FACT SHEET
APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCT OF MS8RF3 OILSEED RAPE FOR SUPPLY OR OFFER TO SUPPLY FOR SALE OR PLACING IN THE MARKET

NBB REF NO: JBK(S) 602-1/1/28

The objective of the Biosafety Act is to protect human, plant and animal health, the environment and biological diversity. Under the Biosafety Act, the National Biosafety Board (NBB) is currently assessing an application for approval submitted by Bayer Co. (Malaysia) Sdn. Bhd. (Bayer CropScience).

1. What is the application for?

The application is for import and release of MS8RF3 oilseed rape and its products for supply or offer to supply for sale or placing in the market.

2. What is the purpose of the import and release?

The aim of the import and release is for direct use as food, feed and processing (FFP) of MS8RF3 oilseed rape and its products. The said oilseed rape event is not intended for cultivation in Malaysia.

3. How has the MS8RF3 oilseed rape been modified?

MS8RF3 is a hybrid product of traditional crossing and possesses the unique hybridization system involving the use of a male sterile line MS8 and a fertility restorer line RF3. The MS8 and RF3 oilseed rape lines (*Brassica napus*) were developed using genetic engineering techniques to provide a pollination control system for production of hybrid MS8RF3 oilseed rape.

The transgenic line MS8 contains the *barnase* gene for male sterility, isolated from *Bacillus amyloliquefaciens*. The *barnase* gene encodes for a ribonuclease enzyme (RNAse) that is expressed only in the tapetum cells of the pollen sac during anther development. The RNAse affects RNA production, disrupting normal cell function and arresting early anther development, thus leading to male sterility. The transgenic line RF3 contains the *barstar* gene isolated from *Bacillus amyloliquefaciens*. The *barstar* gene codes for a ribonuclease inhibitor (Barstar enzyme) that is expressed only in the tapetum cells of the pollen sac during anther development. The ribonuclease inhibitor specifically inhibits Barnase RNAse expressed by the MS8 line. Together, the RNAse and the ribonuclease inhibitor form a very stable one-to-one complex, in which the RNAse is inactivated. As a result, when pollen from the restorer line RF3 is transferred to the male sterile line MS8, the resultant progeny expresses the RNAse inhibitor in the tapetum cells of the anthers allowing hybrid MS8RF3 plants to develop normal anthers and restore fertility.

Both transgenic oilseed rape lines MS8 and RF3 contain the *bar* gene that confers tolerance to the post-emergence, broad-spectrum phosphinothricin herbicides

(Basta®, Rely®, Finale®, and Liberty®). The *bar* gene, isolated from the common soil microorganism *Streptomyces hygroscopicus* encodes a phosphinothricin acetyl transferase (PAT) enzyme. PAT detoxifies glufosinate ammonium by acetylation into an inactive compound, eliminating its herbicidal activity. The herbicide tolerance trait was introduced into the oilseed rape lines as a selectable marker to identify transformed plants during tissue culture regeneration, and as a field selection method to obtain 100% hybrid seed.

4. Characteristics of MS8RF3 oilseed rape

(a) Details of the parent organism

Oilseed rape (rapeseed, canola, *Brassica napus*) is of relatively recent origin (<10,000 years) resulting from the interspecific cross between plant of *B. oleracea* and *B. rapa*. Oilseed rape has an extended history of cultivation and safe use. *Brassica* is a genus of the Brassicaceae (formerly the Cruciferae) family, commonly known as the mustard family, which consists of about 375 genera and 3,200 species of plants mainly found in the northern hemisphere. The mustard family includes crops, condiments, ornamentals and many weeds. The genus *Brassica* contains about 100 species, including cabbage, cauliflower, broccoli, oilseed rape, brussels sprouts, turnip, various mustards and weeds. Many *Brassica* species have been cultivated since prehistoric times for their edible roots, stems, leaves, buds, flowers and seeds. Within a species, crops have been developed for different purposes.

Oils from *B. juncea*, *B. rapa* and later *B. napus* have been part of the Asian diet for centuries. Prior to and during the Second World War, oilseed rape oil was primarily used as lubricant for steam engines and as lamp oil but following the war *B. napus* and *B. rapa* oils became an important constituent of margarine. Breeding and selection within the world's germplasm was successful in developing plants that produced oils with less than 2% erucic acid. This oil was found nutritionally superior to the high erucic oil and proved to be an excellent liquid and salad oil, as well as a suitable ingredient for margarine and shortening manufacture. This new natural oil is called "canola oil" in most countries of the world and is defined as oils from *B. napus*, *B. rapa* or *B. juncea* containing less than 2% erucic acid of the total fatty acid. The fatty acid composition of canola oil met or exceeded the nutritional requirements of a superior edible oil, with the lowest saturate content (6 to 7%) of any edible oil and a high (58 to 60%) level of oleic (18:ln-9) that reduces the undesirable low density lipoproteins (LDLs) without reducing the desirable high density lipoproteins (HDLs).

(b) Details of the donor organisms

Characteristics of *Bacillus amyloliquefaciens*

B. amyloliquefaciens is a commonly occurring bacterium and is frequently used as a source of industrial enzymes such as α-amylase. *B.*

amyloliquefaciens has no known pathogenicity and is used in brewing, bread-making and food industry.

Characteristics of *Streptomyces hygrocopicus*

S. hygroscopicus is a very well-characterized genus within the Streptomycetaceae family. It is a safe common soil saprophytic bacterial species not known to be toxic, allergenic or pathogenic to humans and animals. This *Streptomyces* species is widespread in nature and a common part of the living biosphere all over the world. There are many species of *Streptomyces* similar to *S. hygroscopicus* and many of them are likely to contain *bar* or *pat* homologues. None of these homologues have been reported as being toxic or allergenic in humans or animals. Safety of *S. hygroscopicus* and its protein PAT had been evaluated and demonstrated. Transforming a plant with a coding region derived from *S. hygroscopicus* that encodes a PAT protein is *a priori* expected not to lead to the development of a pathogenic, toxic or allergenic transgenic plant.

(c) Description of the trait(s)and characteristic which have been introduced or modified

Summary of introduced genetic elements

Code	Name	Type	Promoter, other	Terminator	Copies	Form
barnase	barnase ribonuclease	MS	pTa 29 pollen specific promoter from *Nicotiana tabacum*	3' nos	1	Introduced into MS8
barstar	barnase ribonuclease inhibitor	RF	anther-specific promoter	3' nos	1	Introduced into RF3
bar	phosphinothricin N-acetyltransferase	HT	PSsuAra from *Arabidopsis thaliana* chloroplast transit peptide from *A. thaliana*	3' g7	1	Introduced into MS8 and RF3

5. Modification method

The MS8 and RF3 oilseed rape lines were both produced using *Agrobacterium*-mediated transformation of the *Brassica napus* cultivar 'Drakkar'. The T-DNA region of the Ti plasmid was "disarmed" by removal of *virA* genes, normally associated with the pathogenicity and disease-causing properties of *A. tumefaciens*, and replaced with the genes of interest for each transgenic line. During transformation, the T-DNA portion of each plasmid was transferred into the plant cells and stably integrated into the plant genome of MS8 and RF3 respectively.

Line MS8 was produced using plasmid pTHW107, which contained a copy of the *barnase* gene whose transcription was regulated with an anther specific promoter

pTa29 from *Nicotiana tabacum*, terminated by part of the 3´non-coding region (*3´nos*) of the nopaline synthase gene of *A. tumefaciens*. Similarly, line RF3 was produced using plasmid pTHW118, which contained the *barstar* gene under the control of the pTa29 anther-specific promoter from *N. tabacum* and the *nos* termination signal.

In addition, each T-DNA contained a copy of the *bar* gene from *S. hygroscopicus*, which encodes the PAT enzyme. Expression of the *bar* gene was regulated by the PSsuAra promoter from *Arabidopsis thaliana* and post-translational targeting of the gene product to the chloroplast organelles was accomplished by fusion of the 5'-terminal coding sequence with the chloroplast transit peptide DNA sequence from *A. thaliana*.

(a) Characterization of the modification

Southern blot analysis of genomic DNA from lines MS8 and RF3 demonstrated that each line contained a single site of insertion for the T-DNA. The *barnase* and *bar* genes were integrated into MS8 and similarly, the *barstar* and *bar* genes were integrated in RF3.

Based on Southern blots and detailed PCR analyses it was confirmed that no sequences outside of the T-DNA region from plasmids pTHW107 or pTHW118 were integrated into the plant genome. There were no marker genes for antibiotic resistance present in the transformed plants.

(b) Safety of the expressed protein

The human consumption of oilseed rape products is limited to the refined oil. Because virtually no protein is present in the oil extracted from the plants, the potential for human exposure is exceedingly low. Furthermore, the amounts of PAT protein present in seed-meal fed to animals would be too low to cause concern. Additionally, the barnase RNAse and its inhibitor encoded by *barnase* and *barstar* genes, respectively, were not detected in dry seeds. As the introduced gene products were not detectable in the refined oil produced from transgenic canola, there will be no human exposure to these proteins based on normal consumption patterns.

6. Assessment of risks to human health

(a) Nutritional data

The composition of refined canola oil from MS8RF3 hybrid oilseed rape was compared to that for refined oil from non-transgenic oilseed rape. Some statistical differences in fatty acid composition were noted in the comparison, however, the fatty acids for the transgenic lines, including the erucic acid content of the oil, were within the normal range for canola oil fatty acids. Processing according to protocols mimicking industrial practices (including tempering, flaking, cooking, pressing, desolventizing oil and meal, oil blending, degumming, oil refining, water washing, bleaching, hydrogenation and

deodorizing) further demonstrated that the composition and physical characteristics of the oil from MS8RF3 hybrids and control oilseed rape varieties were equivalent. The use of refined oil from MS8RF3 oilseed rape would therefore have no significant impact on the nutritional quality of the food supply. Similarly, the glucosinolate content of seed meal derived from MS8RF3 oilseed rape was the same as that from non-transgenic control cultivars.

(b) Toxicology

Since only the processed oil from transgenic MS8, RF3, or MS8RF3 hybrids derived therefrom are available for human consumption, and the processing removes proteinaceous material, there are no additional toxicity concerns regarding this product. This was further assessed by searching for amino acid sequence homologies with known protein toxins, and by examining the physiochemical characteristics of the introduced RNAse (*barnase*), RNAse inhibitor (*barstar*), and PAT (*bar*) proteins. No homologies with potential toxins were observed. The MS8RF3 oilseed rape is considered as safe and as nutritious as its non-GM counterpart.

(c) Allergenicity

Since only the processed oil from transgenic MS8, RF3, or MS8RF3 hybrids derived therefrom are available for human consumption, and the processing removes proteinaceous material, there are no additional allergenicity concerns regarding this product. This was further assessed by searching for amino acid sequence homologies with known protein allergens, and by examining the physiochemical characteristics of the introduced RNAse (*barnase*), RNAse inhibitor (*barstar*), and PAT (*bar*) proteins. No homologies with potential allergens were observed. The MS8RF3 oilseed rape is considered as safe and as nutritious as its non-GM counterpart.

7. Assessment of risks to the environment

The application does not cover an environment release. The application is intended only to cover the import of the MS8RF3 oilseed rape products from countries where the said oilseed rape event is already approved and commercially grown, and that may enter Malaysia as foodstuffs or as feed or for further food processing.

8. What is the emergency response plan?

The seed derived from MS8RF3 oilseed rape may be imported for processing. However, the MS8RF3 products that likely enter into Malaysia are in highly processed forms like refined oil or oilseed rape meal. The seed may be viable but would not thrive as Malaysia conditions are not optimum for oilseed rape growth. In the rare cases that oilseed rape seeds grow, specific detection tools are already developed and commercially available to enable the identification of products derived from event MS8RF3. As with conventional oilseed rape, the plants from

event MS8RF3 are sensitive to herbicides other than glufosinate and can be controlled or eradicated either by herbicides other than glufosinate or by mechanical destruction.

Seed derived from MS8RF3 oilseed rape is compositionally equivalent to those from conventional oilseed rape. The plants behave agronomically in the same way as conventional oilseed rape except showing the intended tolerance to the herbicide glufosinate. Should adverse effects be reported and verified, appropriate follow up action would be taken to investigate these and if verified appropriate action taken.

(a) First aid measures

No special first aid measures are required with exposure to this product.

(b) Accidental release measures

No special measures are required in response to an accidental release. Spilled seed should be swept, scooped or vacuumed in a manner that avoids dust generation and dust-related hazards. During industrial processing, the seed derived from MS8RF3 is indistinguishable from conventional oilseed rape and needs no specific or additional treatment compared to conventional oilseed rape.

(c) Handling and storage

No special handling procedures are required for this product. For MS8RF3 oilseed rape and its products, the same storage and handling can be applied as for conventional oilseed rape. No special storage procedures are required for this product. Seed is stored as any oilseed rape product.

(d) Disposal considerations

The same measures for waste disposal and treatment as for conventional oilseed rape are valid for seed derived from MS8RF3.

9. How can I comment on this application?

Any member of the public may submit their comment or queries on publicly notified information about the application. Before submission of comments or queries, the person should review the information provided. Your comments and queries on any possible impacts/risks to the health and safety of the people and the environment that may be posed by the proposed release are appreciated. The submission of the comments or queries should be prepared carefully as it will be given the same scrutiny as the application by the NBB. The submission of comments and clarifications of queries should contribute to the NBB's assessment. Even if the submission is not science-based, and focuses on cultural or other values, it should still be developed in the form of a well-founded argument.

Please note that the consultation period closes on 18 February 2016 and written submissions are required by that date. Submissions must be addressed to:

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Please indicate your full name, address and contact details in your submission.