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QUALITY MANAGEMENT
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CERTIFICADO Nº G-91299973/2

BIOLOGICAL TOXICITY ASSAY UNIT

EYES IRRITATION TEST (3)

(In compliance with guidelines for dangerous substances and preparations)

Company: TEXSA, S.A.

Prod./ Sample: LÁMINA POLIMÉRICA VISCOELÁSTICA

Batch/ Reference: Desarrollo 135

Request No. : A7980

CONFIDENTIAL

A7980

EYES IRRITATION TEST (3) IN COMPLIANCE WITH THE GUIDELINES FOR DANGEROUS SUBSTANCES AND PREPARATIONS.

A7980

1. METHOD

-The method used in the rehearsal is the one described in the ROYAL DECREE 363/1995, from March 10th, which endorses the regulations for notification of new substances and classification, bottling and labeling of dangerous substances.

- In compliance the ROYAL DECREE 255/2003, from February 28th , which endorses the regulations for classification, bottling and labeling of dangerous preparations.

2. METHOD SUMMARY.

The intention of this test is to evaluate the potential of the study sample to produce eyes irritation. It is performed on three rabbits because it is not thought that the product may produce serious effects.

The sample is applied in a single dosage to one only eye of each rabbit. The non treated eye is used as control. An observation is made of the importance of each irritation followed by an evaluation after a certain period of time. A detailed description is made of the alterations with the objective of carrying out a complete evaluation of the effects. The reversible character of the observed effects is evaluated. As assessment of the results is made and conclusions are obtained.

3. - SAMPLE OBJECT OF STUDY

Sample: LÁMINA POLIMÉRICA VISCOELÁSTICA

Batch/ Reference: Desarrollo 135

Request No.: A7980

4. - STUDY SITE

The present study was carried out in the Biological Toxicity Assay Unit of the Dr. Echevarne Laboratory located at 29 Cami de Ca la Madrona in the Mata-Rocafonda industrial park of Mataro (Barcelona).

5.- MATERIAL

Animals: 3 male rabbits, white, New Zealand bred, provided by an authorized supplier.

Scale for weighing the animals (COBOS D 6000-SX)

Disposable vinyl or latex gloves.

1 mL syringes (insulin style).

Immobilization rap for rabbits.

Transport car with wheels.

Manual lamp.

Cleaning flask with saline solution.

Colircusí Fluoresceina or equivalent authorized.

6. - ENVIRONMENTAL CONDITIONS

During the acclimatization-quarantine and the experiment, the stable environmental conditions were the following:

Temperature: 21° C (± 2°C).

Relative Humidity: 55% (± 25%)

Air: 15 air renewals per hour and prefiltered a 5µm.

Lighting: Circadian rhythm of 12 hours of light and 12 hours of darkness, regulated by timing devices.

Shelter: Individual cage of stainless steel, brand name Tecniplast

Food: Daily doses of 150g of special diet for experimentation rabbits, provided by an authorized supplier.

Drink: Automatic, as needed, treated and filtered at 5µm.

Cleaning: Automatic

7. - PREPARATION OF ANIMALS

7.1 - INDIVIDUAL CHECK AND IDENTIFICATION

Upon arrival at the Unit, the rabbits were subjected to a sanitary check and subsequently tattooed in the upper part of the left ear for proper identification.

7.2 - ACCLIMATIZATION- QUARANTINE

The animals were observed daily during a period of acclimatization-quarantine of 7 days. They overcame this without any significant observation, the good condition of the eyes was worthy of special attention.

8.- PREPARATION OF SAMPLE

An extract was prepared from the test sample; 0,78 grams of sample was used per mL of extraction medium (=0,78 g/mL P/V).

The extraction process is carried out in a stove to 70ºC during 24 hours.

9. - TEST

Once the rabbits are on the immobilization traps, the sample is applied to the conjunctive bag of one of the eyes by separating the inferior lid. The other eye is used as control.

If liquid samples are used, 0,1 mL is instilled. If solid (crushed into fine powder) or pasty samples are used, 0,1 g is applied.

In order to make the observation of reactions easier, the light of a manual lamp is used and after 24 hours *fluoresceina* is also used to evaluate possible corneal damages.

Observations are made after 1, 24, 48 and 72 hours from the time of application.
If ocular damages are not observed after 72 hours the test is finished.

If ocular damages keep after 72 hours, further observations can be made after 7, 14 or 21 days to evaluate the reversible character or grade of the effects.

If a serious ocular damage is observed (according to approaches on section 9.4) the test is considered finished because of humanitarian reasons.

10. - STUDY PARAMETERS AND ASSESSMENT CRITERION

10-1. - WEIGHT CONTROL

This is carried out at the beginning of the experiment.

10-2. - GENERAL EXAMINATION

The general state of the animal is observed with special attention to the appearance of modifications in the treated eye, that is, cornea, iris, conjunctiva or any other alteration.

10-3.- READING AND EVALUATION OF THE OBSERVATIONS

In each of the observation times (1,24,48 and 72 hours), notes are taken of the alterations to the treated eye (cornea opacity, iris damages, conjunctive irritation and oedema) using the following criterion:

CORNEA.

Opacity: density grade (observations are carried out on densest areas).
No ulceration or opacity ---------- 0
Dispersed or diffuse opacity areas (different from a slight streaming of the normal brightness), iris details clearly visible ---------- 1
Translucent area easily defined, iris details slightly obscured---------- 2
Nacrous area, iris details completely invisible, pupil size hardly perceptible -------3
Opaque cornea, no perceptible iris through opacity ---------- 4

IRIS.

Normal ---------- 0
Markedly deepened rugae, congestion, swelling, moderate pericorneal hyperhemia or injection; any of these symptoms or their combinations, the iris still reacting to light (a slow reaction is positive)---------- 1
No reaction to light, hemorrhages, marked destruction (each of the symptoms or all of them as a whole) ---------- 2

CONJUNCTIVA.

Irritation (it is applied to the most affected area of ocular and eyelid conjunctiva in connection with the control eye).

Blood vessels normal ---------- 0
Well-defined hyperhemia in some blood vessels (injected eyes)---------- 1
Diffuse crimson colour, individual vessels are hardly perceptible ----------2
Strong and diffuse red coloration---------- 3

Conjunctive oedema (nictitate membranes or/and eyelids)
No swelling ---------- 0
Any swelling upper than normal levels (including nictitating membranes) --------- 1
Clear swelling with partial eversion of lids ---------- 2
Swelling with lids about half closed---------- 3
Swelling with lids more than half closed ---------- 4

A7980

10-4. - EVALUATION CRITERIA OF THE RESULTS

In accord with the Royal Decree 363/1995, of the 10th of March, which endorses the regulations on notification of new substances and classification, bottling and labeling of dangerous substances. Published in the BOE No. 133 from Monday June 5th, 1995.

The following sentences of risk will be assigned according to the suitable approaches:

R36 Causes eye irritation.

Substances or preparations which are applied to the animal eye and cause important ocular damages that appear during the first 72 hours after exposure and persist at least 24 hours.
An ocular damage is considered important if the average values of the results of the eyes irritation test described are any of the following:

Cornea opacity: equal to or more than 2 but less than 3.
Iris damage: equal to or more than 1 but less than 1.5.
Conjunctive irritation: equal to or more than 2.5.
Conjunctive oedema: equal to or more than 2.

It is also considered important (in a test made with three animals) if the damages of two or more of the animals used in the test are equivalent to any of these values, except for iris damage (it must be equal to or more than 1 but less than 2) and for conjunctive irritation (its value must be equal to or more than 2.5).
In both cases, when the respective average values are calculated, all the results of each reading period (24, 48 and 72 hours) must be used for each effect.

R41 Risk of serious ocular damages.

Substances or preparations which are applied to the animal eye and cause severe ocular damages that appear during the first 72 hours after application and persist at least 24 hours.
Any ocular damages are considered important if the average values of the results of the eyes irritation test described are any of the following:

Cornea opacity: equal to or more than 3.
Iris damage: more than 1.5.

Other damages are also considered serious (in a test made with three animals) when are equivalent in two or more animals to one of the following values:

Cornea opacity: equal to or more than 3.
Iris damage: equal to 2.

In both cases, when the respective average values are calculated, all the results of each reading period (24, 48 and 72 hours) must be used for each effect.

Ocular damages are also considered serious if persist at the end of the observation period or if the substance or preparation used in this test causes no reversible eye colorátion.

A7980

11. -TABLE OF RESULTS

11-1.- WEIGHT TABLE

RABBIT	WEIGHT in grams.
No. 1	5203
No.2	5205
No.3	5206

A7980

11.2.- TABLE OF ALTERATIONS

RABBIT 1

	1 HOUR	24 HOURS	48 HOURS	72 HOURS	7 DAYS	14 DAYS	21 DAYS
CORNEA OPACITY	...	0	0	0	...	...	...
IRIS DAMAGE	0	0	0	0	...	...	...
CONJUNCTIVE IRRITATION	0	0	0	0	...	...	...
CONJUNCTIVE OEDEMA	0	0	0	0	...	...	...

OBSERVATIONS: -

A7980

RABBIT 2

	1 HOUR	24 HOURS	48 HOURS	72 HOURS	7 DAYS	14 DAYS	21 DAYS
CORNEA OPACITY	...	0	0	0	...	...	...
IRIS DAMAGE	0	0	0	0	...	...	...
CONJUNCTIVE IRRITATION	0	0	0	0	...	...	...
CONJUNCTIVE OEDEMA	0	0	0	0	...	...	...

OBSERVATIONS: -

A7980

RABBIT 3

	1 HOUR	24 HOURS	48 HOURS	72 HOURS	7 DAYS	14 DAYS	21 DAYS
CORNEA OPACITY	...	0	0	0	...	...	...
IRIS DAMAGE	0	0	0	0	...	...	...
CONJUNCTIVE IRRITATION	0	0	0	0	...	...	...
CONJUNCTIVE OEDEMA	0	0	0	0	...	...	...

OBSERVATIONS: -

11.3 - TABLE OF AVERAGE VALUES OF CORNEA, IRIS, AND CONJUNCTIVA OBSERVED AT 24, 48 AND 72 HOURS.

	RABBIT No 1	RABBIT No 2	RABBIT No 3	AVERAGE
CORNEA OPACITY	0.0	0.0	0.0	0.0
IRIS DAMAGE	0.0	0.0	0.0	0.0
CONJUNCTIVE IRRITATION	0.0	0.0	0.0	0.0
CONJUNCTIVE OEDEMA	0.0	0.0	0.0	0.0

11.4.- DESCRIPTION OF THE OBSERVED DAMAGES.

See section 11.2.

A7980

12.-BIBLIOGRAPHY

- ROYAL DECREE 363 /1995, from March 10th, which endorses the regulations for notification of new substances and classification, bottling and labeling of dangerous substances.

- ROYAL DECREE 255/2003, from February 28th , which endorses the regulations for classification, bottling and labeling of dangerous preparations.

- DIRECTIVE 67/548/CEE from the Board, relative to the approximation of the legal guideline, both regulatory and administrative, on the subjects of classification, packaging and labeling of dangerous substances.

- GUIDELINES for Experiments on Chemical Products from the Economic Development and Cooperation Organization (EDCO) No. 405:'A cute Eye Irritation/Corrosion' (enacted 24/02/87)

- JOHN W. DRAIZE and others. Methods for the Study of irritation and toxicity of substances applied topically to the skin and mucous membranes. Journal of Pharmacology and Experimental Therapeutic. 1944.

- DIRECTIVE 86/609 CEE of the Board on the 24th of November, 1986 relative to the approximation of the legal guideline, both regulatory and administrative of the member states with regard to the protection of animals used for experimentation and other scientific ends.

- ROYAL DECREE 22/1988 from the 14th of March on the protection of animals used for experimentation and other scientific ends.

- LAW 5/1995 from the 21st of June on the protection of animals used for experimentation and other scientific ends (DOGC 2073- 10.7.1 1995)

- DECREE 214 /1997 from the 30th of July which regulates the use of animals for experimentation and other scientific ends (DOGC 2450 -7.8-1997).

A7980

13. CONCLUSIONS

Given that the sample " LÁMINA POLIMÉRICA VISCOELÁSTICA Desarrollo 135" (A7980) does not produce important ocular damages, it won't be necessary to assign any sentence of risk of those described in the point 10.4 of the present statement.

This classification does not exclude any other that might result from other experiments.

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Mataro, 17th of january, 2005