A study of acute dermal toxicity for HeberNem® in rats

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TECHNIQUE

ABSTRACT

HeberNem® is a biological nematicide, basically composed by Tsukamurella paurometabola; it can effectively control the phytopathogenicity of nematode populations in several target crops. This study evaluates the skin toxicity of high doses of this compound. Five male and five female Wistar rats of 200 g were shaved in 10% of their body surface, and 24 hours later 4 mL of the product at 108 c.f.u./mL were applied on the skin for 24 hours, carrying out clinical observations for 14 days. Body weight was recorded on days 1, 7 and 14, after which they were euthanized. Heart, lungs, kidneys, liver, stomach, spleen and skin were macroscopically analyzed, finding no clinical signs related to the administration of the product. In the necropsies, no macroscopic alterations were found in the organs. Weight gains were observed in both seres throughout the study. Toxicity was not observed, at least under the conditions of this study; therefore HeberNem® is considered as a potentially non toxic product.

Key words: Tsukamurella paurometabola, HeberNem®, acute dermal toxicity, rat

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RESUMEN

Estudio de Toxicidad Dérmica Aguda de HeberNem® en Ratas. El HeberNem® es un nematicida biológico constituido básicamente por *Tsukamurella paurometabola,* capaz de controlar de manera eficaz la fitopatogenia de las poblaciones de nemátodos en varios cultivos. Se determinó el potencial tóxico del HeberNem® sobre la piel humana, como objetivo fundamental. Para realizar este estudio se utilizaron 10 ratas Wistar (5 de cada sexo) a las cuales se les razuró aproximadamente el 10% de la superficie corporal, para aplicar 24 horas después 4 mL/rata de 200 g con la dosis del producto de 10^8 ufc/mL; el contacto con la piel se mantuvo 24 horas. Durante 14 días, se realizaron observaciones clínicas diariamente; se pesaron los animales los días 1, 7 y 14, y se sacrificaron humanamente el día 14 del experimento. Luego, se analizaron macroscópicamente los siguientes órganos: corazón, pulmones, riñones, hígado, estómago, bazo y piel, en los cuales no se observó ningún signo clínico atribuible a la administración del producto. Se detectó una ganancia de peso corporal entre una y otra semana para uno y otro sexo. En las necropsias no se encontraron alteraciones macroscópicas en ningún órgano. Por lo cual concluimos que bajo las condiciones de este experimento no se observó toxicidad, por lo que el producto se considera potencialmente no tóxico por esta vía.

Palabras claves: Tsukamurella paurometabola, HeberNem®, toxicidad dérmica aguda, rata

*I*ntroduction

Nematodes are widely regarded as one of the most phytopathogenic plagues in agriculture. Their strongest impact is felt mainly in tropical, subtropical and temperate areas [1]; although they can be controlled through chemical or biological pesticides.

The use of biological pesticides for nematode control is usually favored, since they are effective for a longer time and do not pose the risks associated to the use of chemicals, which are often toxic. However, a biological substance can also be highly toxic, as in botulinum toxin D, whose median lethal dose upon intraperitoneal injection in mice (LD50 = 3.2 x 10^{-7} mg/kg) is several orders of magnitude lower than that for reputed chemical poisons such as cyanhydric acid (3 mg/kg). Therefore, several international agencies such as the World Health Organization (WHO) have proposed that any potential biological pesticide must be subjected to the same toxicity testing standards currently used for synthetic products [2].

Several bacterial strains that can potentially be used as biological nematicides have been reported by the Center of Genetic Engineering and Biotechnology at Camagüey, Cuba. The most promising among them due to its effectiveness and ease for industrial scale-up is the Gram-positive bacterium Tsukamurella paurometabola, strain C-924 (No. ATCC 8368) [3], of the Tsukamurella genus from the Nocordiaceae family, and initially identified as Corynebacterium paurometabolum [4], which has been developed into a product named HeberNem®.

This, product may well get in contact with the skin during its use, and must be tested in animals to evaluate their irritating and toxic potential, according to the current international guidelines. Such studies are known as acute toxicity trials [5-7].

The objective of this work is to determine acute dermal toxicity with the biological nematicide HeberNem® using rats as the experimental animals, following the guidelines established by the Environmental Protection Agency (EPA) [5] of the USA, the Organization for Economic Cooperation and Development (OECD) [6], and those outlined by García S. [8].

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Materials and methods

Assay substance

The assay substance evaluated in this study is the final product ready for agricultural use, that is, a preparation containing the microorganism, its culture medium (fodder yeast) and xanthan gum as the preservative.

Characteristics of the tested animals

The study used Wistar rats from the National Center for the Production of Laboratory Animals (CENPALAB). Their weight at the beginning of the study ranged from 175 to 206 g for females, and from 263 to 292 g for males, in compliance with the OECD guidelines for this type of assays [9].

They were split by gender into two groups of 10 individuals each, which received the product under evaluation.

Environmental and housing conditions

The animals were randomly distributed in individual cages. They were fed ad libitum throughout the experiment with ratonin (CENPALAB, Cuba) and water. The temperature was kept at 20 ± 2 ºC and the relative humidity was of between 50 to 70%, under a light-darkness cycle of 12/12 hours.

Experimental design

This study describes a single-dose, dermal toxicity/pathogenicity assay, similar to that designated as 885.3100 by the Office of Prevention, Pesticides and Toxic Substances (OPPTS) [6] and in compliance with the OECD [5] and World Health Organization (WHO) [10] guidelines.

Route and method of administration of the assay substance

Twenty four hours before the beginning of the assay, approximately 10% of the body surface of the animals was shaved. The product was applied at the volume of 4 mL per animal, using patches [8].

The substance was in contact with the skin for 24 hours, after which the patches were removed and the residual product was washed off with a 0.9% sodium chloride solution, using sterile cotton pads.

Dose and treatment groups

Two groups of 5 rats each were used, segregated by gender. Each 200 g rat received 4 mL of the product, containing 108 c.f.u./mL, in compliance with the international guidelines for the evaluation of biological products [6].

Observation of the animals during the experiment

After the administration of the product, the animals were watched for 14 days. The observations were frequent during the first day, and once a day during the rest of the trial.

The experiment lasted 21 days (7 for acclimatizing and 14 for the actual study). The clinical signs for the main organs and systems which could be observed macroscopically (skin, eyes and mucous membranes, respiratory system, circulatory system, central and autonomous nervous systems, somatomotor activity and behavior patterns) were systematically recorded.

Weighing the animals

The individual weights of the animals were taken and recorded on days 1, 7 and 14 of the experiment.

Euthanasia, necropsy and histopathological studies

Fourteen days after the beginning of the study, the animals were anesthetized with ether and slaughtered. Afterwards, several organs (heart, lungs, kidneys, liver, stomach and spleen) were studied, taking samples when necessary together with skin samples. These samples were subjected to a histopathological examination by specialized personnel.

Statistical analysis

The body weights were processed statistically by an analysis of variance with a one-way classification, followed by a Student-Newman-Keuls test for mean comparison with $p < 0.05$.

Results and discussion

Clinical observations

The dermal acute toxicity study is designed to detect effects induced by toxic or non-biological components of the microbiological plague control agent (MPCA) [11]. Its results are used to classify the toxicity of these substances, as well as the selection of the dose to be used in long-term toxicity studies [8].

HeberNem® is an MPCA that may be used of being used as a commercial bionematicide. Its median lethal dose for nematode control is 106 to 107 c.f.u./mL of the final product applied to the soil, particularly around the radicular system of the target plant. Although this study used a dose which is 10- to 100-fold higher than the median lethal dose for phytonematodes, no detectable alterations of the somatomotor activity or behavioral patterns of the experimental animals were observed. There were no alterations of the respiratory, circulatory, or central and autonomous nervous systems, and no problems were detected on the skin, eyes or mucous membranes.

These results agree with those previously obtained in an acute dermal toxicity/pathogenicity study carried out at the Center for Experimental Toxicology (CETEX, Cuba) which evaluated the active principle (The bacterium Tsukamurella paurometabola) on white New Zealand rabbits and found no toxic effects [12]. They are also similar to those previously obtained by other authors during the evaluation of pesticides of microbiological origin such as Bt var kenyae (no toxic effects upon single dose dermal administration in rats) [13], the Metharhizum anisophia fungus (no toxicity in mammals, recently licensed for commercial use) [14], and the Pseudomonas aureofaciens strain Tx-1 (No toxicity upon skin contact) [15].

Body weight

Table 1 shows the evolution of body weight throughout the 14 days of observation. There were statistically significant differences for both genders. Differences

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were found in males between days 1 and 14, but not between days 1 and 7, or 7 and 14.

It should be emphasized that although in some cases there were no statistically significant differences, a steady weight gain was observed between successive measurements that matches the expected weight gain vs. age curve for this rodent line, as reported by IFFA CREDO [16].

During acute dermal toxicity/pathogenicity [12] and ocular irritation [17] studies conducted at CETEX (belonging to CENPALAB) with Tsukamurella paurometabola (the active principle for HeberNem®), it was shown that no toxic effects could be detected either on the skin or the eyes of New Zealand white rabbits, under the conditions of the experiments.

Table 1. Body weight of the rats throughout the 14-day observation period (mean ± standard deviation) (g)

Gender	Time (days)		
	1	7	14
Female	193.0 ± 11.77a	200.8 ± 11.58a,b	214.8 ± 11.43b
Male	278.2 ± 11.48c	292.0 ± 13.06c,d	302.4 ± 10.16 d

a, b, c, d Statistical significance. ($p < 0.05$)

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The present results are also similar to those obtained for the Pseudomonas aureofaciens strain Tx-1 fungicide [15].

Macroscopic findings

The organs of the animals and their skin showed no macroscopic alterations whatsoever. Therefore, no samples were taken for histopathological processing, following the criteria of the pathologist. This decision is also based on previous results obtained during the acute dermal toxicity/pathogenicity studies for the active principle of HeberNem® [12], conducted at CETEX, where no pathogenicity or toxicity was found for the biomodel used. These results, together with the EPA/OPPTS guidelines, guarantee skipping the histopathological analysis of the samples unless there are any previous macroscopic findings.

Conclusions

Under the conditions of the assay, HeberNem® showed no skin toxicity after a topical single administration in rats.

Recommendations

To carry out acute respiratory toxicity studies with HeberNem®.

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