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The Safety of Modified Arabinoxylan from Rice Bran (BioBran/MGN-3)

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Introduction

The ingredients in BioBran/MGN-3 are rice bran and shiitake-fungus culture fluid. Rice bran is classified as a grain food and listed as Food Number 1-57 in the 5th edition of the Japan Food Composition Table. The method of extracting constituents from rice bran in the production process for BioBran/MGN-3 is performed with lukewarm water and no organic solvents are used. The extracted polysaccharides, which consist mainly of hemicellulose, are modified with carbohydrase, derived from the shiitake mushroom. The shiitake mushroom has been used in food since ancient times in Japan. A carbohydrase complex is produced from the secondary mycelium of shiitake mushrooms. Carbohydrase preparations are often produced by biosynthesis using one of any number of species of fungi and purified. Based on the result of a study into cost and safety, we chose to use shiitake-fungus culture fluid, being an enzyme preparation which is sufficiently active even after rough purification, and has no safety problems even if present in the final food product. In short, BioBran/MGN-3 has no safety problems because the production process uses no materials that might raise doubts about food safety. However, since the rice bran ingredient is

concentrated in BioBran/MGN-3 and the hemicellulose is partially modified, ingesting BioBran/MGN-3 is slightly different from taking wholegrain rice or rice bran. For this reason, we have conducted experiments to verify the basic safety of BioBran/MGN-3 as a food.

1. Acute oral toxicity

AMA LABORATORIES, INC.(NY, USA)

(1) Animals

Healthy, young adult Wistar derived aibino rats weighing between 150 to 300 grams were obtained from ACE Animals, Inc., Boyertown, Pennsylvania. 5 male and 5 female rats were selected for each dose level chosen for this study.

(2) Procedure

18 to 24 hours prior to dosing, the rats were fasted. During the fast period water was allowed *ad libitum*. After fasting rats were individually weighed. All body weights were recorded and individual doses calculated based on these weights. The test material was then made available at dose levels chosen to achieve test groups with sufficient mortality rates to permit calculation of the LD_{50}. Once the material had been ingested completely, feed and water were provided *ad libitum*. The rats were individually caged and observed for mortality or other signs of gross toxicity for 14 days. At the end of the test period, all surviving animals were weighed.

(3) Conclusions

The above material (AMA Lab No.:B-4881, Client No.:DAI-001 BioBran/MGN-3) when tested as indicated herein may be regarded as NON-TOXIC according to the reference. $LD_{50} > 36.0$ g/kg.

2. Ames salmonella mutagenicity test

Product Safety Laboratries (NJ, USA)

(1) Assey objective

The purpose of this protocol is to evaluate the ability of a chemical, formulation or extract to induce a mutagenic response in 4 different strains of *Salmonella typhimurium*, namely TA98, TA100, TA1535, TA1537 and one strain of *Escherichia coli* WP2 uvrA (pKM101). Test materials are screened at different dose levels by plating them with the tester strains both directly and along with Aroclor™1254 induced rat liver microsomes (S9) . Mutagenic materials cause an increase in revertant colonies above the spontaneous background (i.e. no test material) level.

(2) Overview

The *S. typhimurium* reversion assay is widely used to evaluate the mutagenic properities of chemicals. The test is based on the work of Dr. Bruce Ames and his coworkers and is generally referred to as the Ames Test. Their studies involved the development of select histidine auxotrophs of *S. typhimurium* which are normally growth arrested due to mutations in a gene needed to produce the essential amino acid histidine. In the absence of an external histidine source, the cells cannot grow to form colonies unless a reversion of the mutation occurs which allows the production of histidine to be resumed. The *E.coli* WP2 uvrA (pKM101) is also used to add a DNA repair-proficient strain; it has an AT base pair at the critical mutation site within the *trpE* gene, which is excision proficient (and thus will detect cross-linking agents) and carries the pKM101 plasmid to enhance error-prone repair. As might be expected, spontaneous reversions occur with each of the strains, usually at a low level (with the exception of strain TA 100).

However, chemical agents can induce a mutagenic response so that the number of revertant colonies is substantially higher than the spontaneous background reversion level. The test involves the analysis of the number of revertant colonies that are obtained with each strain in the presence and absence of the test chemical. Since the mutagenic response of a formulation could vary with the concentration, test materials are routinely dosed over an appropriate concentration range. Samples can also be plated in duplicate or triplicate, at the discretion of the sponsor. In this protocol, a complete set of positive and negative controls is included with each assay, and is plated routinely with all of the tester strains. Aroclor™1254 induced rat liver microsomes are included to mimic the *in vivo* activity of the liver enzymes in activating some pro-mutagens to mutagenic status.

(3) Conclusion

The results show that the test strains are sensitive to the positive control mutagens and had a spontaneous reversion rate well within the accepted values of each strain, indicating that under the test conditions, the strains were sensitive to the detection of potentially genotoxic agents. The metabolic activation using the S9 activation mixture shows an active microsomal preparation. Using the same test conditions, there was no detectable genotoxic activity associated with the sample under testing BioBran/MGN-3 Lot # DAI-001, neither in the presence or absence of the S9 enzyme activation, at the following concentrations: 100,000, 50,000, 10,000, 5,000 and 1,000 μ g/mL.

3. Subchronic toxicity study (90-day dietary study in rats)

Product Safety Laboratries (NJ, USA)

(1) Purpose

To determine the oral toxicity and health hazards likely to arise from continuous exposure to BioBran/MGN-3 in the diet over a 90-day period. The assessment of toxicity was limited to the toxicological endpoints evaluated in this study. A No-Observed-Adverse Effect Level (NOAEL) was also sought for each sex based on these endpoints.

(2) Summary

80 Sprague-Dawley rats (40 males and 40 females) were selected for the test and equally distributed into 8 groups (10 per group, males or females only). Dietary levels of 0, 200, 2,000, and 20,000 ppm of the test article were selected for the test. An appropriate amount of the test article was administered in the diet to each rat for 91 consecutive days. The animals were observed for mortality, signs of gross toxicity, and behavioral changes at least once daily the study. Body weights were recorded once during the acclimation period, prior to test initiation (Day0) and weekly thereafter. Individual food consumption was also recorded weekly. Blood was collected from randomly selected animals (3 per group for all groups except for the male control group in which there were 4) on Day 92 prior to necropsy, and analyzed for a series of blood chemistry and hematology parameters. Necropsies were performed on the decedent and all euthanized animals. Tissues and organs of 5 randomly selected animals from each control and high dietary level group were evaluated histologically.

The average dietary intake of Hydrolysate of BioBran/MGN-3 in male rats administered diets at nominal concentrations of 0, 200, 2,000 or 20,000 ppm was 0, 13, 134, and 1,301 mg/kg/day, respectively. The dietary intake of female rats at the same nominal dietary concentrations was 0, 15, 150 and 1562 mg/kg/day, respectively.

(3) Conclusion

There were no significant effects in rats of either sex after 91 consecutive days of BioBran/MGN-3 presented in the diet. Based on the parameters evaluated (which were limited in scope), the No-Observed-Adverse Effect Level (NOALE) for this study is considered to be 20,000 ppm (1,301 mg/kg/day in males and 1,562 mg/kg/day in females).

4. Guinea Pig Antigenicity Study

Product Safety Laboratries (NJ, USA)

(1) Procedure

The first two group of animals were exposed to the test or control articles over a 3-week sensitization period. During this time, each animal was injected intra-peritoneally (IP) with 10 mg/kg of the test or control article 3 days per week for 3 successive weeks. The test and positive control articles were administered as 1% w/w mixtures in physiological saline. The doses were adjusted weekly based on the most recent body weight. After the last IP injection, the animals were allowed to rest for 17 days. Following the rest period, the guinea pigs were challenged with 2 mg/kg of the test or control articles by intravenous (IV) injection. The test or positive articles were administered as 0.2% w/w mixtures in physiological saline.

In addition to the test and positive control animals, ten native control guinea pigs (5 test and 5 positive control) from the same shipment were maintained under identical environmental conditions and treated with the test or control article at challenge only.

During the challenge injections and the 15 minutes thereafter, all animals (test and control) were observed for the following positive reactions:

i licking the nose or rubbing the nose with forefeet
ii rubbing of the fur
iii abored breathing
iv sneezing or coughing (3 or more times)
v retching

The test article would be considered antigenic if any of the challenged test animals show more than two of the listed symptoms or show rales, convulsions, prostration or die. If the corresponding naïve animals exhibit similar signs, the test is considered inconclusive. In order for the test to be considered valid, at least 3 of the positive control animals need to show at least two of the above listed symptoms.

(2) Results

All test substance, test substance naive control, positive control and positive naive control animals survived following the challenge phase injection.

Group1 Test Substance

All test animals appeared active and healthy throughout the sensitization phase. Apart from chewing noted in 8 of 10 test animals 3 to 15 minutes after challenge, no other positive reactions were observed. Overall, this was considered a negative response.

Group2 Positive Control

All positive control animals appeared active and healthy throughout the sensitization phase. Following challenge, all positive control animals exhibited an antigenic response. Positive reactions including chewing, retching, licking, rubbing, coughing, labored breathing, prone posture and/or ataxia were observed in all

animals after challenge.

Group3 Test Substance Naive Control

No positive reactions were noted in any of the test naive control animals following the challenge injection.

Group4 Positive Naive Control

No positive reactions were noted in any of the positive naive control animals following the challenge injection.

(3) Conclusion

Under the conditions of this study, BioBran/MGN-3 is not considered to be antigenic. The positive response in the positive control group to Egg Albumin, a known antigenic agent, validates the system used.

Summary

The safety of BioBran/MGN-3 has been verified by conducting acute oral toxicity, mutagenicity, subacute toxicity, and antigenicity studies. The safety of BioBran/MGN-3 in humans has also been verified by a 6-month ingestion study in cancer patients (Hiroshi Tsunekawa: Effect of Long-term Administration of Immunomodulatory Food on Cancer Patients Completing Conventional Treatments. Clinical Pharmacology and Therapy 14(3): 295-302, 2004). From the results of the studies, BioBran/MGN-3 has been judged to be a highly safe food.