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Health Canada's Proposal to Amend the *Food and Drug Regulations* to Permit the Use of the Enzyme Asparaginase Sourced From *Aspergillus oryzae* (pCaHj621/BECh2#10) in Certain Food Products – December 2009

Bureau of Chemical Safety
Food Directorate
Health Products and Food Branch

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Canada

PURPOSE

Although this document was previously consulted on with domestic stakeholders in early 2009, this consultation has been re-opened for a period of 75 days in order to fulfill Health Canada's obligation of allowing the international community opportunity to comment.

This document provides information on Health Canada's proposal to amend the *Food and Drug Regulations* ("Regulations") to allow the enzyme asparaginase to be used in the manufacture of certain food products, including wheat dough-based products such as bread, crackers and cookies, cut potato products such as French fries, sliced potato products and fabricated potato chips. It includes a summary of the information considered by Health Canada scientists in their assessment of the safety of asparaginase.

BACKGROUND

Asparaginase is an enzyme that hydrolyses an amino acid, asparagine, to aspartic acid by hydrolyzing the amide in free asparagine. Asparaginase has no activity on asparagine residues in peptides or proteins. Aside from free asparagine, asparaginase only acts on free glutamine. It has no activity on other amino acids.

The purpose for using asparaginase in food manufacture is to reduce asparagine in food, and thereby reduce the risk of formation of acrylamide. Acrylamide is formed as a reaction product between asparagine and reducing sugars when certain foods are baked or fried at temperatures exceeding 120^{o}C. Both asparagine and reducing sugars are commonly found in many raw food materials. Dietary exposure to acrylamide has been identified as of potential concern by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).[1]

In Canada, enzymes used in food applications, such as asparaginase, may be considered food additives, depending on their conditions of use. Permitted food additives are listed in the food additives tables in Division 16 of the Regulations. Table V lists those food additives that may be used as food enzymes in Canada.

[1] *Evaluation of certain food contaminants* (Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series 930, 2006. Available at: http://www.who.int/ipcs/publications/jecfa/reports/en/index.html

Before a new food additive is allowed to be used in Canada, a submission must be filed with Health Canada so the Department can conduct a safety evaluation of the proposed use(s) of the additive. Food manufacturers are not permitted to use the additive until the safety assessment has been completed by Health Canada and the Regulations are amended to formally enable its use.

CURRENT SITUATION

Health Canada received a food additive submission seeking the approval of the enzyme asparaginase for use in the manufacture of wheat dough-based products such as bread, crackers and cookies, cut potato products such as French fries, sliced potato products and fabricated potato chips. Asparaginase meets the regulatory definition of a food additive, but is not currently listed in Table V, Division 16 of the Regulations.

Health Canada has completed a safety assessment of the food additive submission and determined that there are no public health or safety concerns with the use of asparaginase in certain food products, as described in the submission. In addition, efficacy data demonstrated that the use of asparaginase results in less acrylamide in foods. Therefore, Health Canada is proposing amendments to the Regulations to allow asparaginase to be used in the manufacture of bread and unstandardized foods.

SAFETY ASSESSMENT OF ASPARAGINASE

Health Canada scientists conducted a detailed and rigorous pre-market evaluation of the submission that focussed on safety. The evaluation considered the toxicological aspects of the proposed use of the additive, as well as relevant microbiological and nutritional factors, as described in this section.

Product Development and Formulation

Asparaginase is produced by pure culture submerged fed-batch fermentation of a genetically modified strain of *Aspergillus oryzae* (*A. oryzae*) carrying a gene coding for an asparaginase enzyme from *A. oryzae*.

Under the Regulations, food enzyme preparations must meet the quality specifications of the *Food Chemicals Codex* (FCC). With respect to chemical specifications, the FCC limits the amount of lead in an enzyme preparation to 5

mg/kg. The level of lead in a batch of unstandardized liquid asparaginase enzyme concentrate was reported to be less than 1 ppm (i.e. less than 1 mg/kg).

Dietary Exposure

For dough-based products, asparaginase is added to the dough before baking. The recommended dosage of asparaginase preparation is 200-2500 ASNU[2] per kilogram (200-2500 ASNU/kg), on the basis of the final processed food (i.e. after baking or frying).

For French fries, the enzyme treatment will potentially be done by dipping potato pieces in an enzyme bath having a concentration up to 12 000 ASNU per litre of water (12 000 ASNU/L water) for a specified holding time. Potato chips could also be made with this type of enzyme bath.

In a conservative approach for the dietary exposure assessment, Health Canada overestimated the potential intake of total organic solids (TOS) from the asparaginase preparation using a worst-case scenario. This "worst-case"-type scenario involved the calculation of the maximum amount of TOS that could be applied to food, assumed that this amount of TOS will be on all treated foods, and assumed that the preparation will have wider use than will, in fact, be the case. The resultant estimate of TOS intake was considered in the toxicological assessment.

It is expected that the enzyme will be largely heat-inactivated during the manufacture and/or preparation of food (for example, during baking or frying).

Toxicological Assessment

The host organism, *A. oryzae*, is described as having a long history of safe industrial use, being widely distributed in nature and being commonly used for production of food-grade enzymes. The expression plasmid, which contains a gene encoding the *A. oryzae* asparaginase, is well characterized and, according to the petitioner, the introduced DNA does not code for any known harmful or toxic substance.

The petitioner indicated that a sequence homology percentage assessment of the *A. oryzae* asparaginase to known toxins and allergens was performed. The largest homology identified was 16.2%, indicating that the homology to any known toxin

[2] Asparaginase converts the L-asparagine into L-aspartate and ammonia. The unit of asparaginase activity is the ASNU. One ASNU is the amount of enzyme that produces one micromole ammonia per minute under specific reaction conditions.

or allergen sequence in the databases used was very low. The homology of sequence fragments from *A. oryzae* asparaginase was also compared to sequence fragments in known allergens and protein toxins. It has been reported that an immunological significant sequence similarity requires a match of at least eight (8) contiguous identical residues. No significant homology of sequence fragments between asparaginase and known allergens or protein toxins was found.

The safety of the asparaginase enzyme preparation was also evaluated through the toxicological review of studies provided by the petitioner, which included a 13-week oral toxicity study in rats, an Ames Mutagenicity test and a chromosomal aberrations assay using human lymphocytes. In the 13-week study, a No Observable Effect Level (NOEL) was established at 0.88 g TOS/kg bw/day. Asparaginase did not induce point mutation in the bacterial assay and was not clastogenic in the chromosomal aberration assay.

There is a safety margin of more than 300 between the NOEL and the maximum potential intake of TOS estimated in the dietary exposure assessment. Based on this information, Health Canada scientists had no toxicological objections to the use of asparaginase under the proposed conditions of use.

Microbial Assessment

Health Canada scientists conducted a molecular and microbiological review of the production strain and the enzyme manufacturing process to determine the acceptability and safety of the final enzyme preparation. Health Canada scientists noted that the genetic material transferred to the micro-organism is well-characterised and limited in size, and that the production organism is not present in the final enzyme product. Moreover, the asparaginase preparation is subject to further scrutiny by toxicological testing and meets JECFA and FCC specifications for enzyme preparations. Based on this information, Health Canada scientists had no further questions from a molecular biology perspective.

Nutritional Assessment

Assuming that only asparagine and potentially glutamine are being hydrolysed, and since both are non-essential amino-acids (they are not required in the human diet), Health Canada scientists concluded that there are no foreseeable nutritional risks or concerns that would be associated with the introduction of this asparaginase enzyme for the reduction of acrylamide in the food products listed.

RATIONALE FOR ACTION

- Dietary exposure to acrylamide has been identified as a potential concern by the Joint FAO/WHO Expert Committee on Food Additives (JECFA); therefore, Health Canada is supportive, in general, of efforts by industry to find ways to reduce acrylamide in foods;

- The purpose for using asparaginase in food manufacture is to reduce the risk of acrylamide formation in baked or fried food products. Enabling the use of asparaginase would provide the industry with the option of using this enzyme in the production of foods sold in Canada;

- The information provided by the petitioner has satisfactorily met the requirements for a food additive submission outlined in section B.16.002 of the Regulations.

INTERNATIONAL STATUS

Asparaginase is permitted for use in the United States, Australia, New Zealand, and Denmark, and has been given a favourable evaluation by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

RECOMMENDATIONS

Through their evaluation of the submission, Health Canada scientists have concluded that the use of asparaginase as a food additive in the manufacture of wheat dough-based products such as bread, crackers and cookies, cut potato products such as French fries, sliced potato products and fabricated potato chips would not raise any health or safety concerns.

Given that asparaginase is a rather unique food additive that may be of benefit to the health of Canadians insofar as it can reduce acrylamide formation, Health Canada has assigned a high priority to enabling the use of asparaginase. The Department has proposed amendments to the Regulations that will allow asparaginase to be used as a food additive in wheat dough-based products such as bread, crackers and cookies, cut potato products such as French fries, sliced potato products and fabricated potato chips, at a maximum level of use consistent with Good Manufacturing Practice, that is, the minimum amount needed for the intended technical effect.

The proposed listing would appear in Table V, Division 16 of the Regulations as follows:

Column I *Additive*	*Column II* *Permitted Source*	*Column III* *Permitted in or Upon*	*Column IV* *Maximum Level of Use*
Asparaginase	*Aspergillus oryzae* (pCaHj621/BECh2#10)	(1) Bread (2) Unstandardized foods	(1) Good Manufacturing Practice (2) Good Manufacturing Practice

In addition, paragraph B.13.021(h) would be amended to allow asparaginase to be used in breads having a standard of identity and composition in the Regulations.

COMMENTS

Comments on this proposal may be submitted in writing, either electronically or by regular mail. If you are submitting your comments electronically, please use the word "asparaginase" in the subject box of your e-mail. In order to fulfill Health Canada's obligation of allowing the international community opportunity to comment, this consultation has been re-opened for a period of 75 days. **Comments must be received by 12:00 a.m. EDT, on February, 21 2010.**

Please submit your comments to:

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ADDITIONAL INFORMATION

For more information on this initiative, please contact the Chemical Health Hazard Assessment Division.