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# Dermal toxicity, eye and dermal irritation and skin sensitization evaluation of a new formulation of *Bacillus thuringiensis* var *israelensis* SH-14

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ABSTRACT

*Bacillus thuringiensis* (*Bt*) is the best known and most widely used of all pesticidal microbes. The aim of this study was to assess the toxicity of a new formulation of *Bacillus thuringiensis* var *israelensis* SH-14 in rats through acute dermal toxicity, dermal and eye irritation experiments. The acute dermal toxicity and dermal and eye irritation studies were performed using rabbits according to the United States Environmental Protection Agency guidelines 885.3100, 870.2500 and 870.2500, respectively. The skin sensitization study was carried out in accordance to the EPA OPPTS 870.2600 using guinea pigs. There was no mortality and no evidence of treatment-related toxicity in acute dermal toxicity test. No dermal responses, including erythema/eschar or edema, were found in rabbits treated with the new formulation of *Bti* SH-14. Minimum response was observed after eye application of test substance. No skin sensitization reactions were observed after the challenge with the new formulation of *Bti* SH-14 in the *Bti* SH-14-treated guinea pigs. In summary, the present study demonstrated that the new formulation of *Bti* SH-14 is not acutely toxic via dermal route, has low eye irritation and would not cause dermal irritation or hypersensitivity to tested animals.



## 1. Introduction

Increased public concern of the potential adverse environmental effects associated with the heavy use of chemical pesticides has prompted the examination of alternative methods for pest control. Since the 1960s, biological insecticides such as insect-pathogenic microorganisms have received considerable attention as environmentally benign, highly desirable alternatives (Sudakin, 2003).

*Bacillus thuringiensis* (*Bt*) is the best known and most widely used of all pesticidal microbes. *Bt*, first described by Berliner in 1911, is a gram-positive, spore-forming bacterium living in the soil. It is known for its ability to produce crystalline, proteinaceous, delta-endotoxin during sporulation, which are highly toxic to a wide variety of important agricultural and health-related insect pests as well as other invertebrates (Sauka and Benintende, 2008). Around 1976, a new *Bt* strain was discovered with high toxicity to mosquito larvae (Goldberg and Margalit, 1977) which was later identified and designated *Bt* var. *israelensis*, serotype H14 (de Barjac, 1978), since raised to subspecies status as *B. thuringiensis israelensis* (*Bti*). This Diptera-active strain has come to be used extensively for the control of larvae of pest and vector black flies and mosquitoes around the world, providing both medical and environmental benefits (Lacey et al., 2001; Roh et al., 2007).

Grupo Empresarial de Producciones Biofarmaceúticas y Químicas (LABIOFAM, Cuba) is developing a biolarvicide that possesses as active biological agent of *Bti* serotype H14 (endotoxic spores and crystals); this strain is registered in the Culture Collection catalog (IEBC) of the Pasteur Institute as 266/2 9-VII-98. With the aim of satisfying the current requirements on environmental care, the preservative used in the older formulation was changed to a non-toxic and biodegradable preservative.

Information on acute dermal toxicity, skin and eye irritation and sensitization is a fundamental part of the identification of hazardous products. Superficial organs such as the skin and eyes have a high risk of exposure to *Bti* serotype H14, because the deposition of this product to superficial organs has the potential to be a major route of exposure during the manufacturing and use of *Bti*. In the present study, we carried out acute dermal toxicity, dermal and eye irritation experiments in rabbits and skin sensitization

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experiment in guinea pigs using this new formulation of *Bti* SH-14, according to the U.S. Environmental Protection Agency (EPA) guidelines, using some recommendations suggested in the Organization for Economic Co-operation and Development (OECD) Guidelines (OECD, 2013).

## 2. Materials and methods

Studies were accomplished according to the international principles of Good Laboratory Practice (U.S. EPA, 2000), and were approved by the CENPALAB Animal Ethics Committee.

### 2.1. Test materials

The new formulation of *Bacillus thuringiensis* var *israelensis* SH-14 was obtained from Grupo Empresarial de Producciones Biofarmacéuticas y Químicas (LABIOFAM) (Havana, Cuba) (Watery suspension, Batch No. 0507001, concentration: $6.45 \times 10^8$ spores/ml, stability: 2 years).

### 2.2. Animals

Male and female Cenp:NZW rabbits and Cenp:Hartley guinea pigs were obtained from CENPALAB (Havana, Cuba). Animals were housed individually in stainless steel cages and acclimated to the laboratory for 15–30 days prior to the start of the experiment. Only animals found to be in good health were selected for use. Water and feed (CMO:1400 and EMO:1301, ALYco®, CENPALAB) were available *ad libitum*, and animals were maintained in a room environment of 25–28 °C, 60–75% relative humidity, and 12 h photoperiod.

### 2.3. Acute dermal toxicity

One group of 5 male and 5 female Cenp:NZW rabbits was established. The procedure used for determining the dermal toxicity followed the procedures as recommended and documented by U.S. EPA (1996a). Approximately 24 h before the test, an electric clipper was used to expose the skin. Four milliliters of the test substance ($2.58 \times 10^9$ spores) were applied to the intact dorsum of the animals and covered with a dressing. The untreated area was used as control. The product was left in contact with the skin for 24 h and at that time the dressing was removed and any residue was removed by washing the area. The animals were observed daily over 14 days for any irritation and toxicity. Weights of individual animals were determined shortly before the new formulation of *Bti* SH-14 was administered and at 7 and 14 days. All animals were killed after a 14 day observation period and subjected to gross pathological examination.

### 2.4. Dermal irritation experiment

The dermal irritation experiment was performed according to EPA Guideline OPPTS 870.2500 "Acute Dermal Irritation" (EPA, 1998a). Only rabbits with healthy, intact skin were used. Approximately 24 h before the test, an electric clipper was used to expose the skin. The new formulation of *Bti* SH-14 was applied in an area of about 6 $cm^2$ in the left side of the dorsal surface of the trunk of the animals, and covered with a gauze patch, which was held in place with non-irritating tape. The right untreated side was kept as control area. A restrainer device was used to prevent the ingestion of the test substance from the application site. Initially, the test was carried out using one animal. The patches were removed after a 4-h exposure period, and the remaining test article on the application sites was removed by washing the area. No dermal reactions were observed at 1 and 4 h after patch removal. The test was repeated with two additional rabbits to confirm the initial findings, because the rabbit in the initial test did not exhibit any dermal reaction. The dermal responses (erythema and eschar formation, edema) were scored at 60 min, and then at 24, 48 and 72 h after patch removal. Grading of irritation was according to the method of Draize et al. (1944).

### 2.5. Eye irritation experiment

The eye irritation experiment was conducted in accordance to the EPA Guideline OPPTS 870.2400 "Acute Eye Irritation" (EPA, 1998b). Only rabbits with no abnormalities in the anterior eye parts were used. After gently pulling the lower lid away from the eyeball, 0.1 mL of the new formulation of *Bti* SH-14 was instilled into the conjunctival sac of the right eye of each rabbit. The lids were gently held together for about 1 s in order to prevent the loss of the material. The left eye, which remained untreated, served as a control. The initial test was carried using one rabbit. No corrosive effects were observed at 1 h after the ocular instillation. The test was then repeated with two additional rabbits to confirm the initial findings. The eyes of the test animals were washed with distilled water at 24 h following the application of test material, to remove the presence of residual test substance if any. The responses, including corneal opacity, conjunctival redness, abnormality of the iris, and chemosis, were evaluated at 1, 24, 48 and 72 h according to EPA Guideline OPPTS 870.2400. After recording the observations at 24 h, corneal epithelium was stained using sodium fluorescein and lectures were made using a fluorescent lamp.

### 2.6. Skin sensitization experiment

The skin sensitization experiment was carried out in accordance to EPA Guideline OPPTS 870.2600 "Skin Sensitization" (EPA, 2003) and Buehler (1994) method. Only healthy guinea pigs were used. Before the first induction, the guinea pigs were assigned to two groups: a control group ($n = 10$, 5/sex), and a treated group ($n = 20$, 10/sex). Approximately 24 h before the test, an electric clipper was used to expose the skin. On the first day of the first stage of induction, 0.5 ml of sensitizing agent was attached to a patch of test tape. The patch was applied to the left side of the shaved area, and covered with a dressing. Additional hair-removal and induction were carried out once weekly (on days 6–7 and 13–14). The guinea pigs were given sterilized water in the negative control group, and the new formulation of *Bti* SH-14 in the treated group. At 14 days after the third induction (day 28), the test was conducted. The hair on the flank not used for induction was removed using the clippers and shaver. One area of about $6 \times 6$ cm was marked on the right flank of all animals. Two hours later, 0.2 ml of the new formulation of *Bti* SH-14 for challenge was applied to the selected sites in the shaved area of both control and treated groups. The patches were covered with a dressing for a 6-h closed application. At 24 and 48 h after removal of the patches, dermal reactions were evaluated according to Magnusson and Kligman (1969). Weights of individual animals were determined shortly before the new formulation of *Bti* SH-14 was first administered and weekly thereafter. Body weight values were statistically analyzed using the Statistical Package Scientific System 11.5.1 (Statistical Package Scientific System, SPSS for Windows, Copyright SPSS Inc., 2002). Normality of the data was assessed by means of the Kolmogorov–Smirnov test. The statistical significance of differences between treated and untreated control group were determined using Student's $t$ test. Statistical significance was assessed at the $p < 0.05$ level.