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Safety studies conducted on pecan shell fiber, a food ingredient produced from ground pecan shells

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ABSTRACT

Use of pecan shell fiber in human food is presently limited, but could increase pending demonstration of safety. In a 91-day rat study, pecan shell fiber was administered at dietary concentrations of 0 (control), 50 000, 100 000 or 150 000 ppm. There was no effect of the ingredient on body weight of males or females or food consumption of females. Statistically significant increases in food consumption were observed throughout the study in 100 000 and 150 000 ppm males, resulting in intermittent decreases in food efficiency (150 000 ppm males only) that were not biologically relevant. All animals survived and no adverse clinical signs or functional changes were attributable to the test material. There were no toxicologically relevant changes in hematology, clinical chemistry or urinalysis parameters or organ weights in rats ingesting pecan shell fiber. Any macroscopic or microscopic findings were incidental, of normal variation and/or of minimal magnitude for test substance association. Pecan shell fiber was non-mutagenic in a bacterial reverse mutation test and non-clastogenic in a mouse peripheral blood micronucleus test. Based on these results, pecan shell fiber has an oral subchronic (13-week) no observable adverse effect level (NOAEL) of 150 000 ppm in rats and is not genotoxic at the doses analyzed.

1. Introduction

The typical American consumes approximately 15 g fiber/day, well under the recommended 14 g/1000 calories, or 25 g/day for women and 38 g/day for men [17]. Health benefits associated with high fiber intakes include lower risk for developing coronary heart disease, stroke, hypertension, diabetes, obesity, and certain gastrointestinal diseases [13,3,16]. In an attempt to increase the fiber content of the diet, developers of food ingredients have created new fiber ingredients, some of which are produced by genetically modified organisms. However, due to increased demand by consumers for foods containing "natural" ingredients, there is an increased need for new fiber ingredients from "natural" sources.

Pecans, inclusive of several varieties of *Carya illinoensis*, are among the most preferred of all nuts and an economically important crop in the United States [5]. In 2014, approximately 264 million pounds of pecan nuts (in-shell) were produced in the United States [18]. Pecans are often sold without shells, which are removed during processing and often discarded. Based on a 50% shell-out ratio (ratio of kernel weight to the in-shell nut weight) [10], approximately 132 million pounds of pecan shells are produced by the US pecan industry per year. Pecan shell fiber (also known as pecan shell flour or ground pecan shells) is a food ingredient produced from shells of pecans, excluding the husks and nut kernels. Pecan shell fiber is predominantly composed of insoluble fiber (cellulose, lignin and hemicellulose) and contains small amounts of fat (<4%) and protein (<3%). It also contains approximately 4.5% polyphenols and 10% proanthocyanidins, molecules recognized for antioxidant activity [7]. Therefore, pecan shell fiber has potential as both a fiber ingredient and antioxidant in food formulations.

The American Association of Feed Control Officials (AAFCO)[1] includes Ground Pecan Shells (definition 60.110) in the 2015 official publication of animal feed ingredients [1] as a source of fiber. Although pecan shell fiber is Generally Recognized as Safe (GRAS) for use as a "natural" flavor complex for meat products (at a maxi-

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[1] An association which currently provides input to the FDA on the safety and quality of feed ingredients

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mum level of 3000 ppm (0.3%)) [19], pecan shell fiber is not GRAS for other purposes. Under section 201(s) of the Food Drug and Cosmetic Act, the use of a substance, rather than the substance itself, is eligible for a GRAS determination [9]. Although humans may have been ingesting small amounts of pecan shell fiber in the past, they have not been eating the ingredient at the considerably higher levels expected when used as a source of fiber. To support a higher level of consumption in humans, safe consumption of high levels of pecan shell fiber must be demonstrated in experimental animals. Genotoxicity studies should also be conducted because the potential for genotoxicity due to small levels of contaminants would increase with higher levels of consumption. This publication presents results of a bacterial reverse mutation test, an *in vivo* mouse micronucleus test and a 13-week feeding study in rats that were conducted to expand the use of pecan shell fiber in human food. A search of publicly available information indicated that studies of this nature have not been previously conducted for pecan shell fiber.

2. Materials and methods

2.1. Test articles

One batch of pecan shell fiber (Southeastern Reduction Company, Valdosta, GA) was used for the subchronic oral toxicity study (181L21) and another batch for the genetic toxicity studies (119L22). Both batches of test substance complied with the manufacturer's specifications.

The positive control mutagens utilized in the bacterial reverse mutation assay were: 4-nitro-*o*-phenylene-diamine (4-NOPD) (Fluka), sodium azide (NaN_3) (Sigma), 2-aminoanthracene (2-AA) (Aldrich) and methylmethanesulfonate (MMS) (Sigma). The S9 liver microsomal fraction was prepared at Eurofins BioPharma Product Testing Munich GmbH (Eurofins Munich), from male Wistar rats induced orally with phenobarbital (80 mg/kg bw) and β-naphthoflavone (100 mg/kg bw) for three consecutive days. Media and Vogel-Bonner Medium E agar plates were prepared at Eurofins Munich or obtained from an appropriate supplier.

The positive control substance for the micronucleus study was cyclophosphamide (Sigma), dissolved in physiological saline. The solution was stored at ≤15 °C and thawed on the day of use.

2.2. Animals and organisms

Male and female Sprague-Dawley (SD) rats used in the 90-day study were obtained from Charles River Laboratories (Raleigh, NC) and shipped to the study site (Product Safety Labs, Dayton, NJ). The animals were acclimated for six days prior to testing, and were seven to eight weeks old at study initiation. Animals were housed individually in suspended stainless steel cages, per standard practice of the laboratory. The animal room was maintained under a 12 h light/dark cycle, 19–23 °C and 38–60% relative humidity. Litter paper placed underneath the cages was changed at least three times per week. Rats were supplied basal diet (Open Standard Diet D11112221NM, Research Diets Inc., New Brunswick, NJ) and filtered tap water *ad libitum* except for the before study termination, when food was withdrawn. The basal diet consisted of approximately 60% carbohydrates, 17% protein, 7% fat (added fat as soybean oil), and 9% fiber.

Animals were selected for use in the study based on adequate body weight gain, freedom from clinical signs of disease or injury (aside from two animals with minor ophthalmologic findings) and a body weight within ±20% of the mean within a sex. Rats used in the study were randomly distributed into treatment groups according to stratification by body weight. All animals were fasted overnight prior to blood collection. Serum samples from three animals that were housed with study animals but were not part of the study were evaluated for the absence of common rat pathogens (Rat *parvovirus*, Toolan's H-1 Virus, Kilham Rat Virus, Rat Minute Virus, *Parvovirus* NS-1, Rat *Coronavirus*, Rat *Theilovirus*, and *Pneumocystis carinii*) on the last day of the test period. Because the sentinel samples were negative for all pathogens evaluated, the study animals were deemed healthy and reasonably free of common rat pathogens.

Male and female young, healthy adult NMRI mice (minimum seven weeks of age) used in the micronucleus study were supplied by Charles River, 97633 Sulzfeld, Germany and acclimated for at least five days before use. The weight variation of the animals did not exceed ± 20% of the mean weight of each sex. Animals were housed five/sex/cage in IVC, Type II L polysulfone cages with Altromin saw fiber bedding (Altromin Spezialfutter GmbH, Lage, Germany), in a room maintained under a 12 h light/dark cycle, 22 ± 3 °C and 55 ± 10% relative humidity. Food (Altromin 1324 Maintenance Diet) and tap water were freely available to the mice except for a four hour period before the first dose, during dosing, and a two to three hour period after the last dose of test material or vehicle.

Bacterial strains *Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2*uvrA* were used for the mutagenicity study. *S. typhimurium* TA 98 and TA 1535 and *E. coli* strain WP2*uvrA* were obtained from MOLTOX, INC., NC 28607, USA. *S. typhimurium* TA100 and TA 1537 were obtained from Xenometrix AG, Allschwil, Switzerland. Stock cultures were stored in ampoules with nutrient broth (OXOID) supplemented with dimethyl sulfoxide (DMSO) (approximately 8% volume/volume) over liquid nitrogen. Bacterial suspensions were thawed, grown for 12 h at 37 °C in nutrient medium, and used at a concentration of approximately 1×10^9 cells/ml.

2.3. Guidelines

The subchronic oral toxicity study was conducted in accordance with Good Laboratory Practice (GLP) and OECD Guidelines for Testing of Chemicals, Section 4, No. 408, "Health Effects, Repeated Dose 90-Day Oral Toxicity Study in Rodents", dated September 21, 1998.

The bacterial reverse mutation test was conducted in accordance with the Ninth Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 471, "Bacterial Reverse Mutation Test", adopted July 21, 1997; Commission Regulation (EC) No. 440/2008 B.13/14: "Mutagenicity - Reverse Mutation Test Using Bacteria", dated May 30, 2008 and EPA Health Effects Test Guidelines, OPPTS 870.5100 "Bacterial Reverse Mutation Assay" EPA 712-C-98-247, August 1998.

The experimental procedure used in the mouse micronucleus assay complied with OECD Guideline No. 474 "Mammalian Erythrocyte Micronucleus Test", adopted September 26, 2014.

2.4. Experimental design

2.4.1. Subchronic oral toxicity study

Groups of 10 rats/sex were administered 0, 50 000, 100 000 or 150 000 ppm pecan shell fiber in the diet for 91 days, for target exposures of 0, 3571, 7143 and 10 714 mg/kg bw/day test substance, estimated for a 350 g rat consuming 25 g feed/day. The concentrations were based on the results of a fourteen day dose range finding study in rats. Test diets were prepared weekly by thoroughly blending the test substance into the basal diet with a high-speed mixer. All prepared test and control diets were stored under refrigeration until use. At the initial, middle and final diet preparation, a sample of the test (neat) substance was retained for stability analysis. During the first week of the study, samples from each dietary concentration were taken at first presentation of the