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Toxicological Study of Skin Irritation and Skin Sensitization Of LEBAME

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Abstract

The objective of this work is to evaluate the possible adverse effects that could produce reactions of dermal irritation and sensitization in the skin (allergic contact dermatitis) and to determine their potential, it is an important element in the evaluation of the toxicity of a substance. The evaluation of dermal irritation was carried out in Cenp: NZB rabbits, for this study 3 male animals were used, which were distributed in a single experimental group (LEBAME Treaty), body weight was determined at the beginning and at the end of the study. In the curieles, both sexes were used with a Control group (5 females and 5 males) and a LEBAME Treated group with (10 females and 10 males). The curieles received 3 induction applications separated from each other by 7 days and one challenge application at 14 days, weight was determined on a weekly basis. For both studies, observations were made daily at the site to determine if reactions occurred. Both studies concluded with a survival rate of 100%, with no skin irritation or skin reaction (in curials).

Keywords: Efficient microorganisms, Lebame, Skin Irritation Assay, Sensitization, Rabbit and Curiel, Dermal Irritation Test, Guinea pig

Introduction

Efficient Microorganisms (EM) are a combination of beneficial microorganisms of natural origin. Its concept and technology were developed by Dr. Teruo Higa at Ryukyus University, Okinawa, Japan [1]. EMs mainly contain microorganisms from four main groups: phototrophic bacteria, yeasts, lactic acid-producing bacteria, and fungi, as well as metabolites derived from fermentation. These have been widely used in different purposes, in agriculture, where they have been shown to improve the quality of soils, the optimization of crops and harvests. EMs are also known to be used as probiotics and for waste treatment to improve production, control odours and process waste on pig and cattle farms [2]. Due to the likely interaction of this product with animals and humans, it is prudent to conduct the present study with a view to evaluating the possible existence of hazards likely to arise from skin exposure to the test substance.

Materials and Methods

This trial was carried out with the approval of the Institutional Committee for the Care and Use of Laboratory Animals (CICUAL) of CENPALAB. They came from the Directorate of Gnotobiotic Animals of CENPALAB.

Substance: LEBAME (Efficient Microorganisms)

Animals

For the skin irritation test, rabbits from the Cenp: NZB line were used with a weight of approximately 2 and 2.2kg, for skin sensitization curials from the Cenp: HARTLEY line were used, with a weight range between 370-425g, with their corresponding certificates of sanitary and genetic quality. The animals were kept in an experimentation room, with a 12x12 h light regime, water and food "ad limitum", temperature and humidity controlled.

For dermal irritation, a single experimental group of 3 animals was formed, through the LABTOOLS [3] program, two experimental groups were formed for the skin sensitization test.

a) Control Group (Autoclaved Water) 5 females and 5 males

b) Treated Group (LEBAME) 10 females and 10 males

Dermal Irritation

The dermal irritability test was performed on 3 rabbits. The animals were applied 0.5mL on a small surface of the skin (6 cm2), after 24-hour hair removal. Clinical observations were carried out daily in the morning hours where the general condition of the animals was evaluated. 4 hours after the substance was applied (patch removal), the reading was taken to determine the dermal reactions (edema and erythema) during exposure to the test substance. This reading was repeated 24, 48 and 72 hours after the patch was removed. The calculation of the dermal Irritability Index (IID) was determined by summing all the observations and dividing it by the number of observations made. The value obtained was compared with what was reported in the literature, to classify the product and thus approve or reject it according to OECD standards [4].

Skin Sensitization

For this trial, 30 curials (15 of each sex) were used. Approximately 24 hours before each application, the hair was removed from the dorsal area of the back of each animal by shaving. In both groups, three induction applications were performed (day 0, 7 and 14 of the study, on the right side of the loin) and one challenge on day 28 of the study (on the left side of the loin). In all cases, a volume of 0.5 mL was applied, using a 4-6 cm2 gauze patch that remained in contact with the skin for 6 hours. In the challenge, both groups were patched with LEBAME. The concentration used in all cases was the same (100%). Daily individual clinical observation was carried out, and 6 hours after each application of the substances (removal of the patch), observation of the applied area was carried out. At 24 and 48 hours after the removal of the patch, the reading was taken to determine whether or not skin reactions of irritation had occurred. For the interpretation of the results and classification of the substance evaluated, the following evaluation system (Magnusson and Kligman Method) was used as a [5] (Table).

Table 1:

Description	Graduation scale
Non-visible changes	0
Discrete erythema	1
Moderate erythema	2
Intense erythema and inflammation	3

Body weight was determined individually for all animals, prior to the first application on (day -1), and subsequently weekly and at the end of the study. Euthanasia was carried out by contusion and exsanguination.

Results and Discussion

The study culminated in 100% survival. There were no clinical signs of interest. Rabbits and curieles did not show any alteration in the area of application. In all cases, an increase in body weight was obtained at the beginning and at the end of the study.

Dermal Irritability

During the trial and during the following 3 days, skin readings were taken for erythema, edema, and no lesions were observed in the animals. Clinical symptoms were also not observed in the animals tested, so the dermal irritation index for LEBAME was 0.0, which classifies the substance as potentially non-irritating to the skin, according to the scale established in the literature4. Similar studies using other species have obtained negative results5,6 and comply with OECD guidelines.

Skin Sensitization

In the Skin Sensitization study, no visible changes were reported when evaluating dermal reactions, after the three induction applications and after the challenge [7,8]. In other dermal studies using rabbits, negative results were obtained that have allowed this substance to be classified as potentially non-irritating to the skin [5,9]. When the necropsy of the curials was performed, no anatomical-pathological lesions were found.

Conclusion

Under the experimental conditions in which the study of Dermal Irritation and Skin Sensitization was carried out, taking into account that no signs of toxicity were detected, there were no effects on body weight, and no anatomical-pathological alterations were detected. We can conclude that the LEBAME Dermal Irritation

assay obtains the classification of Non-Dermal Irritant. On the other hand, the skin sensitization test. In both the induction and challenge phases, it is classified as NON-SENSITIZING dermally.

Conflicts of Interest

None.

Acknowledgements

None.

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