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-8191Ø-7 Cotton; a new food produced using gene technology.

Dow AgroSciences has developed DAS-8191Ø-7 cotton which is tolerant to the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and glufosinate-ammonium. DAS-8191Ø-7 cotton will provide growers in approved regions with greater flexibility in selection of herbicides for the improved control of economically important weeds; allow an increased application window for effective weed control; and provide an effective weed resistance management solution to the increased incidence of glyphosate resistant weeds.

DAS-8191Ø-7 cotton plants have been genetically modified to express aryloxyalkanoate dioxygenase-12 (AAD-12). AAD-12 is an enzyme with an alpha ketoglutarate-dependent dioxygenase activity which results in metabolic inactivation of the herbicides of the aryloxyalkanoate family. The *aad-12* gene, which expresses the AAD-12 protein, was derived from *Delftia acidovorans*, a gram-negative soil bacterium which can be used to transform ferulic acid into vanillin and related flavour metabolites.

In addition to AAD-12, DAS-8191Ø-7 cotton plants have also been genetically modified to express the phosphinothricin acetyltransferase (PAT) protein. The *pat* gene expressing the PAT protein was derived from *Streptomyces viridochromogenes* and provides tolerance to the herbicide glufosinate-ammonium in DAS-8191Ø-7 cotton plants. The PAT enzyme acetylates the primary amino group of glufosinate-ammonium, also known as phosphinothricin, rendering the herbicide inactive. The *pat* gene has been extensively reviewed by regulatory authorities in over eleven countries, including Australia and New Zealand and has a long history of safe use, being used in over eight crop species representing over twenty-two biotechnology plant events globally.
The *aad-12* and *pat* expression cassettes introduced into DAS-8191Ø-7 cotton are the same as those introduced into DAS-68416-4 soybean and DAS-444Ø6-6 soybean; both have been evaluated and approved by FSANZ.

Australia is the third largest exporter of cotton in the world behind USA and India (USDA 2013). In the period 2011-2012, the Australian cotton industry produced approximately 5 million bales cotton from a planted area of more than 600,000 hectares, generating ca. $2.75 billion AUD in export revenue (ABARES 2012). Only very small quantities of cotton imports to Australia are reported in 2011- 2012, with less than 200 tonnes cotton lint from the USA; and 3,658 tonnes cottonseed oil imported predominantly from Malaysia (FAOSTAT). Only cottonseed oil and linters (fibre) from cottonseed are used in food applications; seeds are mainly used to obtain edible oil and as livestock feed. Due to the harsh conditions used in cottonseed processing, cottonseed oil and linters contain undetectable or negligible amounts of protein, therefore, oil and other products produced from DAS-8191Ø-7 cotton will contain extremely low levels of AAD-12 and PAT protein.

As with DAS-68416-4 soybean and DAS-444Ø6-6 soybean, the data and information presented in this application support the conclusion that food and feed derived from DAS-8191Ø-7 cotton is as safe and nutritious as those derived from non-transgenic cotton. The conclusion was based on 1) detailed molecular characterization of DAS-8191Ø-7 cotton; 2) safety assessment of the introduced AAD-12 and PAT proteins; and 3) nutrient composition analysis of DAS-8191Ø-7 cotton.

Molecular Characterisation of DAS-8191Ø-7 Cotton

The *aad-12* and *pat* genes were introduced into DAS-8191Ø-7 cotton using *Agrobacterium* mediated transformation. Molecular characterization by Southern blot analyses of DAS-8191Ø-7 cotton confirmed that a single, intact DNA insert containing the *aad-12* and *pat* gene expression cassettes was stably integrated into the cotton genome. Southern blot analyses also confirmed the absence of the plasmid backbone DNA in DAS-8191Ø-7 cotton. The integrity of the inserted DNA was demonstrated in five different breeding generations. Data from segregating generations confirmed the predicted Mendelian inheritance pattern. These data confirmed the stability of DAS-8191Ø-7 cotton during traditional breeding procedures.

Biochemical Characterization of DAS-8191Ø-7 Cotton

Microbial-derived AAD-12 and PAT have been extensively assessed to establish the safety of the protein. DAS-8191Ø-7 cotton-derived AAD-12 and PAT proteins were determined to be biochemically equivalent to the corresponding proteins from microbial expression host organisms. A step-wise, weight-of-evidence approach was used to assess the potential for toxic or allergenic effects from the AAD-12 and PAT proteins. Bioinformatic analyses revealed no meaningful homologies with known or putative allergens or toxins for the AAD-12 or PAT amino acid sequences. Both proteins hydrolysed rapidly in simulated gastric fluid. There was no evidence of acute toxicity in mice at a dose of 2000 mg/kg body weight of AAD-12 protein and 5000 mg/kg body weight of PAT protein.

The AAD-12 protein from cotton and soybean event DAS-68416-4 has identical amino acid sequence and N-terminal acetylation. Digestion samples of the transgenic soybean (event DAS-68416-4) leaf extracts were analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and western blot; the AAD-12 protein contained in crude soybean leaf extracts (in both transgenic tissue and nontransgenic tissue fortified with microbe-derived AAD-12) was readily digested by pepsin (not detectable at 30 seconds) under simulated gastric conditions (pH 1.2, 37 °C). Hence, the digestibility of the acetylated AAD-12 protein is established.

AAD-12 and PAT expression levels in DAS-8191Ø-7 cotton were measured using a protein-specific enzyme-linked immunosorbent assay (ELISA). Protein expression was analysed in multiple tissues collected throughout the growing season from DAS-8191Ø-7 cotton plants untreated and treated with 2,4-D plus glufosinate-ammonium. Glycosylation analysis revealed no detectable covalently linked carbohydrates in either AAD-12 or PAT proteins expressed in DAS-8191Ø-7 cotton plants. The low level expression of these proteins presents a low exposure risk to humans and animals, and the results of the overall safety assessment of AAD-12 and PAT indicate that it is unlikely to cause allergenic or toxic effects in humans or animals.

Compositional Assessment of DAS-8191Ø-7 Cotton

A composition assessment was conducted in which levels of key nutrients and anti-nutrients of DAS-8191Ø-7 cottonseed were compared with the appropriate non-transgenic near-isogenic control and non-transgenic reference lines. Samples were analysed for proximates, fibre, minerals, amino acids, fatty acids, vitamins, and anti-nutrients. Fifty-nine cotton analytes were assayed and the analyses conclude that DAS-8191Ø-7 cotton is compositionally equivalent to non-transgenic cotton.

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 herbicide-tolerant DAS-8191Ø-7 Cotton line”, into column 3 of the Schedule of Permitted Foods produced using Gene Technology, immediately after the last cotton entry.