SUMMARY OF SAFETY ASSESSMENT OF CORN T25 FOR FOOD AND FEED

T25 corn 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 corn 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, corn or *Zea mays* L., is the world's third largest grown cereal crop, following wheat and rice and is grown in over 25 countries worldwide including Vietnam. Corn has a long history of safe use for consumption as food and feed. 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.

T25 corn was produced via direct DNA uptake of protoplasts from an embryogenic suspension culture of corn with a pUC/Ac plasmid 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 T25 corn indicated the incorporation of one copy of the *pat* gene cassette and two non-expressible fragments of the *bla* sequences.

T25 corn is as safe as and substantially equivalent to its conventional counterpart. It shows no different allergenic or toxic potential compared to conventional corn currently in the market. Compositional analyses of grains from T25 corn and current commercial corn 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 T25 corn is compositionally and nutritionally equivalent to currently grown conventional commercial corn varieties.

The low potential for toxicity of the PAT protein expressed in T25 corn 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 T25 corn 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.

T25 corn has already been in the market for over 15 years with no reported concerns. T25 corn has been approved for food and feed uses in 17 countries worldwide. There are no adverse effects assumed with use of T25 corn and as a consequence there is no need for a case specific monitoring plan relating to import of herbicide tolerant T25 corn. The grain derived from T25 needs no specific or additional treatment and will be handled in the same way as any other conventional corn commodity.

This application aims for food and feed use of herbicide-tolerant T25 corn in Vietnam. The said corn event is not intended for cultivation in Vietnam.