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Original Submission

000001

# **McKenna & Cuneo, L.L.P.**

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March 9, 2001

**Gary L. Yingling**

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## **VIA HAND DELIVERY**

Office of Premarket Approval  
Center for Food Safety & Applied Nutrition  
Food and Drug Administration  
200 C Street, S.W. (HFD-200)  
Washington, DC 20204

**Re: GRAS Notification - Exemption Claim for a Pullulanase  
Enzyme Preparation that is the Subject of a GRAS  
Affirmation Petition, GRASP 5G0415**

Dear Sir or Madam:

Please find enclosed a GRAS notification exemption claim for a pullulanase enzyme preparation that is the subject of a GRAS affirmation petition. This is being submitted in triplicate. If you have any questions, please do not hesitate to contact me.

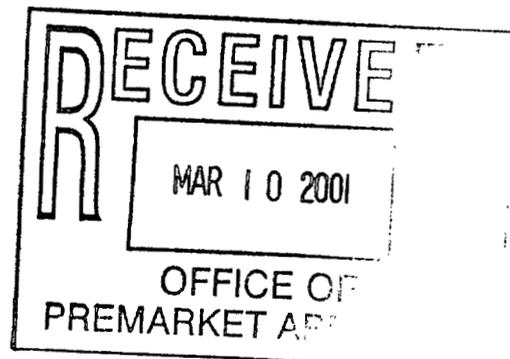
Sincerely,

Gary L. Yingling

GLY/lh

Enclosure(s)

cc: Dr. Linda Kahl, FDA OPA



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Genencor International, Inc.®

925 Page Mill Road  
Palo Alto, California 94304  
650.846.7500  
650.845.6500 fax  
www.genencor.com

March 7, 2001

Office of Premarket Approval  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
200 C Street SW  
Washington, DC 20204

**RE: GRAS Notification - Exemption Claim for a Pullulanase Enzyme  
Preparation that is the Subject of a GRAS Affirmation Petition,  
GRASP 5G0415**

Dear Sir or Madam:

Pursuant to proposed 21C.F.R. §§ 170.36(c)(1), 170.36(g)(2), and the Food and Drug Administration ("FDA") preamble discussion concerning the submission of a Generally Recognized As Safe ("GRAS") notification based on a previously filed GRAS affirmation petition, 62 Fed. Reg. 18938, 18953-18954 (April 17, 1997), Genencor International ("Genencor") is hereby providing FDA with notice that it has determined, based on scientific procedures, that a pullulanase enzyme preparation, a direct food ingredient, is Generally Recognized As Safe ("GRAS") and therefore is exempt from statutory premarket approval requirements. The pullulanase enzyme preparation is also the subject of a GRAS Affirmation Petition (5G0415) submitted by Solvay Enzymes (which was later acquired by Genencor), to the FDA on June 27, 1995.

The following information is provided in accordance with the proposed regulation.

Proposed §170.36(g)(2)(i): Name and address of the notifier.

Genencor International Inc.  
925 Page Mill Road  
Palo Alto, California 94304

Proposed §170.36(g)(2)(ii): The applicable GRAS affirmation petition number.

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A GRAS Affirmation Petition for the pullulanase enzyme preparation was originally submitted by Solvay Enzymes on June 27, 1995, and assigned a petition number, GRASP 5G0415. The FDA filed GRASP 5G0415 on August 18, 1995 (60 Fed. Reg. 43158). Genencor notified FDA on February 27, 1997 of the purchase of Solvay Enzymes and therefore the transfer of GRASP 5G0415 to Genencor.

Proposed §170.36(g)(2)(iii): The common or usual name of the substance (i.e., the notified substance).

The pullulanase enzyme preparation is the common or usual name for the substance for which the GRAS affirmation petition was submitted and this notification is made. Specifically, the pullulanase enzyme preparation is derived from *Bacillus licheniformis* containing the pullulanase gene from *B. deramificans*.

Proposed §170.36(g)(2)(iv): Applicable conditions of use.

As discussed in greater detail in GRASP 5G0415, as amended, the pullulanase enzyme preparation is a direct food ingredient. The pullulanase enzyme preparation's primary functional use is as a processing aid in the manufacturing of starch hydrolysates (maltodextrins, maltose, and glucose) and high fructose corn syrup (HFCS). Pullulanase hydrolyzes the  $\alpha$ -1,6- linkages in amylopectin and pullulan during the saccharification of starch to dextrose and other carbohydrate products. In combination with  $\alpha$ -amylase,  $\beta$ -amylase, and glucoamylase to hydrolyze the  $\alpha$ -1,4- links, pullulanase allows efficient hydrolysis of starch under large scale commercial starch processing conditions.

The pullulanase enzyme preparation is GRAS for use in food at levels not to exceed Good Manufacturing Practices ("GMPs"). Current GMPs results in typical usage level in starch saccharification of 0.023 mg of enzyme protein per gram of starch (= 2.3 ppm in starch).

The pullulanase preparation is not expected to remain as a component of finished starch-derived food ingredients at levels close to its initial concentration in liquefied starch, due to the repeated purification steps to which the food products are subjected. The remaining enzyme residue in HFCS will not exceed 0.01% of the initial concentration (2.3 ppm), which is approximately 0.23 ppb. In glucose syrup and crystalline glucose, the remaining enzyme residue will not exceed 23 ppb, with an estimated total removal of 99%.

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The data and information to support the above uses are contained in GRASP 5G0415, as amended.

Proposed § 170.36(g)(2)(v): Basis for GRAS determination.

This GRAS determination is based on scientific procedures.

Proposed § 170.36(g)(2)(vi): Availability of information.

The complete record that supports the GRAS determination has been submitted to the agency in the above referenced GRASP 5G0415, as amended. The complete file is at FDA.

Sincerely

Cynthia Z. Wang  
Specialist, Regulatory  
and Environmental Affairs

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Submission End

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**McKenna & Cuneo, L.L.P.**

Attorneys at Law

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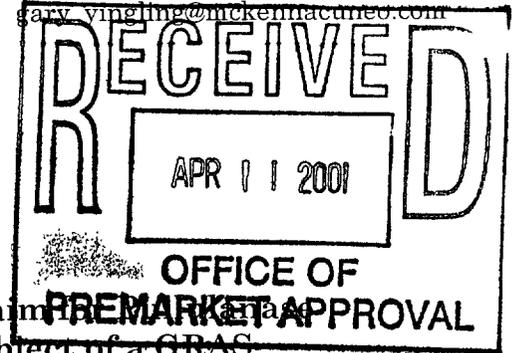
April 5, 2001

Gary L. Yingling

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Office of Premarket Approval  
Center for Food Safety & Applied Nutrition  
Food and Drug Administration  
200 C Street, S.W.  
Washington, DC 20204



Re: GRAS Notification – Exemption Claim for an Enzyme Preparation that is the Subject of a GRAS Affirmation Petition; GRASP 5G0415: Correction of Submission

Dear Sir or Madam:

On March 9, 2001, we submitted on behalf of our client, Genencor International Inc., a GRAS notification for a pullulanase enzyme. Upon review of the submission, it has been called to our attention that there is a typo on page two in the second paragraph under the heading “Proposed § 170.36(g)(2)(iv): Applicable conditions of use.” The sentence reads:

Current GMPs results in typical usage level in starch saccharification of 0.023 mg of enzyme protein per gram of starch (= 2.3 ppm in starch).

The error occurs in that the average level of starch saccharification should be 0.0023 mg of enzyme. In preparation of the document, a zero was omitted. If one looks at the original petition dated June 27, 1995, page 24 (see attachment), the discussion properly reflects 0.0023 mg or 2.3 micrograms (µg).

Please insert the corrected page, which is attached, which has the correct value of 0.0023 mg, into the notification.

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**McKenna & Cuneo, L.L.P.**

Attorneys at Law

Office of Premarket Approval  
April 5, 2001  
Page 2

If you have any questions, please do not hesitate to call.

Sincerely,

Gary L. Yingling

GLY/lh  
Enclosure(s)  
cc: Genencor International

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GRAS AFFIRMATION PETITION  
SOLVAY ENZYMES, INC.

June 27, 1995

Page 24

The pullulanase enzyme is intended to be added to alpha-liquefied starch during saccharification with glucoamylase, which catalyzes the stepwise hydrolysis of  $\alpha$ -1,4-linkages in starch to release glucose units. Pullulanase functions by hydrolyzing the 1,6- $\alpha$ -glucosidic linkages at the branching sites of amylopectin, a major component of starch. Because these branching sites are hydrolyzed more rapidly by pullulanase than by glucoamylase, for which hydrolysis of 1,6- $\alpha$ -linkages is a secondary activity, the use of pullulanase in conjunction with glucoamylase allows the saccharification process to be carried out in a shorter period of time; it also permits the process to proceed at higher dissolved solids levels and with lower dosage levels for glucoamylase, and increases the final glucose yield.

The subject enzyme preparation will be employed during saccharification at a typical usage level of 0.075 PU (pullulanase unit) per gram of starch. The specific activity of the preparation is approximately 33 PU/mg protein (see page 13, Table 3, above). Thus, the enzyme will be added at a level of 0.0023 mg, or 2.3 microgram ( $\mu$ g), of protein per gram of starch [ $0.075 \text{ PU} \div 33 \text{ PU/mg} = 0.0023 \text{ mg}$ ]. This is equivalent to a concentration of 2.3 part per million (ppm) in the starch.

It should be noted that the pullulanase enzyme preparation is not expected to remain as a component of finished

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Pullulanase Enzyme Preparation

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**Kirkpatrick & Lockhart LLP**

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AM



**FAX**

Date • June 6, 2001

No. of Pages, 2  
 including  
 coversheet •

**Transmit To •**

Name	Company	Phone	Fax
Rudaina Alrefai	FDA	202-418-3034	202-418-3131

**From • Gary L. Yingling**

**Phone • 202.778.9124**

**Secretary • Lorraine Higgins**

**Phone • 202.778.9273**

Client/Matter Name	Client/Matter Number	Attorney Number
Genencor	0306453.0100	3052

**COMMENTS:**

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May 25, 2001

Office of Premarket Approval  
Center for Food Safety and Applied Nutrition  
Food and Drug Administration  
200 C Street SW  
Washington, DC 20204

**RE: GRAS Notice (GRN) No. 000072**

Dear Sir or Madam:

Genencor International ("Genencor") is hereby providing FDA with information that all of Genencor's enzyme preparations, including pullulanase enzyme preparation that is derived from *Bacillus licheniformis* containing the pullulanase gene from *B. deramificans* and is the subject of GRAS Notice (GRN) No. 000072, are tested in compliance with the 4<sup>th</sup> edition of Food Chemical Codex (FCC IV). If you have further questions, please contact me at 650-846-7625.

Sincerely

Cynthia Wang  
Regulatory Affairs Specialist  
Genencor International Inc.

Cc: Dr. Rudaina H. Alrefai  
Cc: Mr. Gary Yingling

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