	Altered coding sequence for optimal expression in plant cells.
pat	phosphinothricin N-acetyltransferase (*S. viridochromogenes*)	SM	ubiquitin (ubi) ZM (*Zea mays*) promoter and the first exon and intron	3' polyadenylation signal from ORF25 (*Agrobacterium tumefaciens*)	1 functional; 1 partial, non-expressed;	Altered coding sequence for optimal expression in plant cells.

Characteristics of *Gossypium hirsutum* L. (Cotton)

Center of Origin	Reproduction	Toxins	Allergenicity
Believed to originate in Meso-America (Peruvian-Ecuadorian-Bolivian region).	Generally self-pollinating, but can be cross-pollinating in the presence of suitable insect pollinators (bees). In the U.S., compatible species include *G. hirsutum, G. barbadense*, and *G. tomentosum*.	Gossypol in cottonseed meal.	

Donor Organism Characteristics

Latin Name	Gene	Pathogenicity
Streptomyces viridochromogenes	pat	*S. viridochromogenes* is ubiquitous in the soil. The spore chains are Spirales and the spore surface is spiny. The spore mass is blue, the reverse is green and its pigments are pH sensitive. It exhibits very slight antimicrobial activity, is inhibited by streptomycin, and there have been no reports of adverse affects

		on humans, animals, or plants.
Bacillus thuringiensis subsp. *kurstaki*	cry1Ac	Although target insects are susceptible to oral doses of Bt proteins, there is no evidence of toxic effects in laboratory mammals or birds given up to 10 µg protein / g body wt. There are no significant mammalian toxins or allergens associated with the host organism.
Bacillus thuringiensis var. *aizawai*	cry1F	While target insects are susceptible to oral doses of Bt proteins, no evidence of toxic effects in laboratory mammals or birds.

Summary of Regulatory Approvals

Country	Environment	Food and/or Feed	Food	Feed	Marketing
Australia			2005		
Japan		2005			
Mexico			2004		
United States	2004	2004			

CASE STUDY 10:

A CNA provides information to the BCH about a decision.

Answer Key

Under Article 10(3) "Within two hundred and seventy days of the date of receipt of notification, the Party of import shall communicate, in writing, to the notifier and to the Biosafety Clearing-House the decision referred to in paragraph 2 (a): (a) Approving the import, with or without conditions, including how the decision will apply to subsequent imports of the same living modified organism"

This means that the competent authority will have to provide details about the decision to approve the importation of Widestrike cotton into South Africa under the Advance Informed Agreement process, including whether future importations of Widestrike will be subject to the AIA procedure or the Simplified Procedure of Article 13.

From the Central Portal, the competent authority (who in other circumstances may be a National Focal Point or a National Authorized User of the Management Centre) selects "Management Centre" under "Registering Data" and signs in with his e-mail address and password. From the "Management Centre" home page he selects "Register new information" and then the category of information he wants to enter: "Decisions on LMOs under Advance Informed Agreement procedure (AIA)" under the Decisions and declarations category, and clicks the "create new record" button. This takes him to the common format for registering information in accordance with Annex II which includes:

- Contact details for the exporter and importer
- Information about the LMO, including gene modification, recipient and/or parental organism(s) details, donor organism details, etc.
- Decision status and condition, such as approving the import, prohibiting the import, extending the decision period, etc.
- Reasons on which the decision is based
- Additional information, such as risk assessments, and dates of receipt and decision
- Location of the document text.

- The competent authority fills in the form. Next the record must be validated by the BCH National Focal Point. If the competent authority is not the National Focal Point then he submits the record to be validated.

When entering the exporter, importer and CNA details, a contact reference will need to be created (unless completed in a previous exercise). Clicking on the "Add a reference button" then "Create >>" will open a new contact detail record. After completing and submitting this information (by hitting the "save changes" and "submit for publishing" buttons), the user is taken back to the decision entry page.

Once a record has been submitted for validation by an authorized user, the BCH sends an automatic email notification to the BCH National Focal Point responsible for validating the record. BCH-NFPs can view the record directly by using the link provided in the email, or access it through the Management Centre home page. He selects the record ID from the list of items requiring his attention on the Management Centre home page. He then checks the first of three options:

- Validate the record to make it public
- Edit the record if you wish to make changes
- Reject the record if you do not wish to make it public.

Once the record is validated, the competent authority (as the authorized user who created the record) will receive an email notification that the record is now public. An example of what this record could look like is provided below:

Decision on LMO under Advance Informed Agreement

Record information and status

Record ID	**99999**
Status	**Published**
Date of creation	**2006-04-27 07:59 GMT-5 (case.study@nowhere.za)**
Date of last update	**2006-02-24 03:26 GMT-5 (case.study@nowhere.za)**

General information

Title / Reference number of the decision
Decision on insect protected cotton event, DAS-21023-5 x DAS-24236-5 (trade name Widestrike™)

Country communicating the decision
South Africa

Exporter details

Contacts:

ABC Company 100 Anywhere Street, Cottonville, OH USA Tel: +1 123 555 4567 Fax: +1 123 555 4568 E-mail: info@abc.com	**(Record #9998)**

Responsible authority details

Competent National Authorities

Department of Agriculture	**(Record #5078)**

Dr. Julian Jaftha
Senior Manager
Genetic Resource Management
Department of Agriculture
Harvest House Room
30 Hamilton Street
Arcadia
South Africa, 0002

Tel: +27 12 319 6024
Fax: +27 12 319 6329
Email: SMGRM@nda.agric.za

LMO information

Living modified organism

DAS-21Ø23-5 x DAS-24236-5 (Widestrike™) **(Record #9997)**
Cotton – *Gossypium hirsutum*
Herbicide tolerance, Insect resistance, Resistance to cotton bollworm (*Heliocoverpa zea*), pink bollworm (*Pectinophora gossypiella*), tobacco budworm (*Heliothis virescens*) & phosphinothricin (PPT) herbicide tolerance, specifically glufosinate ammonium
Plasmids carried by *Agrobacterium tumefaciens*

Recipient organism or parental organisms (Annex I (e) and (f))

Cotton – *Gossypium hirsutum*

Donor organism or organisms (Annex I (g))

Streptomyces viridochromogenes
Bacillus thuringiensis subsp. *kurstaki*
Bacillus thuringiensis var. *aizawai*

Decision details

Decision

Permitting the import of DAS-21Ø23-5 x DAS-24236-5 (Widestrike™) (Article 10.3(a)).
Subsequent imports of DAS-21Ø23-5 x DAS-24236-5 (Widestrike™) will require application of the Advance Informed Agreement procedure (Article 8 to 10 and 12)

Reasons

See attached document

Dates

Receipt of notification
2005-06-01
Acknowledgement of receipt

2005-08-01

Communications of the decision

2006-03-01

Additional information

Any other relevant information

DAS-21Ø23-5 x DAS-24236-5 (Widestrike™) has not been approved for commercial release in South Africa. This decision is limited to a single importation of DAS-21Ø23-5 x DAS-24236-5 (Widestrike™) for use in confined, experimental field trials.

Please note that every import into SA is still subject to any other applicable legislation in SA, with particular reference to phytosanitary requirements under the Agricultural Pest Act, 1983.

Relevant documents

EN **Decision on insect protected cotton event, DAS-21023-5 x DAS-24236-5 (trade name Widestrike™)**