RISK ASSESSMENT REPORT

OF THE GENETIC MODIFICATION ADVISORY COMMITTEE (GMAC)

FOR

AN APPLICATION FOR APPROVAL TO RELEASE SINGLE CELL PROTEIN (SCP), LIQUID FERTILIZER AND SOLID FERTILIZER, FROM THE FERMENTATION PRODUCTION OF L-METHIONINE USING *E. coli* KCCM11252P AND *E. coli* KCCM11340P FOR SUPPLY OR OFFER TO SUPPLY FOR SALE/PLACING IN THE MARKET

NBB REF NO: JBK(S) 602-1/1/15

APPLICANT: CJ BIO MALAYSIA SDN. BHD.

DATE: 20 MARCH 2014

I - Summary of Assessment Process

The Genetic Modification Advisory Committee (GMAC, please refer to Appendix 1 for details of GMAC), under the purview of the National Biosafety Board (NBB) was given the dossier by the Department of Biosafety on 27TH December 2013 for an application for approval for release importation for release [sale/placing on the market] of a product of a Living Modified Organism (Single Cell Protein (SCP), Liquid Fertilizer and Solid Fertilizers The application was filed by CJ Bio Malaysia Sdn. Bhd. (hereafter referred to as "the applicant"). GMAC members also took the opportunity to obtain further clarification on certain details of the activity.

A public consultation for this application was conducted from 16 January 2014 to 14 February 2014 via advertisement in local newspapers. There were no comment received.

GMAC had two meetings pertaining to this application and prepared the Risk Assessment Report and Risk Assessment Matrix along with its recommended decision, for consideration by the National Biosafety Board.

II - Background of Application

This application is for approval to release Single Cell Protein (SCP), Liquid Fertilizer and Solid Fertilizers, from the fermentation production of L-Methionine using *E. coli* KCCM11252P and *E. coli* KCCM11340P from *E. coli* K12.

The applicant has established the fermentative production of L-Methionine by using several microorganisms. Origins of these microorganisms are *E. coli* K12 W3110. Some of them have been made by random mutation which is non-GMO and two of them (*E. coli* KCCM 11252P, *E. coli* KCCM 11340P) have been made by genetic modification.

Information about genetically modified *E. coli* KCCM 11252P, *E. coli* KCCM 11340P

The two microorganisms (*E. coli* KCCM 11252P, *E. coli* KCCM 11340P) have been made by genetic modification from *E. coli* K12 W3110 strains in CJ Research Institute of Biotechnology located in Seoul, South Korea. *E. coli* K12 is one of the most well recognised and safe microorganism which has been approved for the production of pharmaceuticals and feed additives in the world. Each of the two microorganisms has one foreign gene from microorganisms which have already been approved as safe. There is no antibiotic resistant marker gene, and no transferable mobile element in the final microorganism and the final microorganisms express no genetic instabilities. These microorganisms are separated, dried and killed to become SCP. Hence there are no remaining viable cells in the SCP. There are also no viable cells in the Liquid Fertilizer and Solid Fertilizer because the microorganisms are separated from the liquid.

These microorganisms also are used for the fermentative production of L-methionine, a feed additive. After the fermentation, the microorganisms are separated and dried. The resultant SCP has a large amount of nitrogen and can be used as a good organic nitrogen source in

the fertilizer market. The liquid is treated further to obtain L-Methionine and Ammonium Sulfate. The final liquid is to be sold as fertilizer (Liquid Fertilizer) as well as dried and sold as Solid Fertilizer.

(a) Details of the parent organism

The parent organism is the wild type of *Escherichia coli* K12 W3110. There is no known precedent for the crossing events of this strain with other species. This strain cannot survive in the environment or in animal. Hence, *E. coli* K12 has a long history of safe use. Its derivatives are currently used in a large number of industrial applications, including the production of specialty chemicals (e.g. L-aspartic, inosinic and adenylic acids), amino acids (L-threonine, L-tryptophane, L-valine, and L-isoleucine) and human drugs such as insulin and somatostatin. Futhermore, *E. coli* K12 is also used for the production of some chemicals such as enzymes. In general, *E. coli* K12 is one of the most extensively studied bacteria, and has been used in genetic studies in laboratories worldwide.

(b) Details of the donor organism

KCCM11252P from *E. coli* K12 W3110 is used in the production of O-acetylhomoserine, the precursor of L-methionine. In this strain, one gene from *E. coli* H155 has been introduced, which is the sucrose utilizing gene. This gene has already been approved as being safe in other amino acid production, such as valine and isoleucine production in Europe.

KCCM 11340P from *E. coli* K12 W3110 is used for the production of conversion enzyme which is used for the conversion of O-acetylhomoserine to L-methionine. The conversion enzyme named O-acetylhomoserine sulfhydrylase is coded by a gene from *Corynebacterium glutamicum*. *Corynebacterium glutamicum* is a Generally Regarded As Safe (GRAS) strain widely used for MSG production and other amino acid production.

III - Risk Assessment and Risk Management Plan

GMAC evaluated the application with reference to the following documents:

(i) CODEX Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants.

(ii) 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).

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

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

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

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

(iii) That any risk identified 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, 2009). 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 'marginal' and 4 to denote a 'major 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 potential hazards, where appropriate. This methodology of assessment follows the procedure of Risk Assessment in Annex III of the Cartagena Protocol on Biosafety.

Although the applicant has applied for an approval to import for the purpose of feed and processing only, GMAC had conducted a thorough assessment and widened the scope of the risk assessment to include the purpose of food as well.

The Risk Assessment was conducted during a meeting and through email consultations among GMAC members. To start with, the possible pathways to risk/hazard arising from release of the products were identified and listed. The potential hazards were identified in three main areas:

(i) **Effects on human health**

Issues pertaining to toxicity, potential allergenicity, and anti-nutritional properties.

(ii) **Effects on animal health**

Issues pertaining to allergenicity, toxicity, anti-nutritional properties, compromised nutritional content, effect on performance, survivability, and horizontal gene transfer.

(iii) **Effects on the environment**

Potential of transgene being transferred to bacterial flora in the gut of animals which eats the SCP, potential of transgene being transferred to soil bacteria in the environment due to decay of GM SCP and other natural conditions, toxic effect on non-target organisms, and transfer of heterologous genes to other related species

Based on the above, a final list of 13 potential hazards was identified. All of these hazards were rated as having an Overall Risk of 1 or "negligible".

IV - Proposed Terms and Conditions for Certificate of Approval

Based on the 13 potential hazards identified and assessed, GMAC has drawn up the following terms and conditions to be included in the certificate of approval for the release of this product:

a) There shall be clear labeling of the products from production to all levels of marketing stating that it is only for the purpose of feed or fertilizer.

b) Should the applicant receive any scientifically proven information that confirms any adverse effects of the produced L-Methionine, Liquid Fertilizer and Solid Fertilizer, the NBB shall be informed immediately.

V - Other Regulatory Considerations

No other regulatory considerations are necessary to the best of our knowledge.

VI - Identification of issues to be addressed for long term use release of this product

Continuous monitoring is required from the applicant to report any unanticipated adverse effect caused by the L-Methionine, Liquid Fertilizer and Solid Fertilizer.

VII – Conclusion and Recommendation

GMAC has conducted a thorough evaluation of the application for approval for release [sale/placing on the market - for use as feed and fertilizer] of a product of a Living Modified Organism (L-Methionine, Liquid Fertilizer and Solid Fertilizer) and has determined that the release of this product does not endanger biological diversity or human, animal and plant health. 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, subject to approval by other relevant agencies.

VIII – Bibliography

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Appendix I

GENETIC MODIFICATION ADVISORY COMMITTEE (GMAC) MEMBERS INVOLVED IN SPECIFIC RISK ASSESSMENT AREAS FOR THE APPROVAL FOR RELEASE OF PRODUCTS OF TC1507 CORN FOR SUPPLY OR OFFER TO SUPPLY

Genetic Modification Advisory Committee (GMAC) members divided the task of looking up more information for the Risk Assessment matrix based on three broad categories. The scope of research aspects for each group is as listed below. Each sub-committee had a nominated leader to coordinate the work and report back to the main GMAC. The respective leader contacted the sub-committee members and discussed the work process with their members. The groupings of GMAC sub-committee members and their assigned tasks are as below:

1. **ENVIRONMENT**

   Effect on ecology of receiving environment due to unintentional release and planting (e.g. weediness, gene transfer to bacteria, accumulation of the PAT protein in the environment, cross pollination and toxic effects on non-target organisms)

   - **Assoc. Prof. Dr. Mohd. Faiz Foong bin Abdullah (Universiti Teknologi MARA) (Leader)**
   - Dr. Sim Soon Liang (Sarawak Biodiversity Centre)
   - Dr. Martin Abraham (Malaysian Society of Marine Sciences)
   - Madam Atikah binti Abdul Kadir Jailani (Department of Agriculture)
   - Dr. Tan Swee Lian (Academy of Science Malaysia)-plant breeding
   - Dr. Mohamed Mohd Salleh (previously Malaysian Agricultural Research & Development Insitute)

2. **HUMAN HEALTH**

   Effect on human health (e.g. acute toxicity of the novel protein, potential allergenicity, mutagenic/tetragenic/carcinogenic effects, reproductive toxicity, potential transfer of antibiotic resistance genes in the digestive tract, the pathogenic potential of donor microorganisms and nutritional equivalence)

   - **Madam T.S. Saraswathy (Institute of Medical Research)(Leader)**
   - Madam Hasimah Hafiz Ahmad (Malaysian Agricultural Research & Development Insitute)
   - Dr. Norwati Muhammad (Forest Research Insitute Malaysia)
   - Dr. Norliza Tendot Abu Bakar (Malaysian Agricultural Research & Development Insitute)
   - Dr. Rahizan Issa (Institute of Medical Research)

- Mr. Jamal Khair b Hashim (Ministry of Health)
- Dr. Adiratna Mat Ripen (Institute of Medical Research)

3. <u>ANIMAL HEALTH</u>

Effect on animal health (e.g. allergenicity, toxicity, anti-nutritional properties, compromised nutritional content, metabolic breakdown of products, survivability, horizontal gene transfer and animal product contamination)

- **Prof. Dr Jothi Malar Panandam (Universiti Putra Malaysia) (Leader)**
- Dr. Ahmad Parveez bin Hj Ghulam Kadir (Malaysian Palm Oil Board)
- Prof. Dr. Helen Nair (Academy of Science Malaysia)
- Dr. Kodi Isparan Kandasamy (Malaysian Biotechnology Corporation Sdn Bhd)