

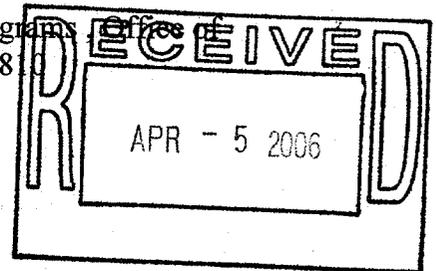
Memorandum

Date: MAR 20 2006

From: Consumer Safety Officer, Division of Dietary Supplement Programs, Office of
Nutritional Products, Labeling and Dietary Supplements, HFS-810

Subject: 75-Day Premarket Notification of New Dietary Ingredients

To: Dockets Management Branch, HFA-305



Subject of the Notification: *Bacillus subtilis* Strain DB9001

Firm: B A U Inc.

Date Received by FDA: 12/20/05

90-Day Date: 3/20/06

In accordance with the requirements of section 413(a) of the Federal Food, Drug, and Cosmetic Act, the attached 75-day premarket notification and related correspondence for the aforementioned substance should be placed on public display in docket number 95S-0316 as soon possible since it is past the 90-day date. Thank you for your assistance.

____Victoria Lutwak____

19955-0316

RPT 324



MAR 3 2006

Jin Hashimoto
B A U Inc.
820 Davis Street
Evanston, Illinois 60201

Dear Mr. Hashimoto:

This is to inform you that the notification, dated December 15, 2005, that you submitted pursuant to 21 U.S.C. 350b(a)(2)(section 413(a)(2) of the Federal Food, Drug, and Cosmetic Act (the Act)) was filed by the Food and Drug Administration (FDA) on December 21, 2005. Your notification concerned the substance that you identified as "DB9011 Spore" which you obtain from endospores of *Bacillus subtilis* strain DB9011. You intend to market "DB9011 Spore" as a new dietary ingredient.

Your notification states that your product will consist of "7.5 x 10⁸ cfu of DB9011-Spore contained in a capsule with lactose". According to your notification, the conditions of use will be "Take one capsule per day", "not recommended for pregnant women and infants" and "A physician should always be consulted for your health problems and symptoms. Discontinue use of this product and immediately consult a medical professional if any adverse reaction occurs."

Under 21 U.S.C. 350b(a), the manufacturer or distributor of a dietary supplement containing a new dietary ingredient that has not been present in the food supply as an article used for food in a form in which the food has not been chemically altered must submit to FDA, at least 75 days before the dietary ingredient is introduced or delivered for introduction into interstate commerce, information that is the basis on which the manufacturer or distributor has concluded that a dietary supplement containing such new dietary ingredient will reasonably be expected to be safe. FDA reviews this information to determine whether it provides an adequate basis for such a conclusion. Under section 350b (a) (2), there must be a history of use or other evidence of safety establishing that the new dietary ingredient, when used under the conditions recommended or suggested in the labeling of the dietary supplement, will reasonably be expected to be safe. If this requirement is not met, the dietary supplement is considered to be adulterated under 21 U.S.C. 342(f) (1) (B) because there is inadequate information to provide reasonable assurance that the new dietary ingredient does not present a significant or unreasonable risk of illness or injury.

FDA has carefully considered the information in your submission and the agency has concerns about the evidence on which you rely to support your conclusion that a dietary supplement containing "DB9011 Spore" will reasonably be expected to be safe.

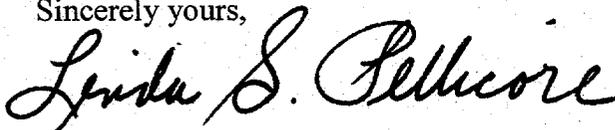
FDA was unable to determine the identity of "DB9011 Spore". For example, it is not clear if your ingredient is composed solely of the organism or also includes culture medium or other substances introduced during production. In addition, the serving level of your ingredient is unclear. Your notification states that each capsule will contain 7.5×10^8 cfu of "DB9011 Spore", however, the serving level is not described in grams or other units of mass. In addition, the ingredients of the products and test materials that are discussed in the information that you submit as evidence of safety are unclear. For example the materials used in the animal feeding studies are not clearly described and are clearly different from one another since, for example, the organism appears to have been prepared at a 50-fold higher density (5×10^9 cfu/g) to produce the feed given to the chickens (Attachment M) compared to the 1×10^8 cfu/g material used to prepare feeds for the calves (Attachment K) and the pigs (Attachment L). Because the identity and serving level of "DB9011 Spore" are unclear, it is unclear how it is quantitatively or qualitatively related to the "DB9011 Spore Dietary Supplement" products and animal feed products that are described in the history of use and other evidence of safety that you provide.

For the reasons discussed above, the information in your submission does not provide an adequate basis to conclude that "DB9011 Spore" when used under the conditions recommended or suggested in the labeling of your product, will reasonably be expected to be safe. Therefore, your product may be adulterated under 21 U.S.C. 342(f)(1)(B) as a dietary supplement that contains a new dietary ingredient for which there is inadequate information to provide reasonable assurance that such ingredient does not present a significant or unreasonable risk of illness or injury. Introduction of such a product into interstate commerce is prohibited under 21 U.S.C. 331(a) and (v).

Your notification will be kept confidential for 90 days after the filing date of December 21, 2005. After the 90-day date, the notification will be placed on public display at FDA's Docket Management Branch in docket number 95S-0316. Prior to that date, you may wish to identify in writing specifically what information you believe is proprietary, trade secret or otherwise confidential for FDA's consideration.

If you have any questions concerning this matter, please contact Linda S. Pellicore, Ph.D. at (301) 436-2375.

Sincerely yours,



for

Susan J. Walker, M.D.

Director

Division of Dietary Supplement Programs

Office of Nutritional Products, Labeling

and Dietary Supplements

Center for Food Safety and Applied Nutrition

BAU, Inc.

820 Davis Street, Evanston, Illinois 60201
Tel: 847 866-1848 Fax: 847 866-0407

December 15, 2005

Division of Standards and Labeling Regulations
Office of Nutritional Products, Labeling, and Dietary Supplements (HFS-820)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Parkway
College Park, MD, 20740-3835



Re: New Dietary Ingredient Notification: *Bacillus subtilis* Strain DB9011 Spore

Dear Madam/Sir:

In accordance with FDA regulations at 21 CFR §190.6, BAU Inc is hereby issuing this 75-day premarket notification for its intent to market *Bacillus subtilis* Strain DB9011 Spore as a new dietary ingredient.

The original notification is enclosed along with two copies and a manuscript for public display.

This notification includes:

1. Notification text
2. Attachment Index
3. Attachments A – M

BAU Inc considers some parts in attachments to be trade secret in accordance with 21 CFR §20.61 and submits the manuscript for public display for your convenience.

Thank you for your attention to this matter. If you have any questions regarding this notification, please do not hesitate to call me at 847-866-1848, or e-mal to hashimoto@bauinc.com. We will respond promptly to any questions you might have.

Very truly yours,
BAU, Inc.


Jim Hashimoto
Secretary

2005-8584
AIMS

Re: Premarket Notification of New Dietary Ingredient

To whom it may concern:

Pursuant to 21 C.F.R. §190.6, BAU Inc submits this premarket notification of its intent to distribute a dietary supplement that contains a new dietary ingredient -- namely *Bacillus subtilis* Strain DB9011 Spore.

1. Name and Complete Address of Manufacturer or Distributor of Dietary Supplement or of the New Dietary Ingredient

Distributor of supplement:

BAU, Inc.

820 Davis Street

Evanston, Illinois 60201

Manufacturer of supplement:

Bio and Medical Informatics, Inc.

4-9-3, Shiba, Minato-ku, Tokyo 108-0014, Japan

2. Name of the new dietary ingredient

Bacillus subtilis Strain DB9011 Spore (DB9011-Spore). A novel bacterium isolated from soil resulted in belonging to *Bacillus subtilis* by the Bergy's manual of systematic bacteriology Vol.2 (Attachement A) and having aflatoxin decomposing ability (Attachment B). This new bacterium was deposited on May 21, 1991 under Fermentation Research Institute Accession Number 3418 (FERM BP-3418) at The National Institute of Advanced Industrial Science and Technology, an Independent Administrative Institution in association with the Ministry of Economy, Trade and Industry (formerly known as Fermentation Research Institute) in accordance with the Budapest protocol. It has also named "*Bacillus subtilis* DB9011". *Bacillus subtilis* is generally known as Gram-positive, aerobic, rod shaped, and spore forming

bacteria. The spore structure is extraordinarily resistant to environmental stresses such as heat, ultraviolet radiation, gamma radiation, chemical disinfectants, and desiccation. The spore formed by *Bacillus subtilis* DB9011, DB9011-Spore, is the new dietary ingredient in this notification.

3. Description of dietary supplement that contains the new dietary ingredient

a. Level of new dietary ingredient in dietary supplement

7.5×10^8 cfu of DB9011-Spore is contained in a capsule with lactose

b. Conditions of use

Take one capsule per day

Not recommended for pregnant women and infants.

A physician should always be consulted for your health problems and symptoms.

Discontinue use of this product and immediately consult a medical professional if any adverse reaction occurs.

4. History of use or other evidence of safety

Bacillus subtilis is widely used for food, livestock food additive, and animal medicine (Attachment C). DB9011-Spore was developed as livestock food additive under the entrustment by AHC Co., Ltd., the patent holder. It was initially approved for the use of hog food additive in August 1995 by the Ministry of Agriculture, Forestry and Fisheries of Japan and then approved for the use of calf and fowl food additives. DB9011-Spore has started to be commercialized in Japan 1995 for pig food additive and extended for calf and chicken in 1997 and 2003, respectively (Attachment D). The recommended feeding amount to calf is $1 - 2 \times 10^9$ cfu/day. To hog, the feed containing DB9011-Spore at 5×10^5 cfu/g is fed ad lib. The product for pig is commonly used to chicken. As of today no adverse event has been reported. DB9011-Spore has been commercialized for dietary supplement for human in Japan since March, 2004 at range of 7.5×10^7 and 1.5×10^{10} cfu per body as the recommended daily amount (Attachment E). As of September 7, 2005, 35 claims have been informed to Bio and Medical Informatics, Inc, a manufacturer and distributor of DB9011-Spore dietary supplement in Japan, at phone number (03-5765-7658) and e-mail (desk@krtpls.net) expressed on the package of the commercial product in Japan. There is just one case where the clinical symptom was claimed. A patient in admission with pulmonary hematoma complied edema in lower limbs. However this symptom

was considered to be appeared by the circular disturbance without any relation to taking the commercial product.

Other data indicating the safety of DB9011-Spore are also available.

Acute oral toxicity study in mice

An acute toxicity study in mice was conducted by Tokyo Food Sanitation Association Food Research Laboratory, which is one of the inspection agencies authorized by the Ministry of Health, Labor and Welfare (formerly known as the Ministry of Health and Welfare) in Japan. The test article provided for the study, named as "Bacillus subtilis DB9011", contained 5×10^6 cfu of DB9011-Spore per gram (Attachment F). ddY mice of 5 week-old received the test article at 6 g/kg bw, corresponding to 3×10^7 cfu/kg bw of DB9011-Spore. No mortality was observed in this study (Attachment G).

Acute oral toxicity and local stimulation studies in mice

These studies were also outsourced to Tokyo Food Sanitation Association Food Research Laboratory with providing another formulation, a suspension containing 1×10^7 cfu of DB9011-Spore per mL, named as "Bacillus subtilis DB9011" (Attachment F).

ddY mice of 5 week-old received the suspension at 20 mL/kg body weight, corresponding to 2×10^8 cfu/kg bw of DB9011-Spore. No mortality was observed in this study (Attachment H).

No symptom was observed on the skin of 5 week-old ddY mice applied with approximately 4×10^6 cfu at three times a day with 1 hour-interval in two consecutive days (Attachment H).

No symptom was observed on the ocular mucous membrane of 5 week-old ddY mice of which an eye ball applied with approximately 2×10^5 cfu at three times a day with 1 hour-interval in two consecutive days (Attachment H).

Acute oral toxicity study in rats

An acute toxicity study in rats was conducted by Nihon Bioresearch Inc (Attachment I). The test article provided for the study, named as "DB9011", contained 1×10^{10} cfu of DB9011-Spore per gram. As mentioned at "test article" in the report, the test article was a lactose formulation containing 1.0×10^{10} cfu/g DB9011-Spore. Sprague-Dawley rats of 5 week-old

received the test article at the dose of 0, 20, 200, 2,000 mg/kg ($0, 2 \times 10^8, 2 \times 10^9, 2 \times 10^{10}$ cfu/kg of DB9011-Spore). No animal deaths occurred in any DB9011 group for both sexes, with no effect of the test substance on general sign, time-course changes in body weight, and necropsy findings. Based on these results, minimum lethal dose of DB9011 was estimated to be at least 2,000 mg/kg in rats and safety was considered to be excellent.

Reverse mutation study

A reverse mutation study was conducted by Nihon Bioresearch Inc (Attachment J). The mutagenic potential of DB9011-Spore was examined by a reverse mutation test using *Salmonella typhimurium* TA100, TA98, TA1535, and TA1537, and *Escherichia coli* WP2uvrA. The study was performed by the pre-incubation method in the absence and presence of S9 mix. There were no mutagenic potential observed.

Long term intake study in calves

A calf intake of DB9011-Spore was studied in order for Japanese government approval of calf food additive products using DB9011-Spore (Attachment K). The test article, DB9011-Spore in lactose, was administered to 5 – 15 day-old milk cows with the daily dose of 1×10^8 cfu/kg of DB9011-Spore for 35 days. For test group, this article was dissolved in Milk substitute at 10% resulting 1×10^7 cfu/g of DB9011-Spore. For control group, lactose was dissolved in Milk substitute at 10%. The Milk substitute was fed 400 g/day for 35 days for both groups. The average body weight of the test group was 39.2 kg at the beginning, 44.0 kg at 14th day, 61.6 kg at 35th day. Therefore, It was assumed that the daily feeding amount of DB9011-Spore during the study period was 1×10^8 cfu/kg bw during 1st – 14th days and $0.6 - 1 \times 10^8$ cfu/kg bw during 15th – 35th days.

In all groups, no mortality, no signs of effect on general condition and weight transition, were observed. Administration of this probiotic significantly improved feed conversion rate. Observation of fecal condition indicated no negative effect of *Bacillus subtilis* DB9011 on clinical condition.

Long term intake study in pigs

A pig intake of DB9011-Spore was studied in order for Japanese government approval of pig food additive products using DB9011-Spore (Attachment L). A 40-day feeding study was conducted in 21 day-old pigs with the test article, DB9011-Spore in lactose, at the daily dose of 6×10^7 cfu/kg of DB9011-Spore. For test group, this article was mixed in feed at 0.5% resulting 5×10^5 cfu/g of DB9011-Spore. For control group, lactose was mixed in feed at 0.5%. The feed was fed 800 g/day for 40 days for both groups. The average body weight of the test group was 6.32 kg at the beginning, 16.4 kg at 20th day, 25.9 kg at 40th day. Therefore, it was assumed that the daily feeding amount of DB9011-Spore during the study period was 6×10^7 cfu/kg bw during 1st – 20th days and $1.5 - 2.4 \times 10^7$ cfu/kg bw during 21st – 40th days.

In all groups, no mortality, no signs of effect on general condition and weight transition, were observed. Administration of *Bacillus subtilis* DB9011 appears to have no negative effect on fecal and general health condition.

Long term intake study in chickens

A chicken intake of DB9011-Spore was studied in order for Japanese government approval of chicken food additive products using DB9011-Spore (Attachment M). An 8-week feeding study was conducted in 0 day-old chunky broiler chickens with DB9011-Spore in a range of 1.5×10^8 cfu/kg/day - 1.4×10^{10} cfu/kg/day, and 1.5×10^9 cfu/kg/day – 1.4×10^{11} cfu/kg/day. This article was mixed in feed to have 1×10^7 cfu/g (Proposed dose group) and 1×10^8 cfu/g (10-fold dose group). The vehicle was prepared for the control group. Animals in three groups were fed for 8 weeks. Daily amount of DB9011-Spore fed was 6.19×10^8 cfu/kg bw in the proposed dose group and 6.20×10^9 cfu/kg bw in the 10-fold dose group.

In all groups, no mortality, no signs of effect on general condition and weight transition, were observed. As no abnormal changes were observed for clinical observation items in the study, hematological, blood biochemistry, pathological, and histopathological tests were not performed.

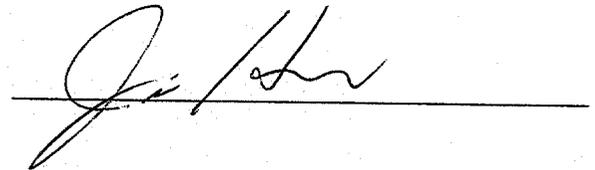
Conclusion

Since this new dietary ingredient is contained 7.5×10^8 cfu in a capsule of the dietary supplement product, the administration range to human is assumed in the range of $8.3 \times 10^6 \sim 7.5 \times 10^7$ cfu/kg bw under the estimation of body weight to be 10 ~ 90 kg. These study results with animals described above suggest the administration at a range of $1.5 \times 10^7 \sim 6.2 \times 10^9$ cfu/kg bw /day can provide no adverse effect to animals studied. Furthermore no adverse effect has been reported yet approximately one and half years in Japanese people. Therefore, the recommended daily amount of DB9011-Spore to human, 7.5×10^8 cfu/day, will reasonably be expected to be safe.

5. Signature of person designated by the manufacturer or distributor

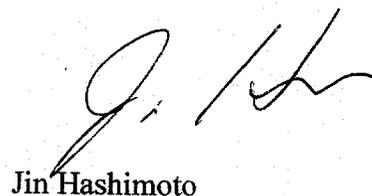
The person designated by the distributor BAU, Inc. is:

Jin Hashimoto
BAU, Inc.
820 Davis Street
Evanston, Illinois 60201
Telephone (847) 866-1848
Fax (847) 866-0407
E-mail : hashimoto@bauinc.com



Should you require any further information or have any questions on this premarket notification, please contact me directly so that I may convey any inquiry from the FDA in a manner to ensure that it is properly understood by BAU, Inc. and its Japanese affiliates.

Very truly yours,



Jin Hashimoto

Attachment Index

- A: (Original in Japanese)
DB9011 株の名称
- A: (Translation)
Name of DB9011
- B: (Original in English)
U.S. Patent
- C: (Original in Japanese)
Bacillus subtilis DB9011 株と類縁の生菌剤に関する資料
- C: (Translation)
Material on a probiotic of *Bacillus subtilis* DB9011 and other relevant strains
- D: (Original in English)
Sales Chart – DB9011-Spore Livestock food additive products
- E: (Original in English)
DB9011-Spore Dietary Supplement Sales Volume
- F: (Original in Japanese)
AHC による送付書 試験品の送付
- F: (Translation)
Shipping Slip of Test article by AHC Co., Ltd.
- G: (Original in Japanese)
急性毒性試験
- G: (Translation)
Acute oral toxicity study in mice
- H: (Original in Japanese)
急性毒性試験+局所刺激性
- H: (Translation)
Acute oral toxicity study in mice, local irritation study (skin, ocular mucosa)
- I: (Original in Japanese)
Bacillus subtilis DB9011 株のラットを用いる単回経口毒性試験
- I: (Translation)
Single Dose Oral Toxicity Study of *Bacillus subtilis* DB9011 in Rats
- J: (Original in English)
Reverse Mutation Test of *Bacillus subtilis* DB9011 with Bacteria

K: (Original in Japanese)

子牛を用いた野外応用試験

K: (Translation)

Field Study Using Calves

L: (Original in Japanese)

子豚を用いた野外応用試験

L: (Translation)

Field Study Using Weaned Pigs

M: (Original in Japanese)

対象家畜等を用いた飼養試験(鶏)

M: (Translation)

Feeding Study Using Target Livestock (Chickens)