SUMMARY OF SAFETY ASSESSMENT OF SOYBEAN A2704-12 FOR FOOD AND FEED

A2704-12 soybean was developed through genetic modification to allow for the use of glufosinate ammonium, the active ingredient in phosphinothricin herbicides (e.g. Liberty®) as a weed control option in soybean crops. The *pat* gene, conferring tolerance to glufosinate ammonium, was cloned from the common aerobic soil actinomycete, *Streptomyces viridochromogenes*, and encodes the enzyme phosphinothricin-N-acetyltransferase (PAT). The PAT protein metabolizes glufosinate to an inactive, acetylated derivative N-acetyl phosphinothricin. N-acetyl phosphinothricin has no herbicidal activity and resistance is therefore conferred through modification of the herbicide rather than the target of its activity. Therefore, when expressed, the *pat* gene confers tolerance to glufosinate-ammonium containing herbicides.

The host organism, soybean or *Glycine max* L., is widely cultivated and has a long history of safe use for consumption as food and feed. Historical and geographical evidence suggests that soybeans were first domesticated in eastern China, between the 17th and 11th century B.C. Today, soybeans are grown as a commercial crop in more than 35 countries throughout the world. The donor organism for the *pat* gene, *Streptomyces viridochromogenes*, is widespread in nature and is not itself known to be a human pathogen nor has been associated with other properties known to affect human health. *S. viridochromogenes* is not known to be an allergen or toxin.

A2704-12 soybean was produced via the particle acceleration method using vector pB2/35SAcK containing a modified form of the *pat* gene under the control of promoter and termination sequences derived from cauliflower mosaic virus. The nucleotide sequence of the *pat* gene was altered via site-directed mutagenesis in order to reduce the high G:C content (typical for bacterial genes but atypical for plant genes) and generate plant-preferred codons. These

sequence modifications did not result in changes to the predicted amino acid sequence of the PAT enzyme. Southern blot analysis of genomic DNA from the A2704-12 soybean indicated the incorporation of two *pat* gene cassettes joined by an inverted 957 bp *Pvu*l fragment from the transforming pB2/35SAcK plasmid. The DNA of the A2704-12 insert was sequenced and is completely identical to the corresponding transforming plasmid DNA sequences.

A2704-12 soybean is as safe as and substantially equivalent to its conventional counterpart. It shows no different allergenic or toxic potential compared to conventional soybean currently in the market. Compositional analyses of beans from A2704-12 soybean and current commercial soybean varieties were compared for compositional and nutritional parameters including moisture, crude fat, crude protein, crude fiber, ash, carbohydrates, mineral content, amino acid profile, and fatty acid composition. The data and findings show that A2704-12 soybean is compositionally and nutritionally equivalent to currently grown conventional commercial soybean varieties.

The low potential for toxicity of the PAT protein expressed in A2704-12 soybean is demonstrated by examining the amino acid sequence homology, chemical characteristics of the protein and by acute oral toxicity testing in rats. The nucleotide sequence of the *pat* gene and the deduced amino acid sequence of the PAT protein were compared with sequences available for known toxins in the GenBank database and showed no significant homology with any known toxins or allergens.

The PAT enzyme expressed in A2704-12 soybean does not possess characteristics typical of known protein allergens and is extremely unlikely to be allergenic. There were no regions of homology when the sequences of the introduced protein were compared to the amino acid sequences of known protein allergens. There was no evidence found of post-translational modifications such as acetylation, glycosylation or phosphorylation of the PAT protein. Unlike known protein allergens, the PAT protein was rapidly degraded by acid and/or

enzymatic hydrolysis when exposed to simulated gastric fluids. *In vitro* digestibility studies, under simulated mammalian gastric conditions, demonstrated that the PAT enzyme was inactivated within one minute and was rapidly degraded. No adverse effects have been reported to be associated with this enzyme.

To date A2704-12 soybean has been approved for food and feed uses in 19 countries worldwide. There are no adverse effects assumed with use of A2704-12 soybean and as a consequence there is no need for a case specific monitoring plan relating to import of herbicide tolerant A2704-12 soybean. The beans derived from A2704-12 soybean needs no specific or additional treatment and will be handled in the same way as any other conventional soybean commodity.

This application aims for food and feed use of herbicide-tolerant A2704-12 soybean in Vietnam.