RISK ASSESSMENT AND RISK MANAGEMENT PLAN FOR JK 1947 & JK 1050

ID CODE:
Insect Resistant Cotton, Mr. S K Gupta Resistance to antibiotics – Kanamycin Resistance to disease and pests – Insects – Lepidoptera (butterflies and moths)

COMMERCIAL RELEASE OF INSECT RESISTANT COTON LINES

APPLICANT: SWAZILAND COTTON BOARD

October 2018

BACKGROUND

1. After the passing of the Biosafety Act of 2012, which got operationalized in December 2013, the Swaziland Environment Authority, as the custodians of the Act, is obligated to receive applications for Genetically Modified Organisms (GMOs) and make the proper decisions (grant/decline permit).
2. Section 9 of the Biosafety Act, 2012 states that applications should be submitted to the Biosafety Registrar of the Competent Authority to screen for completeness of the application and forward the application to the National Biosafety Advisory Committee (NBAC)
3. Section 7 of the Biosafety Act of 2012 states that the National Biosafety Advisory Committee shall conduct risk assessments, review risk assessments provided in applications, review risk management measures, recommend containment measures and provide such other expert advice and assistance to the Management Board of the Swaziland Environment Authority which also serve as the Board for the Competent Authority.
4. Section 4 (3) of the Biosafety Act 2012 then further states that the Board of the Competent Authority (CA) should receive, respond to and make decisions on notifications pursuant to Section 10 and applications pursuant to Section 12 in consultation with the National Biosafety Advisory Committee (NBAC) and in conformity with the requirements of the Biosafety Act.
5. Section 18 (2) (b) states that any decision rendered under subsection (1) shall be based upon the risk assessment report prepared by the Committee in accordance with section 17 (4) and (5)
6. Section 26 (2) (b) states that the Competent Authority shall provide to the Biosafety Clearing House the summaries of the risk assessments generated pursuant to section 17

THE APPLICATION PROCESS AND FLOW OF EVENTS

7. The Swaziland Cotton Board submitted their intent to apply for environmental release in June 2017
8. The Office of the Biosafety Registrar replied the SCB (as advised by the National Biosafety Advisory Committee) by detailing the application requirements as per the legislation
9. The SCB submitted the application in August
10. The specification of the GMO were as follows;

> *This modified cotton contains the insecticidal Cry1Ac gene which imparts resistance against Lepidoptera. The LMO also contains the selectable markers Npt II to isolate transformed seedlings and a GUD gene cassette as a reporter gene.*
> *Bt cotton was generated by using the biolistic method of transformation system. The transformed cotton shoots containing the Npt II gene were selected on medium supplemented with kanamycin. A procedure of biolistic method of transformation of cotton is novel and performed using shoot meristem. Plants were regenerated and ultimately plantlets were grown in soil and assayed for insect resistance.*
> *The Cry1Ac gene in the Indian inbred line behave as a single dominant Mendelian factor and is stably integrated in the plant genome.*

11. After review by the Biosafety Registrar, the application was then sent to the National Biosafety Advisory Committee, to undertake the risk assessment of that particular GM seed
12. The NBAC met on the following dates, which had the following outcomes;

Date	Activities	Outcomes
16 Aug 2017	1. Review of Swaziland Cotton Board's Application	The SCB was instructed to: 1. Work on the outline of the application 2. Correct typos 3. Have the applications signed 4. Re-submit
23 Aug 2017	1. Review of Swaziland Cotton Board's Application	• Discussion of the media publication
20 Sept 2017	2. Review of Swaziland Cotton Board's Application	• Invited the applicants to make a presentation, so to avoid the back and forth communiqué delays

		• Applicants should still improve the application document • Nisela Farms should publicize their application withdrawal
06 Oct 2017	1. Review of Swaziland Cotton Board's Application	• Application document was disappointing ○ Numbering distorted • Have the applications (schedule 1 & 8) synchronized • Provide more information • Conducted the risk assessment of the *Bt* Cotton • Recommendation will be issued once the application document has been improved
	2. Review of Swaziland Cotton Board's Application 3. NBAC's recommendation to the SEA Board	• Document was improved • Additional Human Resource was noted • NBAC issued the recommendation to the SEA Board

INFORMATION PROVIDED

13. Applicant
    - Swaziland Cotton Board

14. Recipient Organism

The parent organism is cultivated cotton (*Gossypium hirsutum*) which is exotic to Swaziland, although sightings of wild cotton species in the country have been reported.

- Common name : Cotton
- Scientific Name : *Gossypium hirsutum*, G. barbadense
- Genus : *Gossypium*
- Family : Malvaceae

15. The Vector

Source: pTi Ach5 strain of *Agrobacterium tumefacieus*. The border sequences of RB and LB of the T-DNA were cloned in the lab and their potentials were judged in the laboratory (Ref: Ind. J. Exptl. Biol. Vol 29, pp 991-1001 (1991).

16. The Gene Construct

Two GM lines are proposed for the release. Both lines have a single introduced trait that is expressed to impart insect resistance on the cotton plant. In creating the gene construct, the following genes were used:

- Cry1Ac gene: This is the insect resistance gene taken from *Bacillus thuringeinsis*

- Gene *nptII* – This is the antibiotic resistance selectable marker that has been derived from kanamycin

- A Constitutive CaMV 35s promoter has been used to express the truncated *Cry1Ac* gene, where as the classical CaMV 35s promoter has been used to express the *npt II* gene in plants.

BIOSAFETY STUDIES REVIEWED

Study #	Study	Conducted at	Status	Report Remarks
1.	Acute Oral Toxicity Study in Rats	Shriram Institute for Industrial Research, New Delhi, India	Completed on 14-11-2003	Found Safe
2.	Sub chronic oral toxicity study in rats (90days)	Shriram Institute for Industrial Research, New Delhi, India	Completed on 8-1-2004	Found Safe
3.	Primary Skin Irritation Test on Rabbits	Shriram Institute for Industrial Research, New Delhi, India	Completed on 14-11-2003	Found Safe
4.	Irritation to mucous membrane in rabbits	Shriram Institute for Industrial Research, New Delhi, India	Completed on 14-11-2003	Found Safe
5.	Dermal Sensitization study in Guinea pigs	Shriram Institute for Industrial Research, New Delhi, India	Completed on 14-11-2003	Found Safe
6.	Feeding Study on birds	Avian Nutrition & Feed Technology Division, (CARI), Izatnagar (UP) ICAR, India	Completed on 29-01- 2005	Found Safe
7.	Study on Soil micro flora	IMTEC, Chandigarh, India	Completed on 28-02-2005	No Effect
8.	Study on Fish	Central Institute of Fisheries Education (Deemed University, ICAR), Mumbai, India	Completed in March,2005	Found Safe
9.	Study on goat	Indian veterinary Research Institute, Izatnagar (UP) ICAR, India	Completed in May, 2005	Found Safe
	Study on Baseline Susceptibility	Indian Agricultural	Completed	No resistance

10.	of Helicoverpa to Bt Cry1Ac toxin	Research Institute, New Delhi, India	Jan,2006	development in Helicoverpa
11.	Study on Cow	Indian veterinary Research Institute, Izatnagar (UP) ICAR, India	Completed on 10-02-06	Found Safe
12.	Study on Beneficial Insects	JKAL Bt Cotton trial Field in South , Central and North Zone, India	Kharif, 2003, Summer and Kharif, 2004 Kharif,2005 Field trial	Found Safe, No effect on Beneficial Insects
13.	Pollen Flow trial	JKAL Bt Cotton trial Field at Ravolkol in Andhra Pradesh.< India	Completed during kharif,2003	No outcross of transgenic pollen beyond 2mts from the periphery of Bt plot

RISK ASSESSMENT AND RISK MANAGEMENT PLAN

Background

17. The risk assessment was conducted in consideration of the fact that the modification that was done on the recipient organism was the introgression of the Cry1Ac gene to impart insect resistance.
18. The first consideration was to assess the overall protection goal that could be affected by the release of the LMO thereby causing any harm to LMO either to the biodiversity or human health. This would then lead to the evaluation of the possible ways which this harm could manifest itself based on the basic information from public concerns, growing conventional cotton, the local management practices, ongoing education campaigns, as well as the local environmental conditions where the cotton will be released.

19. The broad protection goals were then looked at to identify all the possible harms, events or circumstances that could come about as a result of the release. When an event was considered to have some chances of causing harm, it was then identified and that particular chance was then ranked, following the typical principles of probability.

20. Following the risk estimate, risks that were identified to ha a higher risk estimate were then supported with mitigations strategies which would also be adopted as permit conditions or reasons to deny the permit.

Summary Table for the Risk Assessment

PROTECTION GOAL	POSSIBLE HARMS	RISK ESTIMATE	MITIGATION STRATEGY (CONDITIONS)	CONCLUSION
HUMAN & ANIMAL HEALTH	1. The Cry1Ac protein being toxic to human being and animals:	**Negligible** - The Cry1Ac proteins are only toxic to lepidopteran insects - Studies indicate that the proteins do not have any effects on non-target insects		Acceptable
	2. Antibiotic resistance gene (Npt II) being expressed in humans	**Negligible** - Npt II does not contain glycosylation sites - it is also not stable in the mammalian digestive system - it is heat labile - All the above decreases the probability of being allergic		Acceptable
	3. GMO being allergic to humans	**Negligible** - No risk of allergic reactions were identified - this included food materials as well GM cotton fibre		Acceptable
	4. Transfer of genes to humans	**Negligible** - It is unlikely that the transferred genes could be introduced to sexually incompatible plants, animals or human beings - If the plant could be ingested, the		Acceptable

		produced Cry 1Ac plant proteins would be digested and broken down in the digestive tract like any other proteins, rather than to be introduced into the human genome.		
	5. Human getting poisoned	**<u>Negligible</u>** - There is no identified risk of toxicity to people		Acceptable
BIODIVERSITY CONSERVATION	1. Gene-flow to wild relatives	**<u>Negligible</u>** - Swaziland is not a centre of origin for cotton - Although wild relatives have been reported, cytogenetically, the two would not be sexually compatible since the GM cotton is tetraploid		Acceptable
	2. Gene-flow to traditional varieties	**<u>Negligible</u>** - The country does have traditional varieties (CBB 95, Mavolo, etc) that could be polluted by the GM crops - The traditional varieties are bred and multiplied in isolation - Harvested cotton seed is not recycled for re-planting - Possibly polluted cotton seed will be sold along with the other seed to make by-products	• **Maintain the recommended isolation distances**	Acceptable
	3. Loss of local varieties through extensive mono-cropping (gene bank)	**<u>High</u>** - The demand for a particular variety could be high to the extent that all the farmers end up growing it - The inferior varieties could end up becoming obsolete and lost in the process	• Control the GM seed distribution • Always make conventional seed available for the farmers that may not be interested to the GM varieties	Acceptable

		- Saving the local varieties comes as a responsibility of the Ministry of Agriculture	• Keep local accessions at the gene bank	
	4. Development of herbicide tolerant weeds	**Negligible** - The GM varieties in consideration do not have the herbicide tolerance trait	• Maintain the *Bt* event per the application • This permit is specific to *Bt* cotton, not Herbicide Tolerant cotton	Acceptable
	5. Development of *Bt* resistant pests (bollworms)	**Moderate** - The development of resistance can never be overlooked - This could come, mostly, as a result of poor management practices by the farmers - Can be greatly mitigated by the seed or technology suppliers by providing refuge seeds, educating the farmers on the required GM management practices and monitoring the farmers - There should be removal of residual crops from the fields	• Provide the refuge seed for the farmers • Ensure refuge cropping (10%) • The refuge seed should be the non-GM isoline of the GM seed • Ensure continuous training on management practices • Destroy residual crops after harvestings	Acceptable
	6. Effect on non-target insects	**Low** - The Cry1Ac proteins are only toxic to lepidopteran insects - Studies indicate that the proteins do not have any effects on non-target insects		Acceptable

	7. Increased herbicide use	**<u>Negligible</u>** - The GM varieties in consideration do not have the herbicide tolerance trait - There is an insignificant herbicide use		Acceptable
	8. Increased pesticide use	**<u>Negligible</u>** - Insect resistant varieties have reduced pesticide use when compared to the conventional counterparts		Acceptable
	9. Complete eradication of the targeted pest	**<u>Negligible</u>** - As long as there will be the refugia, not all the pests will be exposed to the GM cotton - Complete eradication may be impossible even with heavy pesticide use (conventionally)	• There should always be the refugia	Acceptable
SOCIO-ECONOMIC CONSIDERATIONS	1. Loss of conventional (non-*Bt*) varieties through extensive monocropping	**<u>Moderate</u>** - The demand for a particular variety could be high to the extent that all the farmers end up growing it - The inferior varieties could end up becoming obsolete and lost in the process - Saving the local varieties comes as a responsibility of the Ministry of Agriculture	• There should always be non-GM seed available • Keep the conventional varieties at the gene bank	Acceptable
	2. Loss of intercropped plants and edible weeds (ligusha, umbhidvo, etc)	**<u>Negligible</u>** - Cotton is usually not intercropped due to the toxicity of its conventional pesticides - With insect resistance, even if the GM cotton was to be intercropped there		Acceptable

		would still be weeds since there would be no herbicide use		
	3. Having to buy seed year-after-year	**<u>NA</u>** - Conventional seed is currently bought every year, and has been like that ever since - Farmers do not keep seed	• All cotton seed (GM or Conventional) have to be bought every year	NA
	4. Seed suppliers enjoying monopolies	**<u>High</u>** - Farmers will be given a choice by availing other non-GM varieties - Seed choice solely depends on the farmer - The SCB could source seed from different suppliers, following the dictates of the Biosafety Act, 2012	• There is already a monopoly in conventional cotton seeds • Monopolies are usually caused by farmers demanding a specific variety	Acceptable
	5. Expensive seeds	**<u>High</u>** - Farmers will be given a choice by availing other non-GM varieties - Seed choice solely depends on the farmer - The SCB could source seed from different suppliers, following the dictates of the Biosafety Act, 2012	• SCB should assist farmers to maximise the yields to justify the costs	Acceptable
	6. More drudgery to farmers	**<u>Negligible</u>** - The GM cotton would mean less hard work for the farmers - Fewer sprays due to the insect rsistance		Acceptable
MANAGEMENT PRACTICES	1. Farmers do not understand the management of GM Crops	**<u>Moderate</u>** - The SCB reported to have been educating the farmers for the past three (or more) growing seasons	• SCB should continue with the extension services • The technology	Acceptable

		- Some of the CFTs were used for farmer demonstrations - Farmer extension should is still ongoing	should be given to trained farmers • The technology supplier should provide product stewardship beyond the retail points	
	2. Farmers will not plant the refuge	**Moderate** - The SCB has trained the farmers on the importance of the refugia - Close monitoring	• The Applicant should include refugia seed on the packaging (an isogenic line) • The SCB should monitor the planting of the refugia	Acceptable
	3. Not enough Extension Services available to farmers	**Moderate** - There should be continuous awareness, education and extension for the farmers	• Cotton Board should ensure that the required skills are availed to farmers	Acceptable
	4. Possible miss-use of the technology by farmers	**High** - Farmers may not follow the required management practices or the requirements of the seed suppliers - The Operators may not abide to the terms and conditions of the permit	• Cotton Board should make sure that the technology is only given to trained and certified farmers • SCB should monitor compliance to their permit requirements	Acceptable