EXECUTIVE SUMMARY

Dow AgroSciences LLC is submitting an application to amend the Australia New Zealand Food Standards Code - Standard 1.5.2 to approve the use of DAS-81419-2 Soybean; a new food produced using gene technology.

This submission contains sufficient supporting information to address the requirements identified by FSANZ to approve the use a new food produced using gene technology, as specified in section 3.5.1 of the FSANZ Application Handbook, 1st August, 2011.

DAS-81419-2 soybean is a transgenic soybean that expresses the insecticidal proteins Cry1Ac and Cry1F originally from the naturally-occurring soil bacterium, *Bacillus thuringiensis.* Cry1Ac and Cry1F provide protection against several lepidopteran pests of soybean, including soybean looper (*Chrysodeixis includens,* formerly *Pseudoplusia includens*), velvetbean caterpillar (*Anticarsia gemmatalis*), fall armyworm (*Spodoptera frugiperda*) and tobacco budworm (*Heliothis virescens*). In addition, DAS-81419-2 soybean expresses the phosphinothricin acetyltransferase (PAT) protein from the soil bacterium *Streptomyces viridochromogenes.* The PAT protein provides tolerance to the herbicide glufosinate and was used as a selectable marker during the development of DAS-81419-2 soybean. The transgenes for Cry1Ac, Cry1F and PAT expression were introduced into soybean via *Agrobacterium*-mediated transformation to create DAS-81419-2 soybean.

Cry1Ac consists of 1156 amino acids from three components: the N-terminal toxin core from Cry1Ac1 originating from *B. thuringiensis* subsp. *kurstaki,* followed by a very small portion of Cry1Ca3 originating from *B. thuringiensis* subsp. *aizawai*, and the C-terminal sequence from Cry1Ab1 originating from *B. thuringiensis* subsp. *berliner.* Cry1F consists of 1148 amino acids from three components: the N-terminal toxin core from Cry1Fa2 following by a very small portion of Cry1Ca3 originating from *B. thuringiensis* subsp. *aizawai,* and the C-terminal sequence from Cry1Ab1 originating from *B. thuringiensis* subsp. *berliner.*

Cry1Ac and Cry1F expressed in DAS-81419-2 soybean are 100% identical in amino acid sequence to Cry1Ac and Cry1F expressed in the deregulated events comprising WideStrike® cotton; MXB-7 (also described as 3006-210-23 or DAS-21023-5 expressing Cry1Ac) and MXB-9 (also described as 281-24-236 or DAS-24236-5 expressing Cry1F) (FSANZ 2005). The PAT enzyme, originating from *Streptomyces viridochromogenes*, acetylates the primary amino group of phosphinothricin rendering it inactive. The PAT enzyme expressed in DAS-81419-2 soybean is 100% identical in amino acid sequence to PAT expressed in a number of deregulated events, including LibertyLink® soybean A2704-12 (also described as ACS-GMØØ5-3) (FSANZ 2004), Herculex I corn DAS-01507-1 (also described as TC1507) (FSANZ 2003). MXB-7 (also described as 3006-210-23 or DAS-21023-5 expressing Cry1Ac) and MXB-9 (also described as 281-24-236 or DAS-24236-5 expressing Cry1F) comprising WideStrike® cotton (FSANZ 2005).

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The transgenes *cry1Ac(synpro), cry1Fv3* and *pat* expressing Cry1Ac, Cry1F and PAT proteins were introduced into DAS-81419-2 soybean using *Agrobacterium*-mediated transformation. Molecular characterization by Southern blot analyses of DAS-81419-2 soybean confirmed that a single, intact DNA insert containing the *cry1Ac(synpro), cry1Fv3,* and *pat* gene expression cassettes was integrated into the soybean genome and the intact DNA insert was stably inherited in the five breeding generations tested. Southern blot analyses confirmed the absence of the plasmid backbone DNA in DAS-81419-2 soybean. Analyses of the segregating generations confirmed that segregation of the DNA insert followed the predicted Mendelian inheritance pattern. These data confirmed the stability of DAS-81419-2 soybean during traditional breeding procedures.

Cry1Ac and Cry1F have a long history of safe use. The proteins originate from the naturally-occurring soil bacterium *B. thuringiensis.* The safety of the proteins has been demonstrated in sprayable Bt formulations for pest control in agriculture for over half a century (EPA 2011, Mendelsohn et al 2003, Sanahuja et al 2011). Both proteins are expressed in WideStrike® cotton which is authorized for cultivation in Australia, U.S. and Brazil and for food and feed use in Australia, Brazil, Canada, European Union, Japan, Korea, Mexico, New Zealand and U.S. (www.biotradestatus.com). Bt corn and Bt cotton expressing variations of Cry1Ac or Cry1F have been cultivated for commercial use in the U.S. and other countries for more than a decade. In 1997, the Unites States EPA established an exemption from the requirement of a tolerance for the plant-incorporated protectant Cry1Ac in all plants. The Australian Pesticides and Veterinary Medicines Authority have since determined that a Cry1Ac protein maximum residue limit for human food and animal feed is unnecessary (APVMA 2012). EPA also established an exemption from the requirement of a tolerance for the plant-incorporated protectant Cry1F in cotton (40 CFR §174.504) and in corn (40 CFR §174.520). The exemptions were based on safety assessments of the proteins including digestibility in simulated gastric fluid, lack of homology to known allergens and protein toxins, and lack of mammalian toxicity as demonstrated by mouse acute oral toxicity studies. DAS has filed a petition with EPA for an exemption from the requirement of a tolerance for Cry1F as expressed in soybean in 2012.

An extensive set of biochemical evaluations confirmed the identity of the Cry1Ac and Cry1F proteins produced in DAS-81419-2 soybean. Moreover DAS-81419-2 soybean-derived Cry1Ac and Cry1F were determined to be biochemically equivalent to the corresponding proteins purified from a microbial expression host organism *Pseudomonas fluorescens.* The Cry1Ac and Cry1F purified from *P. fluorescens* had been extensively assessed to establish the safety of the proteins. The assessments included acute oral toxicity in mice and protein digestibility in simulated gastric fluid. The proteins have a very low acute toxicity potential and are rapidly degraded in simulated gastric fluid. Bioinformatics analyses showed that neither Cry1Ac nor Cry1F share meaningful amino acid sequence similarities with known allergens. No significant homology was identified when either protein sequence was compared with known allergens using the search criteria of either a match of eight or more contiguous identical amino acids, or greater than 35% identity over 80 amino acid residues. Likewise, neither protein shares meaningful amino acid sequence similarities with known protein toxins.

The PAT protein was assessed for any potential adverse effects to humans and animals resulting from the environmental release of crops containing the PAT protein. A step-wise, weight-of-evidence approach was used to assess the potential for toxic or allergenic effects from the PAT protein. Bioinformatics analyses revealed no meaningful homologies to known or putative allergens or toxins for the PAT amino acid sequence. The PAT protein hydrolyzed rapidly in simulated gastric fluid. There was no evidence of acute toxicity of the PAT protein in mice. The low level expression of the PAT protein presents a low exposure risk to humans and animals, and the results of the overall safety assessment of the PAT protein indicate that it is unlikely to cause allergenic or toxic effects in humans or animals. The safety of the PAT protein has been assessed previously and it has been approved for use in canola, corn, cotton, rice, soybeans, and sugar beets around the world.

Nutrient compositional analyses of forage and grain were conducted to compare the composition of DAS-81419-2 soybean with that of a non-transgenic soybean control. Compositional analyses were used to evaluate any changes in the levels of key nutrients and anti-nutrients in DAS-81419-2 soybean. A total of 88 analytes were evaluated, nine in forage and the remaining 79 in seed including protein, fat, ash, moisture, carbohydrate, mineral, amino acids, fatty acids, vitamins, and bioactives. Seventeen analytes were excluded from statistical analysis because more than 50% of the samples were below the limit of quantitation. Of the remaining 71 analytes, the results indicate that there were no statistical differences between DAS-81419-2 and the non-transgenic control for 61 analytes based on overall treatment effects and pair-wise comparisons. The statistical differences observed for the remaining ten analytes based on unadjusted P-values were non-existent after adjustment for multiplicity using the false discovery rate method. The numerical differences in mean values observed for the ten analytes between DAS-81419-2 and non-transgenic control were small relative to natural variation. The mean values of DAS-81419-2 soybean were within the literature ranges and/or within the range of the reference varieties included in the study. Nutrient compositional analyses demonstrate that DAS-81419-2 soybean is substantially equivalent to conventional soybean. Endogenous allergen analyses indicate that the genetic modification used to generate DAS-81419-2 soybean did not alter the endogenous allergen content compared to the non-transgenic soybean.

Because DAS-81419-2 soybean is compositionally similar to conventional soybean, and Cry1Ac, Cry1F and PAT proteins have a history of safe use, no significant impact is expected on human or animal health via commodity food and feed soybean products. The availability of DAS-81419-2 soybean is expected to have a beneficial impact on insect pest management by providing another tool to address insect control needs.

Dow AgroSciences is seeking an amendment of the Australia New Zealand Food Standards Code - Standard 1.5.2 Food Produced Using Gene Technology by inserting: “food derived from insect-resistant DAS-81419-2 soybean line”, into column 3 of the Schedule of Permitted Foods produced using Gene Technology, immediately after the last soybean entry.