LIMITED SUMMARY OF DATA FOR CHEMICAL SELECTION

Chitosan
9012-76-4

BASIS OF NOMINATION TO THE CSWG

Chitosan is a cationic carbohydrate polymer derived from chitin. It has gained rapid and widespread acceptance as a diet aid following the 1997 publication of *The Fat Blocker Diet* by Arnold Fox and Brenda Adderly. Chitosan also has many other industrial, pharmaceutical, agricultural, and cosmetic applications. A combination of these uses can result in very large daily intakes of chitosan by consumers.

Although several subacute studies in laboratory animals show that chitosan has hypercholesterolemic properties and may influence weight gain, it is plausible that chitosan also causes a deficiency of minerals and fat-soluble vitamins. These concerns are the reason for requesting consideration of specialized studies relating to metabolic alterations caused by chitosan. The results will determine whether any further evaluation of dietary supplements containing chitosan is warranted.

Because chitosan sold for weight loss is classified as a dietary supplement, it does not need FDA approval; because it is a natural substance, little financial incentive exists for manufacturers to conduct their own studies.

INPUT FROM GOVERNMENT AGENCIES/INDUSTRY

Dr. John Walker, Executive Director of the TSCA Interagency Testing Committee, US Environmental Protection Agency, provided information on annual production of chitosan for 1986, 1990, and 1994.

SELECTION STATUS

ACTION BY CSWG: 6/22/99

Studies requested: Mechanistic studies designed to measure potential for Vitamin E depletion and osteoporosis from ingestion

Priority: High

Rationale/Remarks:

- Significant human exposure, especially through use of dietary supplements promoted as “fat trappers” for weight loss

- Potential for ingestion of large amounts of chitosan

- Suspicion of fat soluble vitamin depletion and other toxic effects based on limited animal studies needing confirmation

- Suspicion of carcinogenic potential very low; not recommended for standard battery of carcinogenicity tests

- Use olestra as a model for testing

- Defined functionally by molecular weight, degree of deacetylation, and viscosity; select a range of chitosans used in the dietary supplement industry for testing; consult FDA Drug Master Files.

CHEMICAL IDENTIFICATION

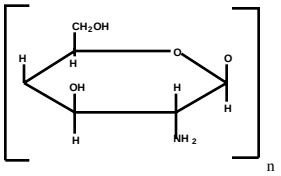
CAS Registry No.: 9012-76-4; 57285-05-0

Chemical Abstracts Service Name: 2-Amino-2-deoxy-- *D*-glucosamine

Synonyms and Trade Names: Chitosan; chitopearl; CTFA 04299; deacetylated chitin; Flonac N; Kytex H; poliglusam; Sea Cure F

Structural Class: Polysaccharide

Structure:



Chemical and Physical Properties:

Description: Chitosan is a modified natural carbohydrate polymer derived from chitin, which occurs principally in animals of the phylum Arthropoda. The primary unit in the chitin polymer is 2-deoxy-2-(acetylamino)glucose. These units are combined by , 1-4 glycosidic linkages, forming a long chain linear polymer. Removal of most of the acetyl groups of chitin by treatment with strong alkalis yields chitosan (Peniston & Johnson, 1980).

Properties: Although chitin is insoluble in most solvents, the properties of chitosan relate to its polyelectrolyte and polymeric carbohydrate character. It is insoluble in water or in alkaline solutions at pH levels above about 6.5, or in organic solvents. It dissolves readily in dilute solutions of most organic acids, including formic, acetic, tartaric, and citric acids. Chitosan is

soluble to a limited extent in dilute inorganic acids except phosphoric and sulfuric acids (Peniston & Johnson, 1980; LeHoux & Grondin, 1993; Sigma-Aldrich, 1999)

Technical Products and Impurities: Chitosan is not one chemical entity, but varies in composition depending on manufacture. Chitosan could be defined as chitin sufficiently deacetylated to form soluble amine salts. The degree of deacetylation necessary to obtain a soluble product must be 80 to 85 percent or higher; *i.e*., the acetyl content of the chitosan product must be <4- 4.5 percent. Chitosan products are highly viscous, resembling natural gums (Peniston & Johnson, 1980).

Chitosan is available in several forms from Sigma-Aldrich (1999). Practical grade chitosan from crab shells has a minimum of 85 percent deacetylation and a viscosity >200 cps (Brookfield, 1% solution in 1% acetic acid); it may contain foreign matter. High molecular weight (600,000) chitosan is a coarsely ground polymer prepared from crab or shrimp shells with a viscosity of 800-2000 cps. Low molecular weight (150,000) chitosan is 75-85 percent deacetylated and has a viscosity of 20-200 cps.

EXPOSURE INFORMATION

Production and Producers: Chitin and chitosan are commercially manufactured by a chemical method. Crab or shrimp shells are deproteinized by treatment with an aqueous 3-5 percent NaOH solution. The resulting product is neutralized and calcium is removed by treatment with an aqueous 3-5 percent HCl solution at room temperature to afford a white or slightly pink precipitate of chitin. The N-deacetylation of chitin is done by treatment with an aqueous 40-45 percent NaOH solution, and the precipitate is washed with water. The crude sample is dissolved in aqueous 2 percent acetic acid, and the insoluble material is removed. The resulting clear supernatant solution is neutralized with aqueous NaOH solution to afford a purified sample of chitosan as a white precipitate (Hirano, 1996).

The main industrial sources of chitin are the shell wastes of shrimp, lobster, and crab. Chitosan is also prepared from squid pens. Squid pen chitosan is synthesized from ß-chitin (amine group aligned with the OH and CH_2OH groups) and crustacean exoskeleton chitosan is synthesized from alpha-chitin (anti-parallel chain alignment) (Shepherd *et al.*, 1997; Felt *et al.*, 1998).

Although chitosan outperforms competitive products in many applications, historical profit margins have been too low to be competitive. Commercial development has been hampered by the corrosive nature of the acids and bases used in the manufacture of chitosan, which destroys equipment, requires careful handling by workers, and presents potential environmental hazards (Peniston & Johnson, 1980; Leffler, 1997).

In 1994, the total world consumption of chitin and chitosan was estimated to be more than 1,000 tons with 800 tons being used in Japan (Hirano, 1996). According to Dr. Walker, based on data submitted to the EPA in 1986, 1990, and 1994, chitosan production was <10,000 lb per annum. By 1997, two US companies were producing chitosan from crab waste (Leffler, 1997). According to the most recent OPD Chemical Buyers Directory, chitosan is now manufactured or distributed by 45 companies; these are AIDP, Inc., ALFA Chem, Allchem Industries, Inc., American Ingredients, Inc., American International Chemical Inc., Arrow Chemical Inc., Ashland Chemical Company, Fine Ingredients Division, Beckmann Chemikalien KG, Belmont

Chemicals Inc., Biopolymer Engineering, Buckton Scott USA, Inc., CPB International, Inc., Chemical Industry Services, Inc., Chugai Boyeki (America) Corp., CitiUSA, Continental Trading Co., DCV Bionutritionals, DNP International Co., Inc., Fabrichem, Inc., G.C.I. Nutrients, H & A Industrial Inc., IRMA Corp., Infinity Marketing Group, Inc., JC Company, Inc., K3 Corp., Kaltron/Pettibone, M.M.P., Inc., Marcor Development Corp., Maypro Indstries, Inc., Mini Star Interrnational Inc., Pharma Nutrients, Pharmline, Inc., Pronova Biopolymer, Inc., RIA International, Paul Schueller International Inc., Schweizerhall, Inc., Seltzer Chemicals, Inc., Serra International Trading Inc., Stauber Performance Ingredients, Inc., Stryka Botanics Co., Inc., Tanabe U.S.A., Inc., Union Carbide Corporation, Universal Preservachem, Inc., Vanson Inc., and Wilke International, Inc. (Tilton, 1998).

Use Pattern: Many potential products using chitosan have been developed, including flocculating agents for water and waste treatment, chelating agents for removal of traces of heavy metals from aqueous solutions, coatings to improve dyeing characteristics of glass fibers, wet strength additives for paper, adhesives, photographic and printing applications, thickeners, and fibers and films (Peniston & Johnson, 1980).

Between 1976 and 1999, 2064 patents involving chitosan were approved by the US Patent and Trademark Office (1999). Some novel applications involving chitosan include biodegradable fish hooks and surgical sutures, coated paper and transparencies for inkjet ink, biodegradable implants and vascular prostheses, and low-fat whipping cream and ice cream.

Chitosan is used as an excipient for oral drug formulations, primarily as a diluent. Type IV Drug Master Files (DMFs) have been submitted to FDA for chitosan by Union Carbide Corporation and Warner Lambert. Chitosan is also being evaluated as a potential vehicle for orally administered controlled-release drugs. Chitosan is among the many materials proposed for preparing microspheres and microcapsules. Interesting applications include the preparation of chitosan-coated liposomes to improve oral absorption of insulin, biodegradable chitosan microspheres for controlled release of antineoplastic agents, and magnetic chitosan microspheres to localize drugs by biochemical and physical means (Felt *et al.*, 1998; FDA, 1999).

Several drug delivery systems based on chitosan for other routes of administration are also being investigated. The good mucoadhesive properties of chitosan make it a promising candidate for development of nasal delivery systems. Chitosan has also been evaluated for the manufacture of ocular bandage lenses and biodegradable surgical implants (Felt *et al.*, 1998).

Wound dressings manufactured from chitosan, are available for clinical use. For example, Tegasorb™ wound dressings by 3M are used with partial and full thickness dermal ulcers, leg ulcers, superficial wounds, abrasions, burns, and donor sites. Tegasorb dressings interact with wound fluid to create a soft, semi-transparent absorbent mass that enhances wound healing (Illum, 1998; McRight, 1998).

Cosmetic compositions based upon chitosan derivatives are suitable for treatment of hair or skin. They can be provided as hair washes, body washes, coloring shampoos, hair dressing creams, hair tonics, blow-dry lotions, hair setting lotions, hair conditioners, agents for permanent hair deformation, and as cosmetic agents for the care, protection, or cleaning of skin. Amerchol, a subsidiary of Union Carbide Corporation, is a supplier of functional ingredients to the personal care industry, including chitosan polymers (Lang *et al.*, 1985; Amerchol, 1998).

Chitosan has several agricultural uses. It acts as a preservative coating and biofungicide when sprayed on fresh fruits and vegetables. Chitosan fertilizers increase the number of useful soil microorganisms and decrease harmful ones. Plant seeds are soaked in aqueous solutions of chitosan to prevent microbial infections and increase plant production. In the US, three products containing chitosan are registered as pesticides, Elexa (0.95% chitosan) by Safescience Products, Inc., and Hygra Yield Enhancing Seed Treating Agent (2.5% chitosan) and Yea! Poly-D-Glucosamine Solution (2.5% chitosan) both by DCV, Inc. (Hirano, 1996; *Anon.*, 1999; USEPA/OPP Chemical Database, 1999).

Cationic chitosan forms polyelectrolyte complexes with polyanioninc polymers and the chelate complexes with metal ions to afford precipitates. These reactions have been used for the clarification of polluted waste water. Chitosan is also usable as an adsorbent for the removal of certain harmful radioisotopes from polluted water and for the recovery of uranium from sea water and fresh water (Hirano, 1996).

The reason for the explosive growth in the number of manufacturers and distributors of chitosan might be its new found acceptance as a diet aide. The chitosan craze was triggered by a book, *The Fat Blocker Diet*, written by Dr. Arnold Fox and Brenda Adderly, and published in 1997 (Amazon.com, 1999). Variously called "the fat magnet," the "fat trapper," or a "sponge," chitosan is though to inhibit fat digestion by dissolving in the stomach, emulsifying fat in the stomach contents, and forming a gel in the intestine which entraps the fat and prevents intestinal absorption (Kanauchi *et al.*, 1995). Some sources of chitosan diet aids available to consumers *via* the Internet are listed in Table 1. Chitosan can also be found in local drug stores and vitamin shops.

Table 1. Some chitosan products sold to consumers over the Internet

Name	Supplier	Recommended Daily Dose	Description
Chito-Maxx	Body Wise	2 grams	500 mg capsules also containing inulin analog and ascorbic acid
ChitoPro	Ultimate Nutrition	3 grams	500 mg capsules
Chitosan	Body Systems Technology		250 mg capsules
Chitosan	Adbiotk		500 mg capsules
Chitosan (90-95% deacetylated)	Natrol	2 grams	500 mg capsules also containing magnesium stearate, silica, and gelatin
Chitosan (93% deacetylated)	Allergy Research Group	1 to 2 grams	500 mg capsules
Chitosan Plus	Nature's RX		500 mg capsules also containing estrified vitamin C, CoQ10, and manganese
Diet Fuel	Twinlab	3 grams	500 mg capsules (90-95% deacetylated chitosan) also containing cellulose, gelatin, water, magnesium stearate, and MCT (undefined)
Fat Zapper	Head Start	up to 1.5 grams	250 mg capsules also containing grapefruit fiber and vitamin C
Mega Chitosan Plus (87-93% deacetylated)	Ultraceutix	2 grams	500 mg capsules also containing Vitamin C (ascorbic acid), lipase, chromium picolinate, and magnesium stearate and calcium phosphate excipients
Southern Blue Chitosan	McFarlane	starting dose of 2 grams	250 mg capsules of chitosan prepared from squid pens

Sources: Adbiotk, 1999; Body Systems Technology, 1999; Bodywise, 1999; Headstart, 1999; Natures RX, 1999; Netrition, 1999; Southern Blue, 1999; Ultranutrition, 1999; Vitanet, 1999

Human Exposure:

Human exposure to chitosan occurs from the uses listed above. Exposure can occur through multiple sources, with individuals often unaware that they are consuming chitosan. A dieter intentionally ingesting chitosan capsules and unintentionally ingesting chitosan as an excipient in a medication, as an ingredient in low-fat dairy products, and as a preservative sprayed on fruits can easily consume a daily dose of 10 to 20 grams.

Environmental Occurrence: Chitosan, itself, is not a product that occurs in nature. Chitosan is biodegraded within 2 months in farming soils in the summer (Hirano, 1996).

Regulatory Status: As a pharmaceutical excipient, chitosan has no regulatory status and may not be sold for use in food or approved drugs unless it can be qualified through FDA approval mechanisms for food components or finished new drug dosage forms. Excipients contained in over-the-counter drug products must be safe in the amounts administered and not interfere with the effectiveness of the preparation or with assays to determine if the product meets professed standards of identity, strength, quality, and purity (IPEC Americas, 1999).

With a new pharmaceutical use for an excipient, it is likely that some necessary data may exist in a confidential drug master file at FDA. Users of the excipient are required to submit a DMF reference letter from the excipient manufacturer to the FDA as part of their drug application (IPEC-Americas, 1999).

Since 1994, dietary supplements, including chitosan used as a diet aide, have been regulated under the Dietary Supplement Health and Education Act (DSHEA). The DSHEA requires no proof of safety for dietary supplements on the market before October 15, 1994. Labeling requirements for such supplements allow warnings and dosage recommendations as well as substantiated “structure or function” claims. All claims must prominently note that they have not been evaluated by the FDA, and they must bear the statement “This product is not intended to diagnose, treat, cure, or prevent any disease” (Croom & Walker, 1995).

EVIDENCE FOR POSSIBLE CARCINOGENIC ACTIVITY

Human Data: No epidemiological studies or case reports investigating the association of exposure to chitosan and cancer risk in humans were identified in the available literature.

Animal Data: No 2-year carcinogenicity studies of chitosan were identified in the available literature.

Two studies of the acute toxicity of chitosan were identified in the available literature. Hirano (1996) reported that the oral LD_{50} was 16 g/kg in mice. The intraperitoneal (ip) LD_{50} of sodium chitosan sulfate in rats was reported to be 208 mg/kg (NLM, 1999).

Short-Term Tests: No *in vitro* or *in vivo* studies evaluating chitosan for mutagenic effects were identified in the available literature. It is possible that such information exists in the Drug Master Files.

Metabolism: Chitosan is a polymer not hydrolyzed by human digestive enzymes (Ormrod *et al.*, 1998). Its function as a dietary supplement is dependent on its lack of absorption in the human body.

Other Biological Effects: *Antigenotoxicity/Anticarcinogenicity.* Chitosan reduced the geno- toxicity of aqueous solutions of the hydrophobic mutagens, 4-nitroquinoline-N-oxide and dinitropyrene, as measured in a sister chromatid exchange assay. Under similar conditions, chitosan did not show antigenotoxic activity for mitomycin C, but it reduced the genotoxicity of adriamycin by 78 percent. These effects were pH dependent for mitomycin C but not for adriamycin (Ohe, 1996).

Dietary chitosan (2%) reduced the incidence of azoxymethane-induced early preneoplastic markers of colon carcinogenesis in female CF1 mice, as indicated by aberrant crypts and mitotic figures and altered crypt morphometrics in the colon (Torzsas *et al.*, 1996).

Weight loss and cholesterol-lowering effects from dietary intake. Interest in the effects of dietary chitosan originated from its use as a coagulation agent for waste treatment systems in food and beverage processing plants. Since the

dried sludge was used as a protein supplement in animal feeds, the potential toxicity of chitosan to the consuming animal had to be investigated (Landes & Bough, 1976).

The following observations have been made.

- Progressive growth reductions occurred in male Sprague-Dawley rats fed 10 or 15 percent chitosan diets for 8 weeks; enlarged liver and kidneys were observed at 15 percent (Landes & Bough, 1976).

- No significant differences in weight gain were seen in male rats fed 1 or 5 percent chitosan for 4 weeks; with 5 percent chitosan, oleic acid and cholesterol absorption was lowered by 58 and 63 percent, respectively (Vahouney *et al.*, 1983).

- No significant differences in growth, food intake, liver weight, and dried fecal weight were observed between control and chitosan-fed (2 or 5%) male Wistar rats after 21 days on a low cholesterol diet. Chitosan feeding (5%) reduced serum cholesterol levels in these rats. Chitosan feeding also suppressed the formation of coprostanol in the intestines. Although chitosan had been reported to increase the excretion of neutral sterols in rats fed a high cholesterol diet, no increase in fecal neutral sterols was observed in rats fed a low cholesterol diet (Fukada *et al.*, 1991).

- High-molecular-weight chitosan (>750 kDa) was a less effective hypocholesterolemic agent than a 70-kDa preparation. A 7.5 percent chitosan formula maintained cholesterol homeostasis in male Long-Evans rats despite a greatly increased cholesterol intake (LeHoux & Grondin, 1993).

- Ascorbate had a synergistic effect on the inhibition of fat digestion by chitosan in male Sprague-Dawley rats (Kanauchi *et al.*, 1995).

- A greater effect on fat digestibility by rats occurred with increases in the viscosity or deacetylation degree of the chitosan preparation (Deuchi *et al.*, 1995a).

- Significant reductions of feed intakes, body weights, total plasma cholesterol, and HDL-cholesterol were observed in broiler chicks fed chitosan for 12 days (Razdan *et al.*, 1997).

- Inhibition of hypercholesterolemia and atherogenesis occurred in gene knockout apolipoprotein E-deficient mice fed 5 percent chitosan for 20 weeks (Ormrod *et al.*, 1998).

Thus, claims that chitosan may help obese individuals to lose weight and lower elevated serum cholesterol appear to have some experimental basis.

Tanaka and coworkers (1997) cautioned, however, that special care should be taken in the clinical use of chitosan over a long time. Chitosan was administered orally and parenterally to mice. When 5 mg of chitosan was injected intraperitoneally, the body weights of the mice decreased significantly and inactivity was observed in the fifth week. Histologically, many macrophages with hyperplasia were observed in the mesenterium. Subcutaneous injection of 5 mg of chitosan did not evoke the general and cellular abormalities. Oral administration of 5 percent chitosan via a casein diet caused mouse body weights to decrease and also decreased the number of *Bifidobacterium* and *Lactobacillus* in normal flora of the intestinal tract.

Concerns have also been raised that chitosan could cause the loss of fat-soluble vitamins and block absorption of medicines such as birth control pills (Orr, 1999). In a study by Deuchi and coworkers (1995b), chitosan feeding for 2 weeks caused a decrease in mineral absorption and bone mineral content in male Sprague-Dawley rats. Moreover, the ingestion of chitosan with ascorbic acid led to a marked and rapid decrease in the serum vitamin E, although such a loss did not occur in rats given glucosamine monomer instead of chitosan.

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