RISK ASSESSMENT REPORT

OF THE GENETIC MODIFICATION ADVISORY COMMITTEE (GMAC)

FOR

AN APPROVAL APPLICATION TO IMPORT TRYPSIN MODULATING OOSTATIC FACTOR _Yeast (TECHNICAL GRADE ACTIVE INGREDIENT, TGAI) TO PRODUCE MOUSTICIDE RH AND MOUSTICIDE WP AND IMPORT OF MOUSTICIDE WP FOR RELEASE

NBB REF NO: JBK (S) 602-1/1/20

APPLICANT: ENTOGENEX INDUSTRIES SDN. BHD.

DATE SUBMITTED: 12 FEBRUARY 2015

I - Summary of Assessment Process

The Genetic Modification Advisory Committee, under the purview of the National Biosafety Board (N B B) was given the dossier by the Department of Biosafety on 22 January 2015 for an application of approval to import TMOF_yeast (Technical Grade Active Ingredient,TGAI) from India to use in the formulation of Mousticide Rice Husk (RH) and Mousticide Wettable Powder (WP) as well as the importation of Mousticide WP from the Philippines (formulated with TMOF_yeast produced in India) to supply, offer to supply for sale or placing on the market. The application was filed by the EntoGenex Industries Sdn Bhd (EntoGenex, hereafter referred to as "the applicant"). Please refer to **Lampiran IB** for Additional Information provided by the applicant.

Previously, the NBB on 26 July 2011 had granted the applicant an approval with terms and conditions to produce the TMOF_yeast at the Institut Pembangunan Bioproduk (IBD), Universiti Teknologi Malaysia (UTM) and to formulate the product Mousticide RH and Mousticide WP in that same facility in order to supply or offer to supply for sale or placing in the market (**Lampiran IC)**. Among the terms and conditions include that a reassessment will have to be carried out if the TMOF is produced in a different facility other than the IBD, as there may be a difference in the effectiveness of the protocol used as well as the identified risks.

This new application involves the importation of the TMOF_yeast from India that will be used in the formulation of the products Mousticide RH and Mousticide WP in a new facility and IBD as well as the importation of Mousticide WP from the Philippines (using the TMOF_yeast from India).

GMAC had a meeting pertaining to this new application and prepared the Risk Assessment Report and Risk Assessment Matrix along with its recommended decision, for consideration by the National Biosafety Board. During the assessment, GMAC also took into consideration data and conditions/requirements that were attached to the applicant's previous approval for the assessment of the locally produced Mousticide WP and Mousticide RH.

Through GMAC assesment, it was concluded that there may be significant consequences to biodiversity through the accumulative effect of long term usage of this product. Therefore the applicant was required to conduct some specific safety studies and report the findings to the NBB.

Taking into consideration the importance of obtaining these safety data for Mousticide RH and Mousticide WP, GMAC has assisted the applicant through a series of meetings and discussions by providing clarifications and guidance on conducting these research studies. The applicant has yet to complete all the required studies and time extensions were granted twice by the NBB.

A public consultation for this application was also conducted from 13 January 2015 to 11 February 2015 via advertisement in local newspapers. The Public Consultation for this application raised some technical and scientific issues regarding the release. Comments were received from Third World Network (TWN) and Consumers' Association of Penang (CAP) regarding the implementation of safety studies commissioned by the NBB through the previous decision for the locally produced Mousticide RH and Mousticide WP, exposure of the product through inhalation or dermal contact, potential risk of amyloid formation, expression of inserted gene, disposal of the product, use of the product in waterways and the proposed control and emergency response plan. GMAC considered these comments and found that all the issues raised were already included in the Risk Assessment and were also being addressed by the applicant via the implementation of the terms and conditions from the previous decision (**Lampiran IC)**.

II - Background of Application

This application is for approval of TMOF_yeast imported from India and Mousticide WP Imported from Philippine (which is formulated with TMOF_yeast produced in India). The aim of the release is to use these products to control the *Aedes aegypti* larval population. These products, in the form of killed dried yeast cells containing TMOF peptide, are mosquito larvicides. They will be used to treat possible mosquito breeding habitats (water bodies) to kill mosquito larvae as a control measure to reduce the population of *Aedes* mosquitoes.

Information about TMOF_ yeast

The parent organism, *Pichia pastoris* is a yeast commonly used for the expression of biological pharmaceutical proteins with no known adverse health effects. It is a non-pathogenic microorganism with decades of safe utilization. Unmodified *P. pastoris* yeast has been approved by the United States Department of Agriculture (USDA) as a food additive in the livestock industry (JBK (S) 602-1/1/5).

P. pastoris KM71H strain was modified with pPICZ B plasmid containing gene sequences for expression of the TMOF peptide, which was originally isolated from ovaries of the female *Aedes aegypti* mosquito. The mode of action of TMOF is by hormonal distruption of transcription and translation of trypsin, a critical enzyme needed by the adult and larval digestive system for the digestion of their protein diet. The lack of free amino acids liberated from the blood meal in adult females causes inhibition of egg development and lack of free amino acids in the larval gut causes anorexia leading to starvation and death of mosquitoes larvae.

The active ingredient, TMOF, is a small protein containing 10 amino acids. The genes for making TMOF have been inserted into *P. pastoris* so that the yeast cells can make large amounts of the protein. The yeast cells are then killed by exposure to high temperature. For this application, killed yeast cells have been formulated to produce Mousticide WP and Mousticide RH. These products are for application onto bodies of water to control mosquito larvae. Once ingested by mosquito larvae, TMOF interferes with the production of trypsin. Exposed larvae are therefore unable to digest food and starve to death.

Supporting information from previous assessment on Mousticide RH and Mousticide WP

Through previous GMAC assesment, it was concluded that there may be significant consequences to biodiversity through the accumulative effect of long term usage of this product. Therefore the applicant was required to conduct these studies:

a) Possibility of toxic effects on the oil palm pollinating weevil
b) Detrimental effect on local fauna
c) Synergistic effect of TMOF with other biopesticides on nontarget organisms
d) Impact of TMOF to human health

The applicant has completed a study on the chronic and acute exposure of Mousticide on tilapia, freshwater prawn (*Macrobrachium rosenbergii*) and freshwater crustacean (*Daphnia sp*.) as in **Lampiran IE**.

III - Risk Assessment and Risk Management Plan

GMAC evaluated the application with reference to the following documents:

(i) Roadmap for Risk Assessment of Living Modified Organisms, (according to Annex III of the Cartagena Protocol on Biosafety produced by the *Ad Hoc* Technical Expert Group (AHTEG) on Risk Assessment and Risk Management of the Convention on Biological Diversity).

(ii) the risk assessment and risk management plan submitted by the applicant.

(iii) United States Environmental Protection Agency Office of Pesticide Programs Biopesticides Registration Action Document for Trypsin Modulating Oostatic Factor (TMOF). This report states that the EPA assessment of TMOF was conducted **for manufacturing use only** and **any end use product using TMOF should be evaluated individually** to determine any risk or labeling issues. This report specifies that in any evaluation of end use product using TMOF, additional information must be provided such as data on food clearances/tolerance, toxicity of end product on non-target organisms, efficacy of product, impact on endangered aquatic invertebrate species (Please refer to **Lampiran IIC** for the full report)**.**

(iv) Report by Universiti Putra Malaysia on the "Study of the chronic and acute exposure of Mousticide on tilapia" submitted by the applicant. The report also included studies on freshwater prawn (*Macrobrachium rosenbergii*) and freshwater crustacean (*Daphnia sp*.) (Please refer to **Lampiran IE** for the full report)**.**

GMAC took cognizance of the following as suggested within the AHTEG guidelines:

(i) That the risk assessment exercise should be specific to the details of this particular application

(ii) That the risk assessment exercise should be specific to the receiving environment in question, and

(iii) That any risk identified should be compared against that posed by the unmodified organism.

A Risk Matrix was prepared based on an assessment mechanism developed by Office of the Gene Technology Regulator, Australia (OGTR, 2005). In applying this matrix, GMAC identified potential hazards, and then added a value/rank for the likelihood of each hazard as well as its consequences. The likelihood of each hazard occurring was evaluated qualitatively on a scale of 1 to 4, with 1 for "highly unlikely", and 4 for "highly likely". The consequences of each hazard, if it were to occur, were then evaluated on a scale of 1 to 4, with 1 for "negligible" and 4 to denote

a "severe consequence". A value was finally assigned for the overall risk from the identified potential hazard. The general formula: Overall Risk = Likelihood x Consequence was employed. GMAC also proposed risk management strategies for some of the potential hazards. This methodology of assessment follows the procedure of Risk Assessment in Annex III of the Cartagena Protocol on Biosafety.

The Risk Assessment was conducted taking into consideration the previous assessment of the locally produced Mousticide RH and Mousticide WP. In this application, assessment was done for the products Mousticide RH and Mousticide WP that was formulated with TMOF_yeast that was imported from India. In addition, Mousticide WP that is imported from the Philippines as a final product was also assessed.The possible pathways to risk/hazard arising from release of these products were identified and listed. The potential hazards identified arose from two main areas:

(i) **Effects on the ecology of the receiving environment**

Issues pertaining to animal health, food chains and toxicity of the gene product/s to non vector mosquito species and other non-target organisms especially important agricultural pollinators.

(ii) **Effects on human health**

Issues pertaining to toxicity and allergenicity through exposure to the products

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Based on the above, GMAC identified a final list of seven potential hazards that were considered to be pertinent to the current application. (please refer to the **Lampiran IIB/Risk Matrix** for details).

GMAC also took extra caution and further discussed pre-emptive mitigation procedures and appropriate management strategies for these hazards where the Overall Risk are estimated to be above the minimal, and also for a few hazards that required further evaluation and data acquisition.

a) Possibility of toxic effects on the oil palm pollinating weevil

TMOF may have toxic effects on important agricultural pollinators, specifically the oil palm pollinating weevil (*Elaeidobius kamerunicus*). EPA reports on the negative effects of TMOF on cotton boll weevil, citrus weevil as well as on minor effects on other insect species. The weevils are of the same family as the oil palm pollinating weevil. Any detrimental impact on the oil palm pollinating weevil will have devastating effects on the oil palm industry. The consequence of this risk is unacceptable unless mitigation is highly effective and feasible, and this is a strong basis as to why GMAC insisted on a comprehensive toxicity study and report from the applicant.

Previously, the applicant submitted a toxicity report to GMAC of a study that was conducted by Malaysian Palm Oil Board (MPOB) demonstrating the low toxicity of the TMOF products to the oil palm weevil. However, after reviewing the report as well as sending it to an independent evaluator for comments, GMAC strongly recommended that more comprehensive experiments be conducted, consistent with the independent evaluator's recommendations. GMAC also lacked confidence in the scientific rigour of the study done, and were of the opinion that the study could be re-designed for better scientific rigour, a view shared by the independent evaluator.

The applicant has appointed Prof. Dr. Idris Abdul Ghani from Universiti Kebangsaan Malaysia (UKM) to conduct the study on the toxicity effect of TMOF on the oil palm pollinating weevil and honey bee. This proposal was reviewed by GMAC as well as two independent evaluators i.e. Dr. A. Sivapragasam from Centre for Agricultural Bioscience International - Southeast and East Asia Regional Centre (CABI) and Prof. Dato' Dr. Mohd. Sofian Azirun from Universiti Malaya (UM).

b) Synergistic effect of TMOF with other larvicides

TMOF may act synergistically with other larvicides when combined in the environment. TMOF has been demonstrated to act synergistically with *Bacillus thuringiensis israelensis* (Bti) which is able to enhance the effect of TMOF. This will result in undesirable widespread effects on other non target organisms due to possible increased target spectrum and toxicity. This risk requires mitigation actions that need to be demonstrated as effective. Users of the Mousticide product should exercise caution when using the product in combination with other larvicidal products. As the applicant claims that Mousticide does not contain any other biopesticides besides the active ingredients, as stated on the product label, the applicant is required to submit an official letter validating the claim before 16 March 2015.

c) Impact of TMOF to human health

There might be an impact on human health through the exposure to insect proteins via inhalation or skin contact during the spraying of wettable powder in an aquatic environments. This is likely if personal protective equipment (PPE) is not used during spraying and other necessary precautions are not taken. Exposure will cause allergic reactions in hypersensitive individuals. The EPA report includes dermal toxicity data which showed allergic reactions in rabbits, slight to moderate erythema, edema, focal eschar and desquamation. These reactions persisted in some animals for more than 7 days. This risk requires actions of mitigation that need to be demonstrated as effective. Product handling and safety instructions should be provided with the product. These instructions should include the need for PPE and proper usage procedure including notification; and follow-up medical monitoring of staff handling the product. The applicant conducted a survey comprising of individuals involved in the TMOF/Mousticide production process as well as the public who are using the Mousticide product (end users) to determine the effect on human health and is in the final stages of completion. The report on this survey will be submitted by the applicant to the NBB before 16 March 2015.

d) Effect of Mousticide on other non target organism

EPA describes significant effects (growth retardation and limited mortality) on some species of Lepidoptera, Hemiptera, Coleoptera and Diptera. The current application using imported TMOF_yeast does not provide any data on local species. Any such effect on local species will result in disruption of the food chain and the ecosystem.This risk requires actions of mitigation that need to be demonstrated as effective. GMAC recommends that the applicant conduct studies on the effects on local key indicator species in representative areas where Mousticide will be applied. GMAC takes note of the study conducted on tilapia, freshwater prawn (*Macrobrachium rosenbergii*) and freshwater crustacean (*Daphnia* sp.) using locally produced Mousticide as well as the pending studies on honey bee and oil palm pollinating weevil. For the honey bee and oil palm pollinating weevil, applicant is requested to test both locally produced and imported Mousticide.

IV - Proposed Terms and Conditions for Certificate of Approval

Based on the seven potential hazards identified and assessed, GMAC has drawn up the following terms and conditions for the certificate of approval for the release of this product:

1. Product handling and safety instructions should be provided with the product. These instructions should include:

   a) the use of PPE and follow up medical supervision of staff handling the product.

   b) caution be exercised when used in combination with other larvicidal products since the safety of the combined effects have not been established.

2. Labelling should include application dosage and frequency, instructions for disposal as well as hazard warning.
3. Imported TMOF_yeast should be accompanied with data sheet that should include information on safety and standardized potency range of the product.
4. Post monitoring survey of end user to determine the effect on human health and environment. The survey should clearly outline the safety objectives to the end user and a brief product description.
5. Continuous environmental safety studies on effects of Mousticide WP and Mousticide RH to the Malaysian fauna are recommended.
6. Mousticide WP and Mousticide RH should not be applied to waterways such as treated drinking water sources.
7. Should the approved person receive any credible and/or scientifically proven information that indicates any adverse effect of Mousticide WP or Mousticide RH either locally or in other countries, the National Biosafety Board authority shall be informed immediately.
8. If the production is to be done in a facility other than IBD, UTM, a notification of that chosen facility for contained use activity must be submitted to the NBB.

V - Other Regulatory Considerations

1. It is recommended that the efficacy of Mousticide WP or Mousticide RH be tested both as a combination of TMOF_yeast and Bti as well as TMOF_yeast and Bti individually.

2. It is recommended that the relevant agencies test the safety of using Mousticide WP or Mousticide RH in combination with other larvicidal products since the safety of the combined effects have not been established.

VII – Conclusion and Recommendation

GMAC has conducted a thorough evaluation of the application for the release activities of:

TMOF_yeast imported from India and Mousticide WP Imported from Philippine (which is formulated with TMOF_yeast produced in India) and has determined that the release of this product is safe to human, animal and plant health in quantities as stated in the final product. However, the long term effect on honey bee and oil palm pollinating weevil have not been established.

GMAC recommends that the applicant conduct studies on the effects on local key indicator species in representative areas where Mousticide WP and Mousticide RH will be applied. This applies to the Mousticide WP and Mousticide RH formulated with TMOF_yeast imported from India and produced in IBD, UTM or any other approved facility. If the final product with TMOF_yeast contains a different formulation from the Mousticide WP and Mousticide RH, a new application for approval must be submitted to NBB.

GMAC recommends that the proposed application for release be **APPROVED WITH TERMS AND CONDITIONS** as listed in section IV - Proposed Terms and Conditions for Certificate of Approval.

VIII - Bibliography

1) Ad Hoc Technical Expert Group (AHTEG). *Final Report of the Ad Hoc Technical Expert Group on Risk Assessment and Risk Management under the Cartagena Protocol on Biosafety*. UNEP/CBD/BS/AHTEG-RA&RM/2/5 document, 5 May 2010

2) Cornell *et al*. "High level methoprene resistence in the mosquito *Ochlerotatus nigromaculis* (Ludlow) in Central". *Pest Manag Sci* 58(2002):791-798

3) Dzolkhifli, O. "Toxicity of TMOF against the oil palm pollinator, *Elaeidobius kamerunicus* Faust." *Unpublished.* Jan 2011

4) Lau YS, Sulaiman, S and Othman, H. "The Effectiveness of Trypsin Modulating Oostatic Factor (TMOF) and Combination of TMOF with *Bacillus thuringiensis serovar israelensis* (Bti) against *Aedes aegypti* Larvae in the Laboratory." *Tehran University of Medical Sciences e Journals.* Jun 2011

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6) OGTR. 2005. Risk Analysis Framework.

7) Ramlah Ali *et al*. "Chronic Effect of TMOF-Bti (WP), TMOF-Bti (RH), MPOB Ecobac-1(EC), on Oil Palm Pollinators, *Elaeidobius kamerunicus."* http://www.researchgate.net/publication/263094521_Chronic_Effect_of_TMOFBti_(WP)_TMOF-Bti_(RH)_MPOB_Ecobac_1(EC)_On_Oil_Palm_Pollinators_Elaeidobius_kamerunicus (Accessed on 10 February 2015)

8) United States Environmental Protection Agency Office of Pesticide Programs. *Biopesticide Registration Action Document Trypsin Modulating Oostatic Factor (TMOF).* PC Code 105403.

9) United States Environmental Protection Agency Trypsin Modulating Oostatic Factor (105403) Fact Sheet (http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/factsheet_105403.htm)