

APPLICATION TO AMEND THE AUSTRALIA AND NEW ZEALAND FOOD STANDARDS CODE TO ALLOW FOR THE USE OF SOY LEGHEMOGLOBIN

EXECUTIVE SUMMARY

Impossible Foods Inc.
400 Saginaw Drive
Redwood City, CA
94063 USA

EXECUTIVE SUMMARY

Impossible Foods Inc. (Impossible Foods) is submitting this application to amend the Australia New Zealand Food Standards Code (the Code) to allow for the use of their soy leghemoglobin derived from *Pichia pastoris* (*P. pastoris*) in meat analogue products. Soy leghemoglobin was developed by Impossible Foods for addition to plant-based meat analogue products, to provide the texture, nutrition, flavour and aroma of their traditional animal-derived counterparts. Soy leghemoglobin itself does not have an extensive history of consumption in the diet, since it is naturally present only within the root nodule of the soy plant (*Glycine max*), and not in the edible soybean seed. Nevertheless, soy leghemoglobin is structurally similar to other heme-containing globin proteins that are readily consumed in the diet (*e.g.*, animal myoglobins, non-symbiotic plant haemoglobins). Impossible Foods has demonstrated that soy leghemoglobin will denature at high temperatures (as applied during cooking), and at low pH (as in the human stomach) to release the iron-containing heme B molecule, thereby serving as a dietary source of iron in a manner that is analogous to the role of animal-based myoglobin in meat.

Impossible Foods produces soy leghemoglobin from the fermentation of *P. pastoris* that has been genetically modified to express the protein. Following fermentation, the cells are lysed, then physical separation techniques are applied to remove the insoluble materials and concentrate the soy leghemoglobin. The resulting liquid concentrate (LegH Prep) is a mixture containing the soy leghemoglobin, residual *P. pastoris* (yeast) proteins, and suitable stabilizers (*e.g.*, sodium ascorbate and sodium chloride). The LegH Prep is specified to contain up to 9% soy leghemoglobin on a wet weight basis and a soy leghemoglobin protein purity of at least 65%. The remainder of the total protein fraction in the LegH Prep is accounted for by residual proteins from the *P. pastoris* production strain. Small amounts of residual *Pichia* DNA (approximately 300 mg/L) may also be present in the LegH Prep.

Impossible Foods intends to use soy leghemoglobin derived from *P. pastoris*, which will be delivered in the LegH Prep, solely as a component within their own meat analogue products (*e.g.*, the Impossible™ Burger) at levels of not more than 0.8% soy leghemoglobin. This proposed level of addition is comparable to the myoglobin content of beef (0.8 to 1.8%). In practice, soy leghemoglobin will be added to meat analogue products only at levels that would be needed to recreate the flavour and aroma contributed by heme proteins (myoglobin) of their animal-derived counterparts. Usage will also be largely self-limiting due to unacceptable organoleptic properties that would result at higher inclusion rates. Soy leghemoglobin will be marketed exclusively as a component of a finished meat analogue products in Australia/New Zealand, which will be available in retail outlets such as grocery stores and restaurants. It will not be made available for purchase and use by other food manufacturers, nor will it be sold directly to consumers, which further limits its use.

The LegH Prep is manufactured by Impossible Foods in compliance with current Good Manufacturing Practices (cGMP), and a number of in-process controls are in place to ensure the purity of the final product. All of the raw materials and processing aids employed in the production of LegH Prep are standard ingredients used in the food/enzyme industry and are suitable for their intended use. LegH Prep is well characterized and meets food-grade specifications, which include acceptable limits on compositional criteria and impurities such as heavy metals and microbiological contaminants. No viable *P. pastoris* strain remains in the LegH Prep as a heat treatment step is included in the final stages of processing. Moreover, considering that the LegH Prep will not be manufactured in Australia/New Zealand, the fermentation substrates, production strain, and processing aids employed will not enter these countries. Impossible Foods has demonstrated that the LegH Prep can be stored as a frozen liquid at -20°C for at least 24 months, with no observable changes in soy leghemoglobin stability observed. Soy leghemoglobin is also shown to be

stable when meat analogue products containing the ingredient are stored for up to 9 days at 4°C, or up to 6 months at -20°C.

As soy leghemoglobin is a protein product, a 2-tier testing strategy for the safety evaluation of protein products of biotechnology developed by International Life Sciences Institute was applied as a tool for part of its safety assessment. Additionally, the safety assessment included a comprehensive evaluation of the safety of the *P. pastoris* production organism, and potential fermentation products derived thereof that may be transferred to the final LegH Prep. This was conducted using an approach similar to that which is commonly employed for the safety assessment of food enzymes, including those produced from modern biotechnology techniques. Overall, the safety of Impossible Foods' soy leghemoglobin for its intended conditions of use in meat analogue products can be supported based on the following:

1. The LegH Prep manufactured by Impossible Foods is produced in a consistent and reliable manner under cGMP, is well characterized and meets food grade specifications, and is free of undesirable substances;
2. Meat analogue products will not contain soy leghemoglobin at levels exceeding 0.8%, which is comparable to the myoglobin content of beef (0.8 to 1.8%);
3. Soy leghemoglobin is produced using a safe and non-toxicogenic strain lineage of *P. pastoris*, a strain lineage that has been the subject of previous premarket safety evaluations;
4. Genetic modifications applied to the host organism are well characterized and do not impart unexpected pleiotropic effects to the organism;
5. The identity of soy leghemoglobin is well defined, and the structure of the protein is homologous to heme proteins with a long history of safe consumption;
6. Amino acid sequence of soy leghemoglobin is not homologous to known or putative allergens and toxins; and
7. Safety is corroborated by the results of preclinical toxicological testing (28-day feeding studies in rats, a bacterial reverse mutation assay, and *in vitro* mammalian chromosomal aberration assay), demonstrating that LegH Prep is without evidence of toxicity or mutagenicity/genotoxicity.

Meat analogue products containing soy leghemoglobin derived from *P. pastoris* are already being marketed by Impossible Foods in other countries. In the United States (U.S.), soy leghemoglobin has Generally Recognized as Safe (GRAS) status for use in ground beef analogues at levels not exceeding 0.8% (GRN 737). Impossible Foods has also obtained market authorization for the use of soy leghemoglobin as a food additive/ingredient in plant-based meat analogue products in Singapore, and soy leghemoglobin meets the food regulatory requirements in Hong Kong and Macao. To date, over 20-million 1/4-pound servings of meat analogue products containing soy leghemoglobin (*i.e.*, the Impossible™ Burger) have been sold in the U.S. since June 2016. In addition to more than 9,000 restaurants in the U.S., at least 300 restaurants in Hong Kong, Macao, and Singapore also serve meat analogue products containing soy leghemoglobin, without any evidence or reports of safety issues. The approval of soy leghemoglobin derived from *P. pastoris* by FSANZ will give consumers access to more choices for nutritious and flavourful plant-based alternative protein products in the Australia/New Zealand marketplace.