Executive Summary

BASF Australia Ltd on behalf of BASF Agricultural Solutions Seed US LLC seeks to vary FSANZ Standard 1.5.2 to allow the use of genetically modified soybean (*Glycine max* L. Merr.) derived from transformation event GMB151 in the Australian and New Zealand food industries. Five food products are derived from soybean: whole soybeans, oil, meal, hulls and protein. Soybean oil is the primary food product consumed by humans in Australia, with the other products used either as food products or as components of animal feed.

Soybean event GMB151 contains the stably integrated *cry14Ab-1.b* and *hppdPf-4Pa* gene cassettes. The incorporation and expression of the GMB151 transgenic locus in the *G. max* genome has been characterised according to international standards for the safety assessment of biotechnology products. This information is included with this application to support the food safety of the Cry14Ab-1 and HPPD-4 proteins.

The GMB151 nematode resistant and herbicide tolerant GM soybean (*G. max*) will be commercialized in the major soybean producing countries of the world. GMB151 soybean produces the Cry14Ab-1 protein, a crystal protein derived from *Bacillus thuringiensis*, which confers resistance to soybean cyst nematode and *Pratylenchus*. GMB151 also produces a modified 4-hydroxyphenylpyruvate dioxygenase (HPPD-4), derived from *Pseudomonas fluorescens*, which confers tolerance to HPPD inhibitor herbicides such as isoxaflutole.

Planting nematode resistant/herbicide tolerant soybean GMB151 varieties provides growers with new options for nematode control. Plant-parasitic nematodes are widely prevalent in soybean agricultural production systems. The HPPD inhibitor herbicides to which GMB151 soybean is tolerant will also provide growers with an alternative class of herbicidal chemistry with which to control weeds in the crop.

GMB151 soybean was developed through *Agrobacterium*-mediated transformation using the vector pSZ8832 containing the *cry14Ab-1.b* and *hppdPf-4Pa* expression cassettes. The OECD identifier is BCS-GM151-6.

(i) The *cry14Ab-1.b* gene encodes for the Cry14Ab-1 protein. The *cry14Ab-1.b* coding sequence was derived from *Bacillus thuringensis*. The Cry14Ab-1 protein is a member of Cry (crystal)-type protein family with fully conserved three-domain structure. Cry proteins are produced by *Bacillus thuringiensis* strains and demonstrate specific toxicity towards insects or nematodes. FSANZ has previously assessed an extensive range of Cry proteins for food safety. This is the first time that the Cry14Ab-1 protein has been assessed for food safety in Australia.

(ii) The *hppdPf-4Pa* gene encodes for the HPPD-4 protein. The *hppdPf-4Pa* coding sequence was developed by introducing four amino acid mutations to the wild type *hppd* gene derived from *Pseudomonas fluorescens* strain A32. Expression of the HPPD-4 protein confers tolerance to HPPD inhibitor herbicides such as isoxaflutole.

Soybean is cultivated primarily for the production of seed that has many food, feed and industrial uses. In the human diet, soybeans are one of the major sources of edible vegetable oil that is used as a purified oil, or utilized in margarines, shortenings and cooking and salad oils. Soybeans may also be consumed directly without any processing as soybean seeds, and many non-fermented and fermented oriental soybean foods such as soy sprouts, milk, tofu, tempeh, miso, natto, and soy sauce. Edible soy protein products including grits and flours, concentrates and isolates are used as food ingredients. The different soy protein products are added in bakery products, snack foods, noodle products and comminuted meat products. Meal derived from soybeans is used as a high protein supplement in feed rations for

livestock. Industrial uses of soybeans range from the production of yeasts and antibodies to the manufacture of soaps and disinfectants.

The GMB151 transgenic locus in the *G. max* genome and the safety of the Cry14Ab-1 and HPPD-4 proteins expressed by the introduced genes, *cry14Ab-1.b* and *hppdPf-4Pa* respectively, have been characterized according to international standards for the safety assessment of biotechnology products. This information is included with this application to support the food safety of the Cry14Ab-1 and HPPD-4 proteins. The GMB151 soybean event will be grown commercially in the soybean producing areas of the USA, Canada and Brazil.

Molecular characterization studies using next generation sequencing (NGS) and junction sequence analysis (JSA) determined that GMB151 contains, at a single locus, a single complete copy of the *cry14Ab-1.b* gene cassette and a single *hppdPf-4Pa* gene cassette that lacks the 5' part of the promoter. Generational stability analysis by NGS/JSA demonstrated that the transgenic locus of GMB151 is stably maintained across multiple generations. Segregation data were consistent with Mendelian principles, confirming that GMB151 has a single insert that is stably inherited over generations.

Bioinformatics analysis of the full DNA sequence revealed no evidence supporting cryptic gene expression or unintended effects resulting from the genetic modification.

Food safety evaluation of the Cry14Ab-1 and HPPD-4 proteins was undertaken utilising guidance provided by Codex. No health-related adverse effects have been associated with the proteins.

A thorough mammalian safety assessment was conducted for both the Cry14Ab-1 and HPPD-4 proteins as expressed in GMB151 soybean. No adverse effects were observed for either protein. The source organism of the Cry14Ab-1 protein, *Bacillus thuringiensis (Bt),* is ubiquitous in the environment, is not known for allergenicity, and has a history of safe use as microbial *Bt*-derived biopesticides. Cry proteins have an established history of safe use and have been used for insect control in crops for over 50 years. The Cry14Ab-1 protein has no amino acid sequence similarity to known allergens or toxins, is rapidly degraded in simulated gastric fluid and exhibited no effects in an acute oral mouse toxicity test. The source organism of the HPPD-4 protein, *Pseudomonas fluorescens,* is a non-pathogenic bacterium which is ubiquitous in nature and has a history of safe use. HPPD proteins are ubiquitous in nature across all kingdoms: bacteria, fungi, plants and animals. The HPPD-4 protein has no amino acid sequence similarity to known allergens or toxins, is rapidly degraded in simulated gastric fluid and exhibited no effects in an acute oral mouse toxicity test.

Food safety for both the Cry14Ab-1 and HPPD-4 proteins are established with data and information within this application for GMB151 soybean - studies confirming the lack of amino acid sequence homology with known toxins and allergens, rapid digestion in simulated gastric fluid, along with complimentary protein expression studies for GMB151 soybean. Acute oral mouse toxicity testing for both proteins and a 90 day toxicity study using GMB151 soybean meal are available and are provided within this submission.

Composition analysis and a comparative assessment demonstrated that GMB151 soybean forage and grain is comparable to that of the non-GM counterpart and reference varieties. These results demonstrate that GMB151 soybean supports the food safety assessment.