



**UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460**

**OFFICE OF PESTICIDES AND TOXIC SUBSTANCES**

**MEMORANDUM**

**DATE:** April 7, 2008

**SUBJECT:** Non-Target Organism and Endangered Species Assessment in Support of the Registration Review of Chitin (Poly-N-Acetyl-D-glucosamine) and Chitosan (Poly-D-Glucosamine). Re-review of Existing Non-target Organism Studies

**Registration Review Case No.:** 6063  
**Chemical Class:** Biochemical  
**PC Codes:** 128991 (Chitin) & 128930 (Chitosan)  
**CAS Numbers:** 1398-61-4 (Chitin) & 9012-76-4 (Chitosan)  
**Tolerance Exemptions:** 40 CFR 180.1089 (Chitin) & 40 CFR 180.1072 (Chitosan)  
**MRID Numbers:** None

**FROM:** Russell S. Jones, Ph.D., Senior Biologist /s/ 04/07/2008  
Biochemical Pesticides Branch  
Biopesticides & Pollution Prevention Division (7511P)

**TO:** Chris Pfeifer, Regulatory Action Leader  
Biochemical Pesticides Branch  
Biopesticides & Pollution Prevention Division (7511P)

**BACKGROUND**

The Food Quality Protection Act of 1996 mandated the continuous review of existing pesticides. All pesticides distributed or sold in the United States must generally be registered by EPA, based on scientific data showing that they will not cause unreasonable risks to human health, workers, or the environment when used as directed on product labeling. The Initial Docket for the Registration Review of Chitin and Chitosan was established for September 2007 and the Final Workplan is scheduled for January 2008. In support of the Registration Review, BPPD has re-evaluated the existing non-target organism studies that have been submitted in support of the registration of products containing Chitin, Chitosan, and Chitosan Hydrolysate (a hydrolyzed form of Chitosan). These data are re-reviewed in this document to support the Endangered Species Risk Assessment for the Registration Review of Chitin and Chitosan. No new studies were submitted.

## EXECUTIVE SUMMARY

Chitin and its closely related active ingredients, Chitosan and Chitosan Hydrolysate, are not expected to cause any adverse effects in any non-target organisms, including threatened and endangered species. A **No Effects (NE)** determination has been made for Chitin and Chitosan (including Chitosan Hydrolysate). Chitin is ubiquitous in nature and is found in many terrestrial and aquatic species (see Effects on Non-Target Organisms and Endangered Species below). Chitosan is a partially deacylated version of Chitin. Chitosan Hydrolysate is an acid hydrolyzed version of Chitosan and is comprised of smaller, more water-soluble chains of Chitosan. Chitin and its derivatives are functionally identical and have a non-toxic mode of action (see Description of the Active Ingredients below). There is no direct activity of the active ingredients against the target pest.

## RECOMMENDATIONS AND CONCLUSIONS

Based on the existing data, adverse effects on non-target organisms are highly unlikely and there will be **NO EFFECTS (NE)** on threatened or endangered species resulting from application of products containing Chitin and Chitosan (including Chitosan Hydrolysate) when products are used in accordance with approved labeling.

## NON-TARGET ORGANISMS AND ENDANGERED SPECIES ASSESSMENT

### Description of the Active Ingredients

Chitin and Chitosan (which includes Chitosan Hydrolysate) are both comprised of a naturally-occurring chain of glucose molecules linked via *B*-1,4-linkages, that is structurally related to cellulose. Both substances are slightly different forms of the same molecule (see Figure 1 below).

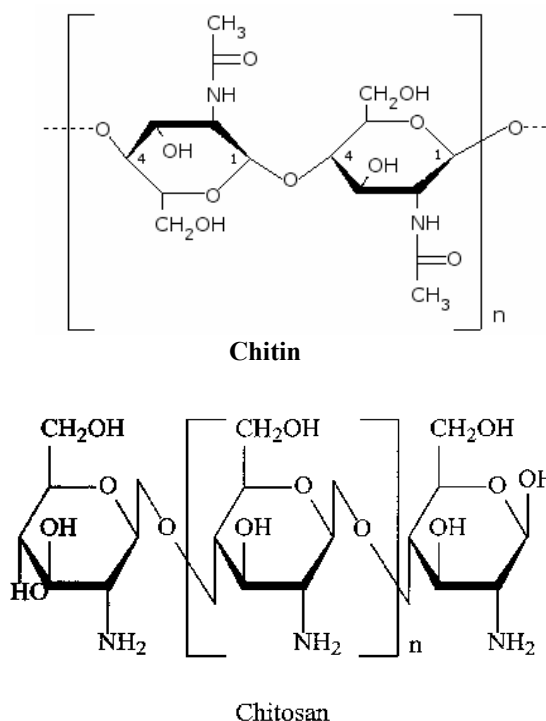
Chitin (Poly- N-Acetyl-D-Glucosamine) is commonly found in nature and is usually derived from crustacean shells, particularly from crabs and shrimp. Historically, it has been used as a food additive and a fertilizer. As a pesticide active ingredient, it acts by stimulating the growth of certain microorganisms in soil, which release substances that kill pathenogenic nematodes and their eggs. The compound is also reputed to play a role as a plant growth regulator by bolstering plant defenses against disease (Systemic Acquired Response, or SAR inducer) (Kramer and Muthukrishnan, 1998). Chitin does not have any direct toxicity to pests or non-target organisms.

Chitosan (Poly-D-Glucosamine) is a synthetic compound prepared through the partial or complete deacetylation of Chitin. Thus, if deacetylation is incomplete, a Chitosan chain will contain molecules of Chitin randomly distributed along its length. Chitosan also differs from Chitin in that it contains an amine group, whereas Chitin contains an amide group. It is

somewhat more water-soluble than Chitin. Chitosan has biomedical applications, as a haemostatic agent that is hypoallergenic and, similar to Chitin, it is known to possess anti-microbial activity. These properties also allow for its use as an active ingredient in anti-microbial pesticides. As a pesticidal active ingredient, Chitosan is best known as a plant growth enhancer that boosts the ability of plants to defend against fungal infections (also an SAR inducer). Similar to Chitin, Chitosan has no direct toxicity to pests or non-target organisms.

Chitosan Hydrolysate is prepared via the acid hydrolysis of Chitosan and is comprised of smaller chains of Chitosan (also an SAR inducer). Biologically, there is little functional difference between Chitin, Chitosan, and Chitosan Hydrolysate.

**Figure 1. The structures of Chitin and Chitosan.**



The actual chemical structure of Chitosan is subject to substantial variations based on the degree of deacetylation of Chitin; the structure above depicts a completely deacetylated chain of Chitosan molecules.

#### Current USEPA-registered Products

There is one USEPA-registered product containing Chitin as its active ingredient: Clandosan 618 [EPA Reg. No. 54137-1 (formerly EPA Reg. No. 58200-9)]. There are two USEPA-registered products containing Chitosan as their active ingredient: ELEXA-4 (EPA Reg. No. 81045-2) and ChitoSante (EPA Reg. No. 81446-1). There are two products containing Chitosan Hydrolysate as its active ingredient that are currently in registration review: Chitosan

Hydrolysate (EPA File Symbol No. 73512-A) and Keyplex 350 (EPA File Symbol No. 73512-L).

Clandosan 618 is registered for non-food use on ornamentals, lawn, and turf grasses. Elexa-4 is registered for use on field crops, ornamentals, turf, home gardens, and nurseries. Chitosante is registered for use non-food use as an antimicrobial pesticide on textiles and surfaces. Chitosan Hydrolysate (registration pending) is intended for manufacturing use only. Keyplex 350 (registration pending) is intended for use food use on commercial crops, ornamentals, and turf.

The amounts of active ingredient in each product are listed in Table 1 below:

**Table 1. Farnesol and Nerolidol Content in USEPA Registered End-Use Products**

Product (EPA Reg. No.)	Percentage Active Ingredients <sup>1</sup>		
	Chitin	Chitosan	Chitosan Hydrolysate
Clandosan 618 (54137-1)	25	-	
Elexa-4 (81045-2)	-	4	
ChitoSante (81446-1) <sup>2</sup>	-	6	
Chitosan Hydrolysate (73512-A) <sup>3</sup>	-		8.33
Keyplex 350 (73512-L) <sup>4</sup>	-	-	0.8

<sup>1</sup> All active ingredients are closely-related forms of poly-D-glucosamine.

<sup>2</sup> An antimicrobial product having no outdoor exposure

<sup>3</sup> Manufacturing-use only.

<sup>4</sup> Also contains 1.5% Salicylic acid as an active ingredient

### Effects on Non-Target Organisms and Endangered Species

Chitin and Chitosan (which includes Chitosan Hydrolysate) are both comprised of a naturally-occurring chain of glucose molecules linked via *B*-1,4-linkages, that is structurally related to cellulose. Both substances are slightly different forms of the same molecule (see Figure 1 above).

Chitin is one of the most common polymers found in nature. It is a major component of the exoskeletons of aquatic and terrestrial arthropods (insects, crustaceans), the radula of molluscs, and the beaks of cephalopods (squid, octopus), and the cell walls of fungi (Campbell, 1996). In addition, many species possess chitinases (Bishop et al., 2000; Kramer and Muthukrishnan, 1998), enzymes that metabolize chitin, particularly those that consume chitin-containing organisms (MacDonald, 2006; Matsumiya et al. 2006). Chitinases are also found in humans (Escott and Adams, 1995; Paolettia et al. 2007).

**Table 2. Summary of Non-Target Organism Data/Information for Chitin (C), Chitosan (CS), and Chitosan Hydrolysate (CSH) End-Use Products (EP)**

<u>Study Type/OPPTS Guideline</u>	<u>LD<sub>50</sub>/LC<sub>50</sub></u>	<u>Active Ingredient</u>	<u>Toxicity Category</u>	<u>MRID</u>
Acute Oral Toxicity (rat) OPPTS 870.1100	>5000 mg EP/kg <sup>1</sup> (200 mg a.i./kg)	CSH	Practically non-toxic for EP <sup>1</sup>	44931205
	>5000 mg EP/kg <sup>2</sup> (40 mg a.i./kg)	CSH	Practically non-toxic for EP <sup>2</sup>	46790003
Freshwater Fish LC50/OPPTS 850.1075 and Freshwater Invertebrate/OPPTS 850.1010	See Non-target Aquatic Organism summary below	C and CS	Practically non-toxic <sup>3</sup>	-
Avian Acute Oral/OPPTS 850.2100 and Avian Dietary/OPPTS 850.2200	See Non-target Bird summary below	C and CS	Practically non-toxic <sup>3</sup>	-
Non-target Plants/OPPTS 850.4000 and Non-target Insects/OPPTS 850.3020; 850.3030; and/or 850.3040	See Non-target Plants and Insects summary below	C	Practically non-toxic <sup>3</sup>	-

<sup>1</sup> Elexa-4 (EPA Reg. No. 81045-2) contains 4.0% CSH.

<sup>2</sup> Keyplex 350 (EPA File Symbol No. 73512-L) contains 0.8% CSH.

<sup>3</sup> Based on the non-toxic mode of action of the active ingredients, the presence of chitin as a naturally occurring component of many non-target organisms, and the presence of chitinases (enzymes that metabolize chitin) in many non-target organisms that consume chitin-containing organisms in their normal diets.

### Non-Target Mammals

An end-use product containing 4% Chitosan hydrolysate has been shown to be practically non-toxic to rats on acute oral basis (see MRID 44931205). No adverse effects are expected to mammalian wildlife when chitin and chitosan-containing products are applied in accordance with approved labeling.

### Non-Target Aquatic Organisms

Chitin is ubiquitous in nature and is a major component of the exoskeletons of aquatic arthropods (insects, crustaceans), the radula of molluscs, and the beaks of cephalopods (squid, octopus), the cell walls of fungi (Campbell, 1996), and the scales of fish (Uawonggul et al. 2002). Chitin is a normal component in the diets of fish (e.g. in the exoskeletons of aquatic arthropods and in the scales of prey fish) and fish possess chitinases that metabolize these dietary chitins (Matsumiya et al. 2006). No mortalities have been observed in fish fed with diets supplemented with chitin and chitosan. No adverse effects are expected to non-target aquatic organisms when chitin and chitosan-containing products are applied in accordance with approved labeling.

### Non-Target Birds:

Chitin is present in the exoskeletons of arthropods and, therefore, is a regular component of the diets of insectivorous birds. Chitin and chitosan have been shown to be relatively indigestible by some birds (Akaki and Duke, 1999; and Razdan and Pettersson, 1994). However, many birds (starlings, raptors, and many seabirds) possess chitinases that aid in the digestion of chitin, which serves as a source of protein (MacDonald, 2006). No mortalities have ever been observed in birds fed with diets supplemented with chitin and chitosan. No adverse effects are expected to birds when chitin and chitosan-containing products are applied in accordance with approved labeling.

### Non-target Plants and Insects

Chitin is ubiquitous in nature and is a major component of the exoskeletons of aquatic arthropods (insects, crustaceans). Chitin, Chitosan, and Chitosan Hydrolysate are intended for use as plant defense "boosters" to protect plants from fungal pathogens via the induction of Systemic Acquired Response (SAR). SAR is a mechanism that stimulates the internal defense mechanisms of plants to resist pathogen infection. In plants, chitin stimulates the production of chitinases which are used to degrade the chitin-containing cell walls of pathogenic fungi (Kramer and Muthukrishnan, 1998). There is no direct activity of Chitin or Chitosan (including Chitosan Hydrolysate) against the pathogen. No adverse effects to non-target insects and plants are anticipated.

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cc: R. S. Jones, BPPD Subject File/IHAD  
R. S. Jones, FT, OPY: 04/08/2008