RISK ASSESSMENT REPORT

OF THE GENETIC MODIFICATION ADVISORY COMMITTEE (GMAC)

FOR

AN APPLICATION FOR APPROVAL FOR RELEASE OF PRODUCTS OF KK179 ALFALFA FOR SUPPLY OR OFFER TO SUPPLY

NBB REF NO: JBK(S) 600-2/1/18

APPLICANT: MONSANTO MALAYSIA SDN. BHD.

DATE: 12 JANUARY 2022

I - Summary of Assessment Process

On 22 September 2021, the Genetic Modification Advisory Committee (GMAC), received from the Department of Biosafety assessment an application for the approval for importation for release [sale/placing on the market for direct use as food, feed and for processing (FFP)] of a product of a Living Modified Organism Reduced Lignin Alfalfa KK179. The application was filed by Monsanto Malaysia Sdn. Bhd. (hereafter referred to as "the applicant"). After an initial review, GMAC requested for additional information from the applicant.

A public consultation for this application was conducted from 24 February 2021 to 25 March 2021 via advertisements in the local newspapers. Comments were received from Consumers Association of Penang (CAP) and Malaysian Agroecology Society (SRI-Mas). GMAC took into consideration the comments that were relevant to the risk regarding genetic contamination of the environment, and impact on human health such as reduced lignin content, as well as animal toxicity studies. GMAC also took into consideration the comment on the risk and uncertainty associated of the genetic modification technology which may pose unintended consequences and need for proper assessment of KK179 alfalfa.

GMAC had four (4) 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 import and release products of a Living Modified Organism reduced lignin alfalfa KK179. The aim of the import and release is to supply or offer to supply for sale/placing on the market for direct use as food, feed and for processing (FFP). According to the applicant, KK179 alfalfa has been registered in a number of countries for cultivation as well as for food, feed and for processing. KK179 alfalfa is approved in a few countries including the United States of America, Australia, New Zealand, Canada, Japan, Korea, Mexico, Philippines, Singapore and Taiwan may be imported, stored and processed for use in food, animal feed and industrial products in the same way as other conventional, non-transgenic alfalfa. The type of expected use of the products derived from KK179 alfalfa in Malaysia will be the same as the expected usage for products derived from conventional alfalfa.

Information about KK179 alfalfa

The recipient or parental plant is alfalfa (*Medicago sativa* L.). Alfalfa is among the most important forage crops in the United States and ranks as the fourth most widely grown crop by acreage, after corn, soybean, and wheat. In certain regions, alfalfa is cultivated as a mixture with perennial grasses where it may be harvested as forage or used for grazing livestock. As a legume, it is also

desired for rotational use to improve soil characteristics such as nitrogen content (Undersander *et al.,* 2011).

Many alfalfa plants exhibit various forms of genetic self-incompatibility or self-sterility and will not successfully self-pollinate (Viands *et al.,* 1988). Alfalfa is adversely affected by inbreeding, i.e., self-fertilized plants commonly demonstrate a dramatic reduction in forage and seed yield potential (Rumbaugh *et al.,* 1988). Inbreeding depression may be due to the loss of heterosis and/or accumulation and unmasking of deleterious recessive alleles that occur as a result of self-pollination and/or pollination among close relatives. Flowers do not shed pollen to the wind. After pollination, alfalfa seed requires four to six weeks of adequate growing conditions to ripen. Rainfall during the ripening period will cause poor seed quality and decrease seed yield (Canada Biology Document, 2005).

Alfalfa has a history of minor use as human food, dietary supplements and herbal remedies (OECD 2005). Vast majority of alfalfa is grown and harvested for animal feed (Higginbotham *et al.,* 2008). In Malaysia, alfalfa is used as feed in the form of pellet, dry blocks or bales of hay. For example, pellets are used as feed for rabbits and guinea pigs.

KK179 was produced by insertion of *caffeoyl CoA 3-O-methyltransferase* (CCOMT) gene segments, derived from alfalfa, assembled to form an inverted repeat DNA sequence. The inverted repeat DNA sequence produces double stranded RNA (dsRNA) that suppresses endogenous CCOMT gene expression via the RNA interference (RNAi) pathway. Suppression of the CCOMT gene expression leads to lower CCOMT protein expression resulting in reduced production of guaiacyl lignin subunits (G lignin) compared to conventional alfalfa at the same stage of growth. The reduction in G lignin subunit production leads to reduced accumulation of total lignin.

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 also referred to the following recommendations 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.

In conducting the risk assessment, 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.

The potential hazards were identified in three main areas:

(i) **Effects on human health**

Relevant scientific publications on the genetic modifications were reviewed for potential human health risks and issues pertaining to acute toxicity of novel protein / altering / interference of metabolic pathways, potential allergenicity of the novel protein, reproductive toxicity, potential transfer of antibiotic resistance genes in digestive tract, pathogenic potential of donor microorganisms, nutritional equivalence and anti-nutritional content.

(ii) **Effects on animal health**

Issues pertaining to allergenicity, toxicity, anti-nutritional content, survivability and animal product contamination.

(iii) **Effects on the environment**

Issues pertaining to accidental release of seeds, unintentional release and planting, potential of transgenes being transferred to bacteria (soil bacteria, bacterial flora of animal gut), increased fitness, weediness and invasiveness, accumulation of the protein in the environment via feces from animals fed with the GM plant/grain and cross pollination leading to transfer of transgenes were examined.

Based on the above, a final list of 20 potential hazards was identified. Most of these hazards were rated as having an Overall Risk of 1 or "negligible".

GMAC also took caution and discussed a few of the hazards that required further evaluation and data acquisition. Some of these risks are expected to be managed effectively with the risk management strategies proposed (please refer to section IV of this document).

Some of the potential hazards are highlighted below along with the appropriate management strategies:

a) Accidental release of viable seeds

Seeds may be accidentally released during transportation. In the conducive warm and humid climate of Malaysia, there is a high likelihood of these volunteers maturing to the flowering and seed-setting stages. However rainfall causes poor seed quality and decrease, seed yield (Canada Biology Document, 2005). Therefore, the likelihood of a feral population is quite low.

b) Planting of seeds

Plants may be grown by uninformed farmers and perpetuated through small scale cultivations. This GM alfalfa may pollinate the non-GM alfalfa. There should also be clear labeling of the product to state that it is only for the purpose of food, feed and processing, and is not to be used as planting material.

c) Compromised nutritional content

Compositional analyses of the forage and grain samples showed no significant difference in nutritional composition between KK179 alfalfa and conventional alfalfa.

However, applicant is required to update the National Biosafety Board immediately if additional information indicate potential adverse effects from the KK179 alfalfa.

IV - Proposed Terms and Conditions for Certificate of Approval

Based on the 20 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 documentation by the exporter describing the product which shall be declared to the Royal Malaysian Customs.

b) There shall be clear labeling of the product from importation to all levels of marketing stating that it is only for the purpose of food, feed and processing, and is not to be used as planting material.

c) Should the approved person receive any information that indicates any adverse effect of KK179 alfalfa, the National Biosafety Board shall be informed immediately.

d) Any spillage (during loading/unloading/transportation) shall be collected and cleaned up immediately.

e) Transportation of the consignment from the port of entry to any destination within the country shall be in secured and closed condition.

V - Other Regulatory Considerations

a) Administrative regulatory procedures shall be arranged between the Department of Biosafety, Royal Malaysian Customs Department and relevant agencies to ensure accurate declaration of product information and clear labeling of the product is implemented.

b) Administrative regulatory procedures shall be arranged between the Department of Biosafety and the Malaysian Quarantine and Inspection Services (MAQIS) to impose post entry requirements for accidental spillage involving the GM product.

c) Administrative regulatory procedures shall be arranged between the Department of Biosafety and the Malaysian Quarantine and Inspection Services (MAQIS) and other competent agencies to impose post entry requirements for food safety compliance.

d) Administrative regulatory arrangements shall be carried out between the Department of Biosafety and the Department of Veterinary Services (DVS) so that any unanticipated adverse effects in animals caused by any consumption of the GM products shall be reported immediately.

e) Administrative regulatory arrangements shall be carried out by Food Safety and Quality of Ministry of Health to monitor compliance to the Food Regulations 1985 for labelling of GM food.

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

a) Continuous monitoring is required from the approved person and any unanticipated adverse effect caused by the KK179 alfalfa shall be reported to the National Biosafety Board.

VII –Conclusion and Recommendation

GMAC has conducted a thorough evaluation of the application for approval for importation for release [sale/placing on the market for direct use as food, feed and for processing (FFP)] of a product of a Living Modified Organism Reduced Lignin KK179 alfalfa 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.

VIII – Bibliography

1. Akin, D. E.; Rigsby, L. L., Theodorou, M. K.; Hartley, R. D., 1988. Population changes of fibrolytic rumen bacteria in the presence of phenolic acids and plant extracts. Anim. Feed Sci. Technol., 19 (3): 261–275
2. Burnside J, Ouyang M, Anderson A, Bernberg E, Lu C, Meyers BC, Green PJ, Markis M, Isaacs G, Huang E, Morgan RW. Deep sequencing of chicken microRNAs. BMC Genomics. 2008 Apr 22;9:185. doi: 10.1186/1471-2164-9-185. PMID: 18430245; PMCID: PMC2375912.
3. Chen F, Srinivasa Reddy MS, Temple S, Jackson L, Shadle G, Dixon RA. Multi-site genetic modulation of monolignol biosynthesis suggests new routes for formation of syringyl lignin and wall-bound ferulic acid in alfalfa (Medicago sativa L.). Plant J. 2006 Oct;48(1):113-24. doi: 10.1111/j.1365-313X.2006.02857.x. PMID: 16972868.
4. Derek M. Dykxhoorn, Carl D. Novina, and Philip A. Sharp. Killing The Messenger: Short RNAS that silence gene expression. Nature Reviews: Molecular Cell Biology: Vol 4: 457-467.
5. EFSA. 2009. Scientific opinion: Opinion on the safety of 'Alfalfa protein concentrate' as food. EFSA Journal 997:1-19.
6. FAO-WHO. 2001. Evaluation of allergenicity of genetically modified foods. Report of a joint FAO/WHO expert consultation on allergenicity of foods derived from biotechnology. Food and Agriculture Organization of the United Nations, Rome, Italy.
7. FSANZ. 2013. Response to Heinemann et al on the regulation of GM crops and foods developed using gene silencing.
8. Higginbotham, G.E., C.L. Stull, N.G. Peterson, A.V. Rodiek, B.A. Reed and J.N. Guerrero. 2008. Alfalfa utilization by livestock. Pages 265-279 in Irrigated Alfalfa Management for Mediterranean and Desert Zones. C.G. Summers and D.H. Putnam (eds.). University of California Agriculture and Natural Resources, Oakland, California.
9. Ivashuta, S.I., J.S. Petrick, S.E. Heisel, Y. Zhang, L. Guo, T.L. Reynolds, J.F. Rice, E. Allen and J.K. Roberts. 2009. Endogenous small RNAs in grain: Semi-quantification and sequence homology to human and animal genes. Food and Chemical Toxicology 47:353-360.
10. Jensen, P.D., Y. Zhang, B.E. Wiggins, J.S. Petrick, J. Zhu, R.A. Kerstetter, G.R. Heck and S.I. Ivashuta. 2013. Computational sequence analysis of predicted long dsRNA transcriptomes of major crops reveals sequence complementarity with human genes. GM Crops and Food 4:90-97.
11. Jonas DA, Elmadfa I, Engel KH, Heller KJ, Kozianowski G, König A, Müller D, Narbonne JF, Wackernagel W, Kleiner J. Safety considerations of DNA in food. Ann Nutr Metab. 2001;45(6):235-54. doi: 10.1159/000046734. PMID: 11786646.

12. Keese P. Risks from GMOs due to horizontal gene transfer. Environ Biosafety Res. 2008 Jul-Sep;7(3):123-49. doi: 10.1051/ebr:2008014.
13. OECD. 2005. Consensus document on compositional considerations for new varieties of alfalfa and other temperate forage legumes: Key feed nutrients, anti-nutrients and secondary plant metabolites. ENV/JM/MONO(2005)13. Organisation for Economic Co-operation and Development, Paris, France.
14. Parrott W, Chassy B, Ligon J, Meyer L, Petrick J, Zhou J, Herman R, Delaney B, Levine M. Application of food and feed safety assessment principles to evaluate transgenic approaches to gene modulation in crops. Food Chem Toxicol. 2010 Jul;48(7):1773-90. doi: 10.1016/j.fct.2010.04.017. Epub 2010 Apr 23. PMID: 20399824.
15. Petrick, J.S., B. Brower-Toland, A.L. Jackson and L.D. Kier. 2013. Safety assessment of food and feed from biotechnology-derived crops employing RNA-mediated gene regulation to achieve desired traits: A scientific review. Regulatory Toxicology and Pharmacology 66:167-176.
16. Reddy AM, Zheng Y, Jagadeeswaran G, Macmil SL, Graham WB, Roe BA, Desilva U, Zhang W, Sunkar R. Cloning, characterization and expression analysis of porcine microRNAs. BMC Genomics. 2009 Feb 5;10:65. doi: 10.1186/1471-2164-10-65. PMID: 19196471; PMCID: PMC2644714.
17. Rodrigues Thais B. and Petrick Jay S. 2020. Safety Considerations for Humans and Other Vertebrates Regarding Agricultural Uses of Externally Applied RNA Molecules. Frontiers in Plant Science. Vol 11. Article 407.
18. Rumbaugh, M. D., Caddel, J. L., and Rowe, D. E. 1988. Breeding and quantitative genetics. In: Alfalfa and Alfalfa Improvement. pp. 777–808. Hanson, A.A., Barnes, D. K. and Hill, Jr. R. R., Eds. American Society of Agronomy,Inc. Publishers, Madison, Wisconsin, USA.
19. Sara E. Heisel, Yuanji Zhang, Edwards Allen, Liang Guo, Tracey L. Reynolds, Xiao Yang, David Kovalic, James K. Roberts. Characterization of unique small RNA populations from rice grain. PLoS One. 2008; 3(8): e2871. Published online 2008 Aug 6. doi: 10.1371/journal.pone.0002871
20. Siomi H, Siomi MC. On the road to reading the RNA-interference code. Nature. 2009 Jan 22;457(7228):396-404. doi: 10.1038/nature07754. PMID: 19158785.
21. Jonathan W. Snow, Andrew E. Hale, Stephanie K. Isaacs, Aaron L. Baggish, and Stephen Y.Chan. 2013. Ineffective delivery of diet-derived microRNAs to recipient animal organisms. RNA Biology 10:7, 1107–1116.
22. Undersander, D., D. Cosgrove, E. Cullen, C. Grau, M. Rice, M. Renz et al. 2011. Alfalfa management guide. https://www.agronomy.org/files/publications/alfalfa-management-guide.pdf
23. U.S. FDA. 1992. Statement of policy: Foods derived from new plant varieties. Federal Register 57:22984-23005.
24. U.S. FDA. 2001. Premarket notice concerning bioengineered foods. Federal Register 66:4706-4738.
25. USDA-APHIS. 2010. Glyphosate-tolerant alfalfa events J101 and J163: Request for nonregulated status. Final environmental impact statement – December 2010. U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Washington, D.C.

26. Viands, D. R., Sun, P., and Barnes, D. K. 1988. Pollination control: Mechanical and sterility. In: Alfalfa and Alfalfa Improvement. pp. 931–960. Hanson, A.A., Barnes, D. K. and Hill, Jr. R. R., Eds. American Society of Agronomy, Inc. Publishers, Madison, Wisconsin, USA.
27. Zhou X, Sunkar R, Jin H, Zhu JK, Zhang W. Genome-wide identification and analysis of small RNAs originated from natural antisense transcripts in Oryza sativa. Genome Res. 2009 Jan;19(1):70-8. doi: 10.1101/gr.084806.108. Epub 2008 Oct 29. PMID: 18971307; PMCID: PMC2612963.

Appendix 1

GENETIC MODIFICATION ADVISORY COMMITTEE (GMAC) MEMBERS INVOLVED IN SPECIFIC RISK ASSESSMENT AREAS FOR THE APPROVAL FOR RELEASE OF PRODUCTS OF KK179 ALFALFA 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 which were environment, human health and animal health. Each sub-committee had a nominated leader to coordinate the work and report back to the main GMAC. The GMAC members involved in the risk assessment are as below:

- **Prof. Dr. Mohd. Faiz Foong bin Abdullah (Universiti Teknologi MARA) (GMAC Chairman)**
- **Dr. Kodi Isparan Kandasamy (Industry Representative) (Environment sub-committee Leader)**
- **Madam T.S. Saraswathy (Institute of Medical Research - retired) (Human Health sub-committee Leader)**
- **Prof. Dr Jothi Malar Panandam (Universiti Putra Malaysia - retired) (Animal Health sub-committee Leader)**
- **Dr. Rahizan Issa (Institute of Medical Research - retired) (Notification Assessment sub-committee Leader)**
- Dato' Dr. Sim Soon Liang (Academy of Sciences Malaysia)
- Prof. Dr. Abd Rahman Milan (Universiti Malaysia Sabah - retired)
- Prof. Dr. Chan Kok Gan (Universiti Malaya)
- Assoc. Prof. Dr. Choong Chee Yen (Universiti Kebangsaan Malaysia)
- Dr. Adiratna Mat Ripen (Institute of Medical Research)
- Dr. Norliza Tendot Abu Bakar (Malaysian Agricultural Research & Development Institute)
- Dr. Norwati Muhammad (Forest Research Institute of Malaysia)
- Dr. Saifullizam bin Abdul Kadir (Department of Veterinary Services)
- Dr. Teo Tze Min (Entomological Society of Malaysia)
- Dr. Mohd Hefni Rusli (Malaysian Palm Oil Board)
- Madam Shafini Abu Bakar (Ministry of Health)
- Madam Sabariah Kamis (Department of Agriculture)
- Mr. Harun bin Ahmad (Department of Chemistry Malaysia)