EXECUTIVE SUMMARY
to
Application to Food Standards Australia New Zealand for the Inclusion of Soybean MON 87769 in Standard 1.5.2 - Food Derived from Gene Technology

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EXECUTIVE SUMARRY

Monsanto has developed biotechnology-derived soybean MON 87769 that contains stearidonic acid (SDA), a sustainable alternate source of an omega-3 fatty acid to help meet the need for increased dietary intake of long chain omega-3 fatty acids. In mammals, SDA is a metabolic intermediate in the production of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from alpha linolenic acid (ALA), a common dietary constituent. SDA is an eighteen carbon fatty acid with four double bonds (18:4) and as such is found in fish and fish/algal oil products.

Refined oil produced from MON 87769 contains approximately 20 to 30% SDA (wt% of total fatty acids) and can be used for the production of margarine, mayonnaise, shortenings, salad dressings, ready-to-eat foods, and other food products. Since SDA has fewer double bonds than either EPA (20:5) or DHA (22:6), SDA soybean oil is more stable to oxidation (i.e., less prone to fishy or rancid odors and taste) than fish oils thereby expanding the potential formulation options for food companies and consumers. Fish and plant oils rich in omega-3 fatty acids are used currently in feed applications such as aquaculture and poultry feeds. SDA soybean oil from MON 87769 may be used in aquaculture and feed applications as an alternative to fish oil and other omega-3 rich feed components. MON 87769 soybean meal is compositionally similar to other commodity soybean meal and will be used in a manner similar to conventional soybean meal.

The development of MON 87769 involved the introduction of two desaturase genes, *Primula juliae* Δ6 desaturase (*Pj.D6D)* and *Neurospora crassa* Δ15 desaturase (*Nc.Fad3)* that encode for the PjΔ6D and NcΔ15D proteins. Soybean plant lacks Δ6 desaturase gene, which is a minimal requirement for the production of SDA. However, Δ6 desaturase also converts linoleic acid (LA) to gamma linoleic acid (GLA). The addition of a Δ15 desaturase with temporal expression similar to the Δ6 desaturase increases the flux of ALA to SDA and lowers the substrate pool for GLA production. To produce SDA in soybean, the conventional soybean variety A3525 was transformed with vector PV-GMPQ1972 that contained the *Nc.Fad3* and the *Pj.D6D* genes driven by promoters that are known to be spatially and temporally active only in the developing soybean seed.

SDA soybean oil contains increased levels of SDA (approximately 20-30%) and GLA (~7%). SDA is produced from the abundant dietary fatty acid ALA during mammalian fatty acid metabolism. The safety of SDA is based on its long-standing history of consumption from several marine and plant sources. Similarly, GLA is an intermediate in fatty acid metabolism and is present in oats, barley, meats, fish, and human breast milk establishing a history of consumption in food and feed. The safety of SDA soybean oil is supported by the results of a published 90-day/one-generation reproductive toxicity study in rats with a reported no observable adverse effect level (NOAEL) of 1 g SDA/kg body weight/day (4 g SDA soybean oil/kg body weight/day). There were no adverse effects observed in this study. Finally, published nutritional and toxicological studies from other researchers corroborate the safety of SDA and GLA. Based on the known SDA:EPA conversion ratio of approximately 3:1 in humans, the total intake of EPA and DHA (i.e., resulting from the intake of SDA from the proposed food uses of SDA soybean oil plus the baseline per capita daily intake) would not exceed the maximum FDA established level of 3 g/day per person combined EPA and DHA intake. SDA soybean oil containing 20 to 30% SDA is generally recognized as safe (GRAS). Therefore, SDA soybean oil is safe for human and animal consumption.

The genetic modification in MON 87769 was comprehensively characterized. The results confirm that MON 87769 contains a single insert with the intended sequence, where the insert is stably maintained over multiple generations, and that the insert will not result in unintended gene products with similarity to known allergens or toxins. The strategy used to characterize the genetic modification included: 1) Southern blot analyses to assay the entire soybean genome for the presence of DNA derived from the transformation plasmid, PV-GMPQ1972, to confirm that a single copy was inserted at a single site in the genome and that the insert was stably inherited; 2) DNA sequencing analyses to determine the exact sequence of the inserted DNA and allow a comparison to the transfer DNA (T-DNA) sequence of the transformation vector to confirm that only the expected sequences were integrated; and 3) a segregation analysis to confirm that the introduced traits are inherited according to Mendelian laws of genetics. Additionally, open reading frame bioinformatic analyses of the junction site between the soybean genomic DNA and the insert containing the *Nc.Fad3* and *Pj.D6D* cassettes confirm that no relevant similarities exist between any putative polypeptides and known toxins or allergens. Taken together, the characterization data demonstrate that a single copy of the T-DNA was inserted at a single locus in the genome of MON 87769. The stability of the integrated DNA and absence of the backbone sequences in multiple generations of MON 87769 was also confirmed. These results are consistent with a single site of insertion that segregates in subsequent progeny according to the Mendelian laws of genetics.

A multistep approach was used to characterize the PjΔ6D and NcΔ15D proteins expressed in MON 87769 resulting from the genetic modification. This detailed characterization confirms that both proteins are safe for human and animal consumption. The assessment involved: 1) characterizing the physicochemical and functional properties of both proteins; 2) quantifying protein levels in plant tissues; 3) examining the similarity of each of these proteins to known allergens, toxins and other biologically-active proteins known to have adverse effects on mammals; 4) evaluating the digestibility of both proteins in simulated gastrointestinal fluids; 5) documenting the history of safe consumption of these proteins or their structural and functional homologues that lack documented adverse affects on human or animal health; and 6) investigating potential mammalian toxicity through a oral gavage assay. The proteins share sequence similarity to several proteins that are ubiquitous in the human diet and are directly consumed in many common foods. The proteins are expressed at low levels in the seed. Both proteins lack biologically-relevant amino acid sequence similarities to known allergens, toxins and anti-nutritional proteins known to have adverse effects on mammals. Additionally, both proteins are rapidly digested in simulated gastric and intestinal fluids and do not exhibit any signs of toxicity when administered to mice via oral gavage. Ultimately, the safety assessment supports the conclusion that dietary exposure to either the PjΔ6D or the NcΔ15D proteins derived from MON 87769 poses no meaningful risk to human or animal health.

Detailed compositional and nutritional comparisons of MON 87769, a conventional soybean control, and ten commercially available soybean varieties were conducted. These compositional comparisons were made by analyzing the seed and forage harvested from five replicated field sites across the United States during the 2006 field season. The analysis included protein, fat, carbohydrates, fiber, ash, moisture, amino acids, fatty acids, vitamins, and anti-nutrients. The compositional analyses confirmed that MON 87769 had the intended change in fatty acid composition, while the other components analyzed in MON 87769 were compositionally equivalent to conventional soybean. As intended, MON 87769 seed had increased levels of SDA (26%) and GLA (7.1%) and small changes in two minor fatty acids, trans-SDA (0.18 %) and trans-ALA (0.44%). Considering linoleic acid (LA) is the starting material from which SDA and GLA are produced, the LA levels were significantly different in MON 87769 compared to conventional soybean. As anticipated, the LA values were also outside the 99% tolerance interval for the population of conventional references as well as the range of values found in the published literature and the International Life Science Institute (ILSI) Crop Composition Database. In addition to LA, combined-site statistical differences were found in five other fatty acid levels (palmitic, oleic, linolenic, arachidic, and behenic acid). The difference in level of each of these five fatty acids were relatively small in absolute magnitude (<4 wt% of total fatty acids) and/or their mean values and ranges in MON 87769 harvested seed were within the 99% tolerance interval for the population of the conventional reference varieties. Given the intended shift in the fatty acid metabolism toward an increase in SDA content in MON 87769, differences in fatty acid levels were expected.

Twenty-two non-fatty acid analytes in harvested seed were significantly different ($p<0.05$) between MON 87769 and the conventional control in the combined-site analysis. Most of the statistical differences were seen in amino acids (>75%, seventeen out of the twenty-two significant differences) and the magnitude of the amino acid differences between MON 87769 and conventional soybean control were small (<10%), where the mean and range values were within the calculated 99% tolerance interval for the population of commercial conventional soybean varieties and also within the range of values found in the published literature and the ILSI Crop Composition Database. The mean and range values for three isoflavones, genistein, daidzein, and glycitein, were lower in MON 87769 harvested seed compared to the values in the conventional soybean control. However, the range of values for these three analytes in MON 87769 were all within the 99% tolerance interval for the population of conventional reference varieties, and were also within the range of values found in the published literature and the ILSI Crop Composition Database. It is well-documented that the soybean isoflavone levels are greatly influenced by many factors, ranging from environmental conditions, variety, and agronomic practices. The remaining two differences were in carbohydrates and protein, and their magnitude of differences between MON 87769 and conventional soybean control were very small (<6%). Furthermore, the mean and range values of carbohydrates and protein were within the calculated 99% tolerance interval for the population of commercial conventional soybean varieties and also within the range of values found in the published literature and the ILSI Crop Composition Database. Therefore, these differences were not considered to be biologically meaningful from a food and feed safety and/or nutritional perspective. Combined-site analysis of forage showed no significant differences ($p<0.05$) between MON 87769 and the conventional soybean control.

In addition to the compositional analysis of harvested seed and forage, four soybean processed fractions (oil, meal, lecithin, and protein isolate) were produced from MON 87769 seed and subjected to compositional analysis in accordance with OECD guidelines. As expected, apart from the intended fatty acid changes, the composition of the soybean processed fractions from MON 87769 is equivalent to the composition of soybean processed fractions from conventional soybean control. Thus, the processed fractions from MON 87769 are concluded to be as safe and nutritious as the processed fractions prepared from conventional soybean.

Based on the information provided in this document, it is reasonable to conclude that with the intended increase in omega-3 fatty acid content, the food and feed derived from MON 87769 are as safe and nutritious as conventional soybean. This conclusion is based on several lines of evidence including:

1. The detailed molecular characterization of the inserted DNA, which confirmed the presence of an intact *Pj.D6D* and *Nc.Fad3* gene cassette stably integrated at a single locus of the soybean genome,
2. The history of safe use and the biochemical characterization of the PjΔ6D and NcΔ15D proteins produced in MON 87769,
3. A safety assessment of the PjΔ6D and NcΔ15D proteins, which shows the lack of allergenic potential and acute toxicity,
4. Compositional and nutritional assessments demonstrating that MON 87769 is equivalent in composition to conventional soybean, except for the intended fatty acid changes (i.e., the presence of SDA and GLA),
5. The established safety of SDA and GLA for food and feed use, and
6. The GRAS status of the SDA soybean oil from MON 87769 that contains approximately 20-30% of SDA and 7% of GLA.

It is, therefore, concluded that the consumption of MON 87769 and the food and feed derived from it will be fully consistent with FDA's Policy (FDA, 1992), the scope of the GRAS assessment of SDA soybean oil, and in compliance with all applicable requirements of the Federal Food, Drug and Cosmetic Act.