Executive Summary

Using the techniques of modern molecular biology, maize (*Zea mays* L.) has been genetically modified to produce Event ZZM032 maize, which provides tolerance to glyphosate and glufosinate-ammonium in herbicide products. Maize plants derived from Event ZZM032 contain the transgenes *bar* and *epsps*. The transgene *bar* encodes phosphinothricin acetyltransferase (PAT). PAT acetylates glufosinate-ammonium, thus inactivating it and conferring tolerance to glufosinate-ammonium in herbicide products. The transgene *epsps* encodes 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). The protein EPSPS confers tolerance to glyphosate in herbicide products.

The host organism is *Zea mays* L. commonly known as maize or corn, is a large annual monoecious grass grown globally for diverse applications including animal feed, silage, human grain, vegetable oil, sugar syrups, bioethanol, and numerous industrial products.

Event ZZM032 contains two bacterial-derived genes that confer tolerance to specific herbicides.

1. The ***bar*** **gene** is derived from *Streptomyces hygroscopicus*, encoding phosphinothricin acetyltransferase (PAT), which confers tolerance to glufosinate-ammonium herbicides.

2. The ***epsps*** **gene** is derived from *Agrobacterium* sp. strain CP4, encoding 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS), which confers tolerance to glyphosate herbicides.

Both donor organisms have been extensively reviewed during regulatory evaluations of multiple herbicide-tolerant events globally.

Transformation of *Zea mays* to produce Event ZZM032 maize was accomplished using *Agrobacterium tumefaciens*-mediated transformation of immature embryos from inbred line 'Xiang 249'. Using this method, the genetic elements within the left and right border regions of the transformation plasmid pZHZH15032, which contains the genes *epsps* and *bar*, were efficiently transferred and integrated into the genome of the plant cell, while genetic elements outside these border regions were not transferred.

Molecular characterization of ZZM032 maize confirms that the genome contains a single copy of the T-DNA insert, integrated at a single locus on chromosome 9. Comprehensive sequencing and Southern blot analyses verified the integrity of the insert and confirmed the absence of any plasmid backbone sequences, including antibiotic resistance marker genes such as *aph(3')-IIIa*. Furthermore, bioinformatic analyses demonstrated that the integration of the ZZM032 T-DNA insert did not disrupt any known endogenous maize genes.

The genetic stability of the event was demonstrated through inheritance studies and protein expression analysis. Chi-square analysis across multiple segregating generations confirmed that the transgenes follow standard principles of Mendelian inheritance. Additionally, protein quantification via ELISA across several generations established that the expression levels of the PAT and EPSPS proteins remain phenotypically consistent over time.

Safety assessments using bioinformatic tools identified no biologically relevant similarities between hypothetical open reading frames spanning the insert or junction regions and any known or putative toxins or allergens.

Based on these collective findings, it is concluded that the molecular characterization of ZZM032 maize revealed no unintended changes or safety concerns, supporting the stability and safety of the event.

The safety and exposure histories of the PAT and EPSPS proteins in foods are characterized by their natural origins, widespread regulatory acceptance, and decades of safe consumption.

The weight of evidence supports the conclusion that the PAT protein expressed in ZZM032 maize does not exhibit characteristics typically associated with food allergens or protein toxins. Bioinformatic analyses indicate that PAT does not share biologically relevant amino acid sequence similarity with known or putative toxins or allergens of mammalian concern. These results are consistent with previous assessments of PAT. Together with the established mode of action and history of safe use for PAT proteins, PAT is unlikely to be toxic or allergenic to human or animal consumers. Accordingly, no adverse health impacts are anticipated from dietary exposure to PAT expressed in ZZM032 maize.

The weight of evidence supports the conclusion that the EPSPS protein expressed in ZZM032 maize does not exhibit characteristics typically associated with food allergens or protein toxins. EPSPS was shown to be readily digested under simulated mammalian gastric conditions and sensitive to elevated temperatures relevant to common food processing, reducing the likelihood that intact protein would persist through processing and digestion. Bioinformatic analyses indicate that EPSPS does not share biologically relevant amino acid sequence similarity with known or putative protein toxins or allergens of mammalian concern. These findings, together with the established mode of action and history of safe use for EPSPS proteins, support the conclusion that EPSPS is unlikely to be toxic or allergenic to human or animal consumers. Accordingly, no adverse health impacts are anticipated from dietary exposure to EPSPS expressed in ZZM032 maize.

As part of a food and feed nutritional assessment, levels of key food and feed nutrients and antinutrients in forage and grain from Event ZZM032 maize were compared to those in forage and grain from nontransgenic, near-isogenic control maize.

In conclusion, forage and grain from Event ZZM032 maize are not materially different in nutrient composition from that of nontransgenic, conventional maize.