Poly-D-Glucosamine (Chitosan)

Crops

Identification of Petitioned Substance

Chemical Names:
poly-D-glucosamine

Other Name:
Chitosan

Trade Names:
Chito-stik

CAS Numbers:
9012-76-4

Other Codes:
128930 (EPA/OPP Chemical Code)

Characterization of Petitioned Substance

Composition of the Substance:
Chitosan (poly-D-glucosamine) is a polymer[1] of glucosamine sugars, specifically glucosamine and N-acetyl-glucosamine (Hadwiger 2004). Its structure and composition are similar to both cellulose (i.e., the primary structural component of plant fiber) and chitin. Like chitin, chitosan is found naturally in the shells of all crustaceans and insects, as well as certain other organisms such as many fungi, algae, and yeast. Chitosan is one of the most common polymers found in nature (EPA 2003).

Properties of the Substance:
Chitosan is a chemically stable, white to pale yellow powder or flake (Polysciences 2003). Chitosan has a strong positive charge, which is the basis of its use as a "sticking" agent (i.e., an adhesive adjuvant). The positively charged molecules adhere to negatively charged pesticides and plant surfaces.

Chitosan is not soluble in water. It can be made soluble in water, however, by treating it with an acid to form soluble chitosan ions (Rabea et al. 2003). See Evaluation Question #1 form more information the production of chitosan.

Specific Uses of the Substance:
The petitioned use of the substance is as an adhesive adjuvant for use in organic crop production (Hadwiger 2004). As an adhesive adjuvant, the substance would be used to make a pesticide or fungicide stick to plant surfaces. Specifically, the petitioner seeks approval to test chitosan as a sticking agent for the fungicide copper sulfate pentahydrate for the control of potato late blight.

Approved Legal Uses of the Substance:
Chitosan is a registered pesticide (OPP No. 128930) that is used in crop production as a plant growth enhancer and plant defense booster (EPA 2003). In these uses, chitosan is applied to treat field crops, ornamentals, turf, home gardens, and nurseries. Target pests include early and late blight, downy and powdery mildew, and gray mold. Proposed application rates for the petitioned use as a sticking agent are much lower than the application rates for use as a pesticide/fungicide. Chitosan is exempt from the requirement for a pesticide tolerance (EPA 1995). See Evaluation Question #6 for more information on chitosan application rates and Evaluation Question #8 for more information on the modes of action for approved legal uses as a biopesticide.

According to the petition, chitosan is listed as an animal feed component in the Official Publication of the Association of American Feed Control Officials (Hadwiger 2004).

[1] A polymer is a large molecule that is a chain of linked, identical or similar molecular units called monomers.

Chitosan is used as a human dietary supplement for weight loss and cholesterol reduction (Rabea et al. 2004).

Chitosan is also used as a flocculating (i.e., settling) agent in wastewater treatment systems, a hydrating agent in cosmetics, a pharmaceutical agent in biomedicine, and an antimicrobial food wrap (Rabea et al. 2003). The State of Oregon has approved the use of chitosan in unrestricted amounts as a soil amendment (fertilizer). This use is not regulated by EPA under the Federal Insecticide, Fungicide, and Rodenticide Act (EPA 1995).

Status

Action of the Substance:

Chitosan has a positive chemical charge, which causes it to attract negatively charged materials. This property is the mode of action for the petitioned use as an adhesive adjuvant. Specifically, chitosan would be used to adhere to negatively charged copper sulfate particles and plant surfaces.

International

Chitosan is not specifically listed for the petitioned use or other uses in the following international organic standards:

- Canadian General Standards Board
- CODEX Alimentarius Commission
- European Economic Community (EEC) Council Regulation 2092/91
- International Federation of Organic Agriculture Movements
- Japan Agricultural Standard for Organic Production

Evaluation Questions for Substances to be used in Organic Crop or Livestock Production

Evaluation Question #1*: Is the petitioned substance formulated or manufactured by a chemical process? (From 7 U.S.C. § 6502 (21))*

According to the petition, the raw material for chitosan is crab shell waste byproduct (Hadwiger 2004). Other potential raw material sources for commercially produced chitosan include shrimp shells (e.g., FDA 2002), lobster shells (EPA 1995), and cultured fungi (Rabea et al. 2003).

The process used to formulate chitosan is shown in Figure 1. The process begins with chitin obtained from seafood byproducts. Non-chitinous components of the seafood byproduct are stripped with a hydrochloric acid (not shown in Figure 1). Next, sodium hydroxide (NaOH), which is a base, and heat are used to remove residual meat attached to the shell material. Next, a stronger sodium hydroxide solution is used (Step 1 in Figure 1), in a step called deacetalation, to convert some N-acetyl glucosamine (the primary component of chitin) to glucosamine (the primary component of chitosan) (Rabea et al. 2003).

Following deacetalation, the chitosan is rinsed with water to remove remaining sodium hydroxide and impurities (Step 2 in Figure 1). A mild organic acid, such as lactic or acetic acid, is then applied (Step 3 in Figure 1) to adjust the pH of the chitosan below neutral (i.e. from basic to acidic). This step is required to make the chitosan soluble in water (Rabea et al. 2003). In the last manufacturing step, the chitosan is dried.

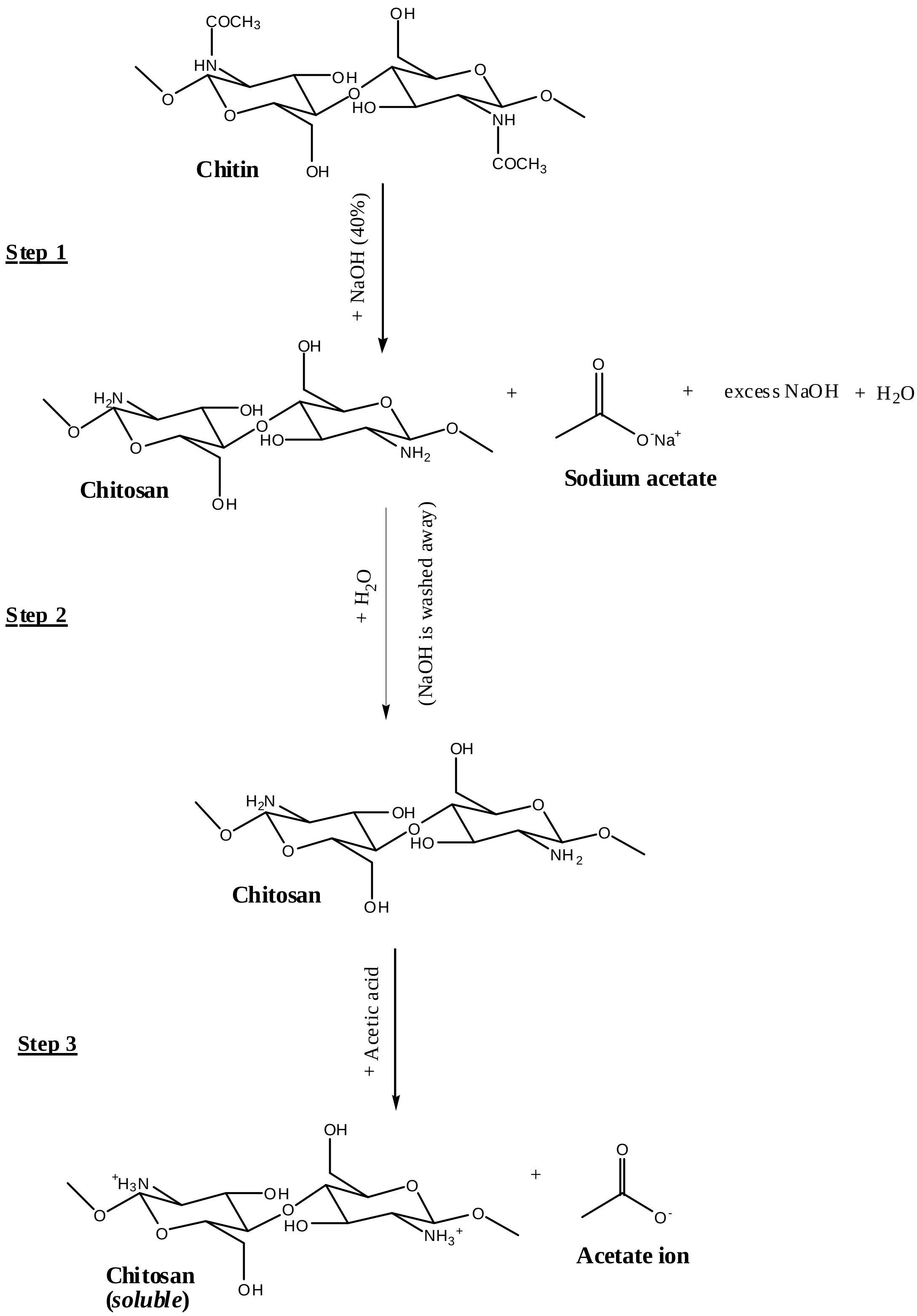

Figure 1. Formulation of Chitosan

<u>Evaluation Question #2:</u> Is the petitioned substance formulated or manufactured by a process that chemically changes the substance extracted from naturally occurring plant, animal, or mineral sources? (From 7 U.S.C. § 6502 (21).)

In the chitosan manufacturing process, the primary component of chitin (i.e., N-acetyl glucosamine) is chemically changed to the primary component of chitosan (glucosamine). Both the starting and ending chemicals in this process are natural components of both chitin and chitosan and are present in the natural animal byproduct source (e.g., crab shells). The proportion of the two chemicals determines whether a mixture is chitin or chitosan. According to the petitioner, chitosan is approximately 80 percent glucosamine and 20 percent N-acetyl glucosamine (Hadwiger 2004). There are no precise definitions, however, of chitin and chitosan based on the percentage composition of glucosamine and N-acetyl glucosamine (Rabea et al. 2003). In both chitin and chitosan, these two chemicals are linked together in chains, called polymers, of as many as 5,000 glucosamine and N-acetyl glucosamine molecules (i.e., monomers).

Although N-acetyl glucosamine is converted to glucosamine in nature, the conversion does not occur by the controlled process used for commercial production (Figure 1).

<u>Evaluation Question #3:</u> Is the petitioned substance created by naturally occurring biological processes? (From 7 U.S.C. § 6502 (21).)

In nature, N-acetyl glucosamine may be deacetylated to glucosamine. The natural deacetalation process, however, does not occur as a result of the specific process (i.e., application of NaOH) used for commercial manufacturing.

<u>Evaluation Question #4:</u> Is there environmental contamination during the petitioned substance's manufacture, use, misuse, or disposal? (From 7 U.S.C. § 6518 (m) (3).)

There is no information available from EPA or FDA to suggest that environmental contamination results from the manufacture, use, misuse, or disposal. Chitosan is a registered pesticide, which implies a potential for misuse or improper disposal. It is a naturally occurring and biodegradable chemical, however, and EPA exempted it from the requirement for a tolerance limit when used as a pesticide in the production of any raw agricultural commodity (EPA 1995). In exempting chitosan from the requirement for a tolerance limit, EPA cited the following considerations:

> "Chitosan (1) is not toxic, as demonstrated in acute toxicity studies in mice, rats, and rabbits; (2) is naturally occurring in the environment in large concentrations; (3) has been exempted from the requirement of a tolerance in or on barley, beans, oats, peas, and wheat (40 CFR 180.1072) when used as a seed treatment at an application rate of 4 oz./100 lbs. seed; (4) has been approved by the State of Oregon for use in unrestricted amounts as a soil amendment (fertilizer), a use not regulated by EPA under the Federal Insecticide, Fungicide, and Rodenticide Act." (EPA 1995)

In addition, according to EPA's pesticide fact sheet for chitosan, it is not expected to harm people, pets, wildlife, or the environment when used according to label directions due to its low potential for toxicity and abundance in the natural environment (EPA 2003).

The petitioner notes that manufacturing chitosan from crab shell waste reduces the potential for environmental contamination associated with disposal of the wastes (Hadwiger 2004).

<u>Evaluation Question #5:</u> Is the petitioned substance harmful to the environment? (From 7 U.S.C. § 6517 (c) (1) (A) (i) and 7 U.S.C. § 6517 (c) (2) (A) (i).)

Chitosan is a naturally occurring chemical and is one of the most common polymers found in nature (EPA 2003). EPA exempted chitosan from the requirement for a tolerance limit due to its low potential for toxicity and abundance in the environment. EPA concluded that chitosan is not expected to harm people, pets, wildlife, or the natural environment, in part because chitosan was found to be nontoxic in acute toxicity studies in mice, rats, and rabbits (EPA 1995).

Evaluation Question #6: Is there potential for the petitioned substance to cause detrimental chemical interaction with other substances used in organic crop or livestock production? (From 7 U.S.C. § 6518 (m) (1).)

The adhesive property of chitosan that is the basis of its petitioned use could cause negatively charged particles other than the co-applied organic pesticide to stick to plant surfaces. Potential examples of other particles that may be attracted to the chitosan adhesive adjuvant include other agricultural products or fine soil particles. No information sources reviewed for this report described or evaluated potential adverse impacts of this nature.

Biochemically, however, chitosan is unlikely to cause detrimental chemical interaction with other substances used in organic crop or livestock production. As a component of the shells of insects and crustaceans, as well as certain other organisms such as many fungi, algae, and yeast (EPA 2003), chitosan is naturally present in agroecosystems. In addition, plants and microbes (e.g., in soil) have enzymes called chitinases and chitosanases that can break chitosan down to utilizable carbohydrates (Hadwiger 2004; Brzezinski and Neugebauer 2004).

Evaluation Question #7: Are there adverse biological or chemical interactions in the agro-ecosystem by using the petitioned substance? (From 7 U.S.C. § 6518 (m) (5).)

For the petitioned use, chitosan is unlikely to cause adverse biological or chemical interactions in the agroecosystem. Chitosan is found naturally in agroecosystems, and it may be broken down and utilized by plants and microbes (Hadwiger 2004; Brzezinski and Neugebauer 2004).

Although chitosan attracts positively charged particles, it is not highly reactive and it is not known to be toxic (e.g., EPA 1995). EPA has approved the use of chitosan as a pesticide and plant growth promoter at much higher application rates than proposed for its use as an adhesive adjuvant (see Evaluation Question #6).

Evaluation Question #8: Are there detrimental physiological effects on soil organisms, crops, or livestock by using the petitioned substance? (From 7 U.S.C. § 6518 (m) (5).)

Chitosan has documented physiological effects on plants and soil organisms, including plant growth enhancement, and antimicrobial ability. These effects, which are regarded as beneficial to crop production, are not involved in the petitioned use of chitosan as an adhesive adjuvant. The rate at which chitosan would be applied for the petitioned use (i.e., 5 to 10 grams per acre) is well below the recommended application rates for these other uses (e.g., 180 to 1,080 grams per acre [EPA 2001]). The known physiological effects of chitosan are described further below.

Chitosan has been shown to have antimicrobial, antifungal, and antiviral effects, and it is also known to be a plant growth enhancer (Rabea et al. 2003). The antimicrobial and antifungal effects are influenced by the length and composition of the chitosan polymers, environmental conditions, and other factors (Rabea et al. 2003). For example, very short chitosan polymers have the strongest antimicrobial and antifungal effects. Although Rabea et al. (2003) summarized several hypotheses about chitosan's mode of antimicrobial action, the exact mode of action is still unknown.

As a plant growth enhancer, the mode of action is believed to be that chitosan is taken up by plant cells where it enters the cell nucleus and stimulates messenger RNA and enzyme production. This action stimulates the plant to produce more lignin in the stems, resulting in stronger stems (EPA 1995, Rabea et al. 2003).

At the proposed application rates, chitosan is unlikely to create unacceptable changes in soil temperature, water availability, pH levels, nutrient availability, or salt concentration.

Evaluation Question #9: Is there a toxic or other adverse action of the petitioned substance or its breakdown products? (From 7 U.S.C. § 6518 (m) (2).)

Breakdown products of chitosan include smaller chitin and chitosan polymers (i.e., shorter chains of glucosamine and N-acetyl-glucosamine monomers), unlinked monomers, and other glucose-related molecules. These breakdown products are all nontoxic. Glucose is a sugar that can be utilized by many organisms.

Evaluation Question #10: Is there undesirable persistence or concentration of the petitioned substance or its breakdown products in the environment? (From 7 U.S.C. § 6518 (m) (2).)

Chitosan and its breakdown products are not persistent in the environment, and significant environmental accumulation of chitosan and its breakdown products would not result from repeated use of chitosan at the proposed application rate. Chitosan is biodegradable. For example, plants and microbes (e.g., in soil) have enzymes called chitinases and chitosanases that can break chitosan down to utilizable carbohydrates (Hardwiger 2004).

Evaluation Question #11: Is there any harmful effect on human health by using the petitioned substance? (From 7 U.S.C. § 6517 (c) (1) (A) (i), 7 U.S.C. § 6517 (c) (2) (A) (i)) and 7 U.S.C. § 6518 (m) (4).)

Chitosan is not known to be toxic to humans. Chitosan is marketed as a human dietary supplement for control of obesity and high cholesterol. The scientific evidence of these benefits is questionable, however, and FDA sent a warning letter concerning unsubstantiated claims to the maker of one chitosan supplement (FDA 2004).

In 2001, Primex Ingredients, ASA, submitted a Generally Regarded as Safe (GRAS) notification to FDA for chitosan produced from shrimp. Primex subsequently withdrew the GRAS notification (FDA 2002), and chitosan is not currently GRAS.

Evaluation Question #12: Is there a wholly natural product that could be substituted for the petitioned substance? (From 7 U.S.C. § 6517 (c) (1) (A) (ii).)

The availability of alternative products was investigated by consulting organic industry resources, researching sources cited by the poly-D-glucosamine petition, and conducting Internet searches. This investigation identified one adhesive adjuvant product formulated with the functional agents lactose, bentonite, and casein. These ingredients are recognized as natural by organic industry sources (e.g., OMRI, 2005). The investigation also identified two similar adjuvant products formulated with pine-based functional agents (i.e., di-1-P-menthene, poly-1-P-menthene). However, it is unknown whether these products are synthetic or not.

ATTRA (Kuepper and Sullivan 2004) published a guide to organic alternatives for late blight control in potatoes. Although this source does not discuss adhesive adjuvants, it does describe alternative late blight control practices (e.g., application of compost tea) that do not involve fungicides and thus would not require an adhesive adjuvant.

Evaluation Question #13: Are there other already allowed substances that could be substituted for the petitioned substance? (From 7 U.S.C. § 6518 (m) (6).)

Based on a review of organic industry resources, there are at least three products marketed as organic adhesive adjuvants. As described in Evaluation Question #12, one of the products contains the functional agents bentonite, lactose, and casein. The two other products are pine-based. However, it is unknown whether the pine-based functional agents of these closely-related products are synthetic. No information about the manufacturing process for these products was found and National List petitions have not been submitted for any uses of the functional agents.

<u>Evaluation Question #14:</u> Are there alternative practices that would make the use of the petitioned substance unnecessary? (From 7 U.S.C. § 6518 (m) (6).)

The petition proposes the use of chitosan as an adhesive adjuvant for use with an approved organic fungicide, such as copper sulfate (7 CFR 205.601(i)(2)). The petitioned use would enhance the ability of the fungicide to stick to plant surfaces, thereby improving effectiveness and reducing fungicide application rates. Potential alterative practices include application of the organic fungicide without the adhesive adjuvant or use of an alternative organic adhesive adjuvant (see Evaluation Questions #12 and #13).

Although the petitioner states that there is no effective control currently available for potato late blight, a publication by ATTRA (Kuepper and Sullivan 2004) provides guidance on organic control of late blight in potatoes. The ATTRA guidelines include cultural controls, such as:

- Field scouting and inspection of tubers going into storage to catch outbreaks in their earliest stages;
- Avoiding piling and leaving culls;
- Using certified seed and mixing seed lots;
- Using an AireCup® planter;
- Carefully monitoring seed planting depth and hilling operations;
- Using organic contact herbicides to kill infected plants;
- Managing irrigation to regulate leaf wetness;
- Destroying green vines;
- Harvesting tubers two weeks after destroying green vines; and
- Minimizing damage to tubers and keeping regulated air flow through storage piles.

In addition, ATTRA identified foliar feeding (e.g., with products made from kelp or horsetail) and application of compost tea as a method of enhancing disease resistance (Kuepper and Sullivan 2004).

<u>References</u>

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