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TOXICOLOGICAL-BIOLOGICAL TESTING UNIT

STUDY OF ACUTE DERMAL TOXICITY (LD_{50})

ACCORDING TO METHOD OF THE OFFICIAL DIARY OF THE EUROPEAN COMMUNITIES IN COMPLIANCE WITH THE DANGEROUS PREPARATIONS DIRECTIVE

Company: TEXSA,S.A.

Prod./Sample: LÁMINA POLIMÉRICA VISCOELÁSTICA

Request Nº : A7980

Batch/Reference: Desarrollo 135

CONFIDENTIAL

ACUTE DERMAL TOXICITY (LD_{50}) ACCORDING TO THE METHOD OF THE O.D.E.C. (D.P.)

1.- METHOD

- The method employed in the study is described in the Official Diary of the European Communities L383: Directive and Annex 92/69/EEC of the Commission, 31 July 1992, which is adapted, for the seventeenth time, on technical progress, Directive 67/548/EEC of the Council, relative to the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.

- For the evaluation and interpretation of the results the criteria used are defined in Order 9, December 1992, of the Ministry of Relations with the Courts and the Secretary of the Government, by which the technical annexes "Regulations on the Declaration of New Substances and Classification, Packaging and Labelling of Dangerous Substances" are updated and approved by Royal Decree 2216/1985, of 23 October.

- All this in compliance with the Royal Decree 255/2003, of 28 February, by which "The Regulations for the Classification, Packaging and Labelling of Dangerous Substances" are approved.

2.- METHOD SUMMARY

The study substance is administered subcutaneously (hair shaven previously) to various batches of rats, at one dose per batch. The effects and the mortality caused by the substance are observed.

Autopsies are performed on the dead animals during the study, as well at the end of it. A report of the study, with the conclusions, is prepared.

PRELIMINARY STUDY: 2000 mg/Kg of the product is administered topically to 2 rats, they are repeatedly observed for 48 hours. If there are no deaths, the limit test is performed.
LIMIT TEST: 2000 mg/Kg of the product is administered topically to 5 males and 5 females. If after a period of observation of 14 days. If there are no deaths, the study will be finalised. If there is mortality due to the substance, a complete study will be carried out and the 50% Lethal Dose (LD_{50}) calculated.

3.- SAMPLE OBJECTIVE OF THE STUDY

Sample: LÁMINA POLIMÉRICA VISCOELÁSTICA

Request Nº : A7980

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4.- MATERIAL

.- Animals: Sprague Dawley (CFY) rats provided by an authorised supplier.

.- Balance for weighing the animals (COBOS D 6000-SX).

.- Single use vinyl or latex gloves.

.- Electric shaver (OSTER model GOLDEN A5).

.- 2 mL syringes.

.- Gauze strips (Melolín) or authorised equivalent.

.- Surgical tape.

.- Hypoallergenic adhesive dressing (Fixomull Stretch) or authorised equivalent.

.- Standard uniflex bandages or authorised equivalent.

.- Scissors.

.- Receptacle for weighing the animals.

.- 1 mL syringes (insulin type).

.- Insulin type needles (25G x 5/8 0.5 x 16).

.- Ketamine.

..- Transport cart with wheels.

5.- ENVIRONMENTAL CONDITIONS

During acclimatisation-quarantine and the study, the environmental and stabling were the following:

Temperature: 21ºC (± 2ºC).

Relative Humidity: 55% (± 25%).

Air: 15 renewals of air per hour and pre-filtered at 5µm.

Illumination: circadian rhythm of 12 hours light and 12 hours darkness, regulated by means of timers.

Accommodation: Techniplast Makrolon cages (48 x 27 x 20 cm), with bed of wood shavings.

Feeding: free access to an RMM diet for rats, provided by an authorised supplier.

Drink: *ad libitum*, using Makrolon bottles.

6.- PREPARATION OF THE ANIMALS

6-1.- IDENTIFICATION OF THE GROUPS

On their arrival at the Unit, the rats were subjected to health inspection and subsequently housed in groups of 2 and 5 animals, each group identified, with their data on a label on each cage.

6-2.- ACCLIMATISATION -QUARANTINE

The rats were observed daily for a period of acclimatisation-quarantine for 7 days, which passed without any significant observations.

6-3.- PREPARATION FOR THE STUDY

The shaving of the rats is carried out in the retroscapular dorsum region, forming a rectangle of 8 cm long and 5 cm wide.

6-4.- INDIVIDUAL IDENTIFICATION

Before the administration, the rats were identified individually by black felt-tip markings on the tail, which were maintained throughout the whole study.

7.- PREPARATION AND APPLICATION OF THE SAMPLE

7-1.- PREPARATION

200 mg of the remitted sample/100 g weight of rat was applied.

7-2.- APPLICATION

On the shaved zone the prepared sample is applied, topically, at a single dose, on an area equivalent to 10% of the total body surface of the animal.

Next, it was covered by a gauze strip and a hypoallergenic bandage which allowed the animals to move, but prevented access to the application zone.

After 24 hours of application, the bandage was removed, eliminating the remains of the study substance. Next the study data corresponding to each cage was noted oneach label and on the worksheet.

8.- STUDY

8-1.- PRELIMINARY STUDY

2000 mg/Kg of the product is administered topically to 2 rats, they are repeatedly observed for 48 hours. If there are no deaths due to the application of the sample, the limit test is proceeded to be carried out.

8-2.- LIMIT TEST

2000 mg/Kg of the product is administered cutaneously to 5 males and 5 females. If after a period of observation of 14 days. If there are no mortality is observed due to the application of the sample, the study will be finalised.

If there should be mortality due to the substance, it would be necessary to carry out a wider study and the 50% Lethal Dose (LD_{50}) calculated.

8-3.- SACRIFICE

At the end of the study, the animals were sacrificed by a humanitarian method (spine dislocation).

9.- DOSES ADMINISTERED

All the animals in the study were administered a single dose:

- 2000 mg remitted sample / Kg body weight.

10.- PARAMETERS STUDIED AND EVALUATION CRITERIA

10-1.- WEIGHT MONITORING

It is carried out on starting the study, before the administration and later once a week and at the time of death of each individual.

10-2.- GENERAL EXAMINATION

A clinical examination is carried out each working day in which are observed, among others, changes in:

Skin, hair, eyes, mucosa, respiratory apparatus, circulatory system, central and autonomous nervous system, somato-motor activity and behaviour rules.

Paying special attention to: shivering, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

The first day the observations were carried out frequently, on the following days they were observed twice daily up to the14 days.

10-3.- CRITERIA FOR THE EVALUATION OF THE RESULTS

According to the Order 9, December 1992, of the Ministry of Relations with the Courts and the Secretary of the Government, by which the technical annexes "Regulations on the Declaration of New Substances and Classification, Packaging and Labelling of Dangerous Substances" are updated and approved by Royal Decree 2216/1985, of 23 October. Published in the Official State Bulletin on Thursday 17 December 1992 (Nº302):

The substances are classified and placed in the category "very toxic on contact with the skin", "toxic on contact with the skin" or "harmful on contact with the skin" using the determination of the oral LD_{50} and in accordance with the following criteria:

CATEGORY	Cutaneous LD_{50}	RISK PHRASE TO ASSIGN
Very toxic	$LD_{50} \leq 50$ mg/Kg	R27
Toxic	50 mg/Kg $< LD_{50} \leq 400$ mg/Kg	R24
Harmful	400 mg/Kg $< LD_{50} \leq 2000$ mg/Kg	R21

11.- RESULTS TABLES

11.1.- RESULTS OF THE PRELIMINARY STUDY (2000 mg/Kg– 48 hours.)

RAT	INITIAL WEIGHT (in grams)	DEATH DUE TO TOXICITY
P1	285	Negative
P2	213	Negative

OBSERVATIONS:

On not presenting with mortality due to the application of the sample the limit test is performed.

See chapter 12 (Symptoms observed).

11-2.- RESULTS OF THE LIMIT TEST (2000 mg/Kg – 14 DAYS.)

11-2-1.- TABLES OF THE WEIGHTS OF THE RATS IN THE LIMIT TEST

MALES	Weight (in grams)		
	Initial	7th day	Final
L 1	345	370	384
L 2	368	389	408
L 3	381	406	425
L 4	316	340	358
L 5	292	304	314

Observations: The variation in recorded weights, can be considered within the normal ranges for this breed.

FEMALES	Weight (in grams)		
	Initial	7th day	Final
L 6	226	238	261
L 7	215	235	248
L 8	242	248	269
L 9	205	213	231
L 10	215	213	224

Observations: La variation in recorded weights, can be considered within the normal ranges for this breed.

11-2-2.- LIMIT TEST RAT MORTALITY TABLES

MALES	Mortality due to toxicity	Initial date	Date sacrificed (or death)
L1	negative	23/12/04	07/01/05
L2	negative	23/12/04	07/01/05
L3	negative	23/12/04	07/01/05
L4	negative	23/12/04	07/01/05
L5	negative	23/12/04	07/01/05

Observations: All the animals survived up to 14 days observation without any deaths being produced due to toxicity. See chapter 12 (symptoms observed).

FEMALES	Mortality due to toxicity	Initial date	Date sacrificed (or death)
L6	negative	23/12/04	07/01/05
L7	negative	23/12/04	07/01/05
L8	negative	23/12/04	07/01/05
L9	negative	23/12/04	07/01/05
L10	negative	23/12/04	07/01/05

Observations: All the animals survived up to 14 days observation without any deaths being produced due to toxicity. See chapter 12 (symptoms observed).

12.- SYMPTOMS OBSERVED

No anomalous behaviour was observed or signs of toxicity attributable to the administration of the sample.

All the administered animals, survived without problems up to 14 days of observation.

13.- AUTOPSIES

Autopsies were carried out on the study animals, without observing macroscopic changes in any of them.

14.- BIBLIOGRAPHY

(1).- ROYAL DECREE 1078/93, of 2 July, of the Ministry of Relations with the Courts and the Secretary of the Government, by which the Regulation on the classification, packaging and labelling of dangerous substances is approved.

(2).-ROYAL DECREE 2216/1985, of 28 October, by Presidency of the Government, by which it approved the Regulation on the Declaration of New Substances and Classification, Packaging and Labelling of Dangerous Substances, and its subsequent modifications and updates of its annexes.

(3).- ORDER of 14 March 1988, Ministry of Relations with the Courts and the Secretary of the Government, by which study methods are developed for the determination of the properties of dangerous substances.

(4).- ORDER of 13 November 1989, Ministry of Relations with the Courts and the Secretary of the Government, by which new methods for the determination of the properties of dangerous substances are added to those approved by ORDER of 14 March 1988.

(5).- ORDER 9 December 1992, Ministry of Relations with the Courts and the Secretary of the Government, by which the technical annexes "Regulations on the Declaration of New Substances and Classification, Packaging and Labelling of Dangerous Substances" are updated and approved by Royal Decree 2216/1985, of 23 October.

(6).- COMMISSION DIRECTIVE AND ANNEX 92/69/EEC, of 31 July 1992, by which it is adapted, for the seventeenth time, due to technical progress, COUNCIL DIRECTIVE 67/548/EEC, relative to the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances: B.1. Acute toxicity by oral route.

(7).- COMMISSION DIRECTIVE 93/21/EEC AND ANEXES I, II, III and IV, of 27 April 1993, adapted, for the seventeenth time, due to technical progress, COUNCIL DIRECTIVE 67/548/EEC, relative to the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.

(8).- COUNCIL DIRECTIVE 92/32/EEC, of 30 April 1992, by which it modifiesfor the seventh time the COUNCIL DIRECTIVE 67/548/EEC, relative to the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances.

(9).- TEST GUIDELINES for Studies of Chemical Products of the Organisation for Economic Cooperation and Development (OECD): nº 401. Acute Oral Toxicity. (Posted on 24.02.87)

(10).- COUNCIL DIRECTIVE 86/609/EEC, of 24 November 1986, in relation to the approximation of laws, regulations and administrative provisions of the Member States as regard the protection of animals used for experimentation and other scientific purposes.

(11).- ROYAL DECREE 223/1988, of 14 March, on the protection of animals used for experimentation and other scientific purposes

15.CONCLUSIONS

The preliminary study and the limit test carried out on the remitted sample, showed no signs of toxicity in the administered animals and on the autopsies no significant macroscopic changes were found.

The sample **"LÁMINA POLIMÉRICA VISCOELÁSTICA desarrollo 135"** (A7980) had an $LD_{50}^{'}$ via the dermis of greater than 2000 mg/Kg.

(Note: 2000 mg/Kg. is the maximum administrable according to the rules used)

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