



**Application to Food Standards Australia New Zealand
for the Inclusion of
Glyphosate-Tolerant Canola DP-Ø73496-4
in Standard 1.5.2 - Food Produced Using Gene Technology**

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Part 1 General Information

Executive Summary

Pioneer Hi-Bred International, Inc. has developed a transgenic canola product that provides tolerance to glyphosate herbicides. This event has an OECD identifier of DP-Ø73496-4 and is hereafter referred to as GM canola line 73496. GM canola line 73496 will provide a market alternative to currently available herbicide-tolerant canola lines. Pioneer is submitting information for review to the FSANZ about the food safety of GM canola line 73496. GM canola line 73496 has been genetically modified to express the GAT4621 (glyphosate acetyltransferase) protein. The *gat4621* gene is a variant of three *gat* genes from the common soil bacterium *Bacillus licheniformis*. The GAT4621 protein is equivalent to the protein expressed in maize event DP-Ø9814Ø-6, approved by FSANZ in May 2010, and is encoded by the *gat4621* gene which confers tolerance to glyphosate-containing herbicides by acetylating glyphosate and thereby rendering it non-phytotoxic. In GM canola line 73496, the expression of the *gat4621* gene is driven by the *Arabidopsis* polyubiquitin constitutive promoter.

GM canola line 73496 was generated using biolistic transformation of canola microspores with a gel-purified fragment isolated from plasmid PHP28181 containing the *gat4621* gene cassette. Molecular characterization of GM canola line 73496 by Southern blot analysis confirmed that a single, intact PHP28181A DNA fragment was inserted into the genome. Southern blot analysis verified the absence of plasmid backbone DNA. Segregation analysis of GM canola line 73496 confirmed Mendelian inheritance of the *gat4621* gene.

The potential for allergenicity and toxicity of GM canola line 73496 was evaluated by examining the allergenic potential of canola as a crop and by assessing the allergenic and toxic potential of the GAT4621 protein. Canola is not a common allergenic food and the modification in GM canola line 73496 is not expected to alter the allergenic potential of canola. The allergenic potential of the GAT4621 protein was assessed using a weight-of-evidence approach using guidance from the Codex Alimentarius Commission. Bioinformatic analyses revealed no biologically significant identities to known or putative protein allergens or toxins for the GAT4621 protein sequences. The GAT4621 protein is non-glycosylated and is rapidly digested (within 30 seconds) in simulated gastric fluid. In simulated intestinal fluid, the GAT4621 protein hydrolyzed within five minutes. There was no evidence of acute toxicity in mice for GAT4621 at a dose of 1640 mg protein per kg of body weight. Based on the GAT4621 protein levels in 73496, exposure levels would be exponentially lower than the tested doses. These data support the conclusion that the GAT4621 protein is unlikely to cause an allergic reaction in humans or be a toxin in humans and therefore support the food safety of GAT4621. Finally, the applications of soybean event DP-356Ø43-5 and maize event DP-Ø9814Ø-6 which also express GAT4621 protein were assessed by FSANZ and concluded no potential health and safety concern in 2009 and 2010, respectively (FSANZ, 2009; FSANZ, 2010).

Extensive nutrient composition analysis of seed was conducted to compare the composition of GM canola line 73496 to that of a near-isoline control and to commercial canola lines. Compositional analysis of GM canola line 73496 was used to evaluate any changes in the concentrations of key nutrients, anti-nutrients and secondary metabolites. Compositional comparability is a general indicator that GM canola line 73496 does not exhibit characteristics of relevance that impact the food safety of these canola lines in comparison to commercially available canola. Based on the compositional evaluation, the seed of GM canola line 73496 was considered to be nutritionally comparable to conventional canola.

Although the primary substrate of the GAT4621 protein is glyphosate, GAT4621 is also known to N-acetylate certain free amino acids (L-aspartate, L-glutamate, glycine, L-serine, and L-threonine) resulting in production of *N*-acetylaspartate (NAA), *N*-acetylglutamate (NAG), *N*-acetylglycine (NAGly), *N*-acetylserine (NAS), and *N*-acetylthreonine (NAT). The efficiency of acetylation of free amino acids by GAT proteins is considerably lower than the activity displayed toward glyphosate. Therefore, the concentrations of these five acetylated amino acids were measured in seed, whole plant, and processed product samples derived from GM canola line 73496. Findings demonstrated elevated concentrations of NAA and NAG, and in some instances NAGly, NAS, and NAT when compared with the corresponding concentrations of these substances in unmodified canola. Low but quantifiable amounts of each *N*-acetylated amino acid were found in each sample type except refined, bleached, deodorized (RBD) oil, where levels were either not detectable or below the limit of quantification. These five acetylated amino acids are not novel substances as they are present in conventional canola as well as in other plants.

Commercial canola seed for consumption is processed into oil and meal fractions. Oil is the major fraction consumed by humans. Processed fractions derived from GM canola line 73496 were analyzed for the presence of NAA, NAG, NAS, NAT, and NAGly. None of these substances were detected in refined, bleached, deodorized oil (the major fraction that is consumed by humans) obtained from GM canola line 73496 ; therefore, the increased concentration of these acetylated amino acids in GM canola line 73496 will not affect human exposure to any of these substances.

Based on the food safety assessment of GM canola line 73496, Pioneer concludes there will be no adverse effects to human health resulting from the consumption of GM canola line 73496. Commercialization of GM canola line 73496 and consumption of oil derived from GM canola line 73496 would be fully consistent with FSANZ's Food Policy and in compliance with all applicable requirements of the Standard 1.5.2 of the *Australia New Zealand Food Standards Code*.