CASE STUDY 10:

Competent national authority provides information to the BCH about a decision

Objective:

- To understand what information should be registered with the BCH after a country makes a decision on the importation of LMO and how it is done through the BCH Management Center.

References:

- Cartagena Protocol on Biosafety (Go to http://bch.cbd.int > Cartagena Protocol > Text of the Cartagena Protocol).
- Common format "Country's Decision or any other Communication" (Go to http://bch.cbd.int > Resources > Common formats).
- BCH Training Site (Go to http://bch.cbd.int > Help > Training Site of the BCH).

Scenario:

Competent National Authority of the Republic of South Africa approves importation of modified cotton DAS-21023-5 x DAS-24236-5 (trade name Widestrike™; see **Case Study 09**) for intentional release into the environment. What information must the South African competent authority provide to the BCH about its 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 can be submitted to the Biosafety Clearing House and which article(s) of the Cartagena Protocol are pertinent to this activity. Identify what information is missing in the attached decision.

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

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 from pTiAch5	3' polyadenylation signal from ORF25 (*Agrobacterium tumefaciens*)	1 functional;	Cry1F active insecticidal core and non-toxic portions of the Cry1Ab1 and Cry1Ca3 proteins. Sequence 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			

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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 may be a BCH National Focal Point or a National Authorized User) selects "Registering Information" from the horizontal navigation bar and signs in to the "Management Centre" with his/her e-mail address and password. From the "Management Centre" home page he/she selects "Register new information" and then the category of record to be created : "Country's decision or any other communications", and clicks the icon "submit the record online". This opens the common format for registering information 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 (for all cases except approval without conditions)
- Additional information, such as risk assessments, and dates of receipt and decision
- Location of the document text.

When entering the exporter, importer and CNA details, a contact reference will need to be created (unless completed earlier). It will also require registering a summary of risk assessment on which the decision is based. Clicking on the "create a new record" button" will open a new 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.

When the form is completed and submitted for publishing it's either automatically published (if CNA and BCH National Focal Point is the same person), or sent for validation to the BCH National Focal Point (if CNA is registered as National Authorized User). Once record has been submitted for validation, 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/she selects the record ID from the list of items requiring attention on the Management Centre home page, and after reviewing the record checks one of the following 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.

Off-line registration of information in the BCH is possible for users with no Internet access or poor Internet connectivity. In this case, the user should download common format in MS Word, complete it and submit, duly signed, to the Secretariat. The SCBD will register information in the BCH on behalf of the user.