

FACT SHEET

APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF TMOF_YEAST FOR SUPPLY OR OFFER TO SUPPLY

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

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 the Entogenex Industries Sdn. Bhd.

1. What is this application for?

To import the technical grade active ingredient (TGAI) of Trypsin Modulating Oostatic Factor (TMOF_ yeast) from India and final products containing TMOF_ yeast (genetically-modified *Pichia pastoris*) from Philippines for supply or offer to supply. The two final products that are being assessed are Mousticide RH and Mousticide WP. These products will be used in Government programmes to eradicate the dengue disease.

2. What is the purpose of the release?

The aim of the release is to control the *Aedes aegypti* larvae population. These products, in the form of killed dried yeast cells containing TMOF peptide, are mosquito larvicides.

3. How is this application different from the previous application JBK(S) 602-1/1/5 that was approved on 26 July 2011?

This application is to gain approval to import the final product of Mousticide WP from the Philippines. In addition, the TGAI of TMOF_ yeast will be imported from India and formulated into Mousticide RH at the Institute of Bioproduct Development in Universiti Teknologi Malaysia (IBD UTM). Assessment is being done again as the TMOF_ yeast and final product (Mousticide WP) are produced in another facility and there may be variables in the effectiveness of the protocols used as well as variations in the risk exposure pathways. The National Biosafety Board decision on the previous application is available at www.biosafety.nre.gov.my.

4. How has the TMOF_yeast strain been modified?

Pichia pastoris KM71H strain was modified with pPICZ B plasmid containing gene sequences for expression of TMOF peptide, from ovaries of female *Aedes aegypti* mosquito. The mode of action of TMOF is hormonal disruption of transcription and translation of trypsin, resulting in reduced digestion of mosquito diet ultimately leading to starvation of mosquito larvae. The lack of free amino acids liberated from the blood meal in adult females or larval gut causes inhibition of egg development to adult, anorexia and death of larval mosquitoes.

5. Information about the release of TMOF_yeast products?

The two products (Mousticide RH and Mousticide WP) will be distributed throughout Malaysia in Government programmes to eradicate the dengue disease. They will be used as larvicides to treat possible mosquito breeding habitats (water bodies) to kill mosquito larvae as a control measure to reduce population of *Aedes* mosquitoes.

6. Characteristics of the TMOF_Yeast

(a) Details of the parent organism

Pichia pastoris yeast is commonly used for the expression of biological pharmaceutical proteins with no adverse health effects. It is a non-pathogenic microorganism with decades of safe utilization to establish utility and safety. Unmodified *Pichia pastoris* yeast has been approved by the United States Department of Agriculture (USDA) as a food additive in the livestock industry.

(b) Trypsin Modulating Oostatic Factor (TMOF)

TMOF is used against mosquito larvae in aquatic environments. TMOF is a small protein that interferes with digestion in mosquito larvae, leading to their starvation.

(c) Description of the Active Ingredient

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 extremely high temperatures. Killed yeast cells are then formulated to produce Mousticide WP and Mousticide RH. These products are then applied to bodies of water to control mosquito larvae. Once eaten by mosquito larvae, TMOF interferes with the production of trypsin,

a critical enzyme needed by the larval digestive system. Exposed larvae are unable to digest food and therefore starve to death.

(d) Use Sites, Target Pests, and Application Methods

Use Sites: Habitats for mosquito larvae such as ponds, streams, ditches, puddles, and other sources of standing water.

Target Pests: Mosquito larvae.

Application Methods: Mousticide WP and Mousticide RH is applied directly to water or other places mosquito larvae may be found.

(e) Assessment of Risks to Human Health

No harmful health effects to humans are expected from the use of TMOF as an active ingredient in pesticides. No evidence of toxicity or infectivity was found in animal laboratory studies, largely because digestive systems in humans and other mammals contain many enzymes besides trypsin. Also, the TMOF protein is broken down quickly in the human gut and doesn't have the opportunity to inhibit trypsin synthesis (please refer to the United States Environmental Protection Agency [EPA] TMOF Biopesticides Registration Action Document as link below:

[http://www.epa.gov/opp00001/chem_search/reg_actions/registration/decision_PC-105403_26-Jan-05.pdf])

(f) Assessment of Risks to the Environment

No adverse environmental effects are expected when products containing TMOF are used according to label instructions. Laboratory studies and a literature search found no evidence indicating that TMOF is harmful to birds, mammals, plants, marine species, or insects other than mosquitoes (please refer to the United States Environmental Protection Agency [EPA] TMOF Biopesticides Registration Action Document

[http://www.epa.gov/opp00001/chem_search/reg_actions/registration/decision_PC-105403_26-Jan-05.pdf])

Studies were conducted by the Malaysian Palm Oil Board (MPOB) researchers to determine the acute effect and chronic effect of Mousticide WP and Mousticide RH against oil palm pollinators, *Elaeidobius kamerunicus*; and they found that these products did not harm or kill the pollinators.

Acute toxicity: [<http://goo.gl/uwt3y7>]

Chronic toxicity: [<http://goo.gl/CmCTlw>]

(The report of this study is available upon request).

In addition, studies have been conducted by University Putra Malaysia (UPM) to assess the acute toxicity of Mousticide WP and Mousticide RH against red hybrid tilapia fish (*Oreochromis sp.*), freshwater prawn (*Macrobrachium rosenbergii*), and *Daphnia sp.* The results showed that Mousticide WP and Mousticide RH have no acute toxicity against tilapia fish and *Daphnia sp.* Meanwhile, Mousticide WP and Mousticide RH showed a very low toxicity against freshwater prawn. (The report of this study is available upon request).

(g) Assessment by the Genetic Modification Advisory Committee (GMAC) for the previous application (JBK(S) 602-1/1/5)

The GMAC has previously done an assessment for Mousticide WP and Mousticide RH that were produced at IBD UTM and found that a limited amount does not endanger biological diversity or human, animal and plant health. The full report may be accessible at www.biosafety.nre.gov.my. The National Biosafety Board has imposed a post-release environmental monitoring on the use of this product as well as a requirement to conduct additional studies to negate long term cumulative effects.

(h) Existing country registration for MOUSTICIDE

MOUSTICIDE has been registered in Malaysia under the National Pesticide Board, Agricultural Department. In other countries, MOUSTICIDE has been registered in Singapore, Ghana, the Philippines and Myanmar. In Singapore, MOUSTICIDE has been used since April 2014 as a measure to control dengue in various locations with high diversity of flora and fauna. Among them are:

- Singapore Zoological Gardens
- Night Safari
- River Safari
- Jurong Bird Park
- Gardens by the Bay
- Resorts World Sentosa

Thus far, no adverse effects to the environment or human health has been reported. Additionally, the active ingredients of MOUSTICIDE are also approved by the US Environmental Protection Agency.

7. What controls are proposed for this release?

Through their risk management, the applicant declared that the products have an extremely high safety level. The products should be stored under dry conditions. No special handling instructions are necessary other than to avoid breathing dust particles (as is recommended for any product capable of producing fine airborne particles and is not dependent on any safety issues associated with the modified TMOF yeast.)

Waste disposal (containers used for the products) can be disposed of as normal waste. Accidental spills can be gathered and discarded as normal waste. Surfaces can be washed with water and disposed for complete removal.

8. What is the Emergency Response Plan?

In the event of any adverse occurrences, the products can be gathered and discarded using normal waste handling techniques as proposed by applicant, no special precautions need to be taken.

9. How can I comment on this application?

Any member of the public may submit their comments 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 **11th February 2015** and written submissions are required by that date. Submissions must be addressed to: Director General, Department of Biosafety, Ministry of Natural Resources and Environment, Level 1, Podium 2, Wisma Sumber Asli, No. 25, Persiaran Perdana, Precinct 4, 62574 Putrajaya, MALAYSIA. E-mail: biosafety@nre.gov.my. Fax: 03-88904935.

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