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DATA EVALUATION RECORD

Study Type: Subchronic 90-Day Dermal Toxicity - Rats (82-3)

EPA Identification No.s: EPA MRID (Accession) No. 416145-03
EPA ID No.059820-00001
EPA Record No. S383994
EPA Pesticide Chemical Code 009501
Caswell No. 082
HED Project No. 0-2005,formerly 01843

Test Material: Acarosan Moist Powder (5% Benzyl Benzoate as the a.i.), batch No. Prod. Sept. 1987.

Synonyms: Acarosan-Feuchtpulver

Sponsor: Werner and Mertz GMBH, Rheinallee 96, D-6500 Mainz 1, Germany

Study Number(s): 099540

Testing Facility: RCC Research & Consulting Company, AG 1, Rte de Troinex, CH 1229, Carouge, Switzerland

Title of Report: Acarosan Moist Powder: Subchronic 90-Day Repeated Dose Dermal Toxicity in Rats

Author(s): Ph. Thevenaz, H. Luekemeier, G. Pappritz, and P. Mladenovic

Report Issued: 03- 30-1988

Conclusions: Based on the results of this study, the dermal NOEL for Acarosan moist powder is 100 mg/kg/day (mid dose) in rats of both sexes. The dermal LEL is 1000 mg/kg/day (highest dose tested) based on incidences of slight to well defined erythema in males and of slight to transiently severe erythema in females. The systemic NOEL is greater than 1000 mg/kg/day.

It is noted that this study was conducted on the end-product Acarosan moist powder (5% benzyl benzoate as the a.i.), and not on technical grade benzyl benzoate.

Core Classification: MINIMUM

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A. Materials

Test animals were 120 Wistar rats (60 males and 60 females), weighing from 166 to 242g for the females and from 201 to 253g for the males.

The test material was Acarosan Moist Powder, a white solid end-product, containing 5% Benzyl Benzoate as the active ingredient. The vehicle was distilled water containing 4% carboxymethyl cellulose. Samples prepared for treatment weeks 2,5,9, and 13 were analysed for content (determined as the a.i. benzyl benzoate) and homogeineity.

B. Methods:

The test animals were housed individually and were acclimated for 8 days. During this period, they were observed for clinical signs and administered an opthalmoscopic examination. The rats were randomly alloted to one of the following groups:

GROUP	DOSE (mg/kg/day	VOLUME (ml/kg/day)	# ANIMALS	
1	0	4 ml vehicle	15 M	15 F
2	10	2 ml test material	15 M	15 F
3	100	2 ml test material	15 M	15 F
4	1000	4 ml test material	15 M	15 F

A dorsal skin area corresponding to about 10% of the total body surface was shaved. The vehicle/test material was applied to the shaved skin, 5 consecutive days/week for 13 weeks. The daily exposure time was 6 hours. During this period, the application sites were covered with semi-occlusive dressings. The application sites were washed with water and blotted dry after every exposure and were shaved every week. Ten males and 10 females of each group were killed at the end of the 13-week treatment period, and the remaining 5 rats/sex/group were sacrificed after an additional treatment-free period of 28-29 days (recovery period).

C. Observations:

All rats were checked for mortality twice daily. Clinical signs were observed at least once daily. The application sites were examined daily, prior to each exposure, and treatment related dermal reactions were scored according to the Draize scoring system.

Individual body weight and food consumption were recorded weekly. Groups 1 and 4 animals received an additional opthalmoscopic examination at the end of the treatment/recovery periods.

At the end of the study, retro-orbital plexus blood samples were drawn from all animals after an 18-hour fast, in the period between 6.30 and 9.00 a.m., to minimize any circadian induced-variations. Urine samples were collected through the 18-hour fast. The following parameters were evaluated:

Hematology:

Erythrocyte count
Hemoglobin
Hematocrit
Mean corpuscular volume
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Platelets count
Reticulocyte count
Nucleated erythrocyte normoblasts
Total leukocyte count (WBC)
Differential WBC
RBC morphology
Thromboplastin time (TP)
Partial TP

Clinical Biochemistry:

Creatinine
Total Bilirubin
total Cholesterol
Triglycerides
Aspartate aminotransferase
Alanine aminotransferase
Lactate dehydrogenase
Creatinine kinase
Alkaline phosphatase
Gamma-glutamyltransferase
Sodium
Potassium
Chloride
Albumin
Calcium
Phosphorus
Total Protein
Glucose
Urea

Urinalysis:

Urobilinogen
Urine Sediment
Volume (18-hour)
pH
Protein
Glucose
Ketone
Bilirubin
Blood

D. Pathology:

All animals were necropsied and all macroscopic abnormalities were recorded. At the end of the treatment/ recovery periods, all animals were killed by an IP injection of sodium pentobarbital and representative specimens of the following tissues/organs were collected and fixed in 4% phosphate buffered neutral formaldehide solution:

xx Adrenals
x Aorta
Bone (femur)
Bone marrow (femur, sternum)
xx Brain
x Prostate gland
x Salivary glands
x Sciatic nerve
x Seminal vesicle
Skeletal muscle
x Skin (treated and

x Epididymides
x Esophagus
Eyes (with optic nerves and Harderian glands)
xx Heart
xx Kidneys
x Large intestine (caecum, colon, rectum)
Larynx
Lacrymal glands
xx Liver
x Lungs
x Lymph nodes (mandibular and mesenteric)
Nasopharynx
xx Ovaries
x Pancreas
x Pituitary glands
untreated areas)
x Small intestine (ileum, duodenum, jejunum)
xx Spleen
x Stomach
xx Testes
x Thymus
x Thyroid gland (with Parathyroids)
Tongue
Trachea
xx Urinary bladder
x Uterus
Vagina
x Gross lesions

All specimens were processed, sectioned, and stained with hematoxylin and eosin. Tissues specimens CHECKED (x) were examined histologically for all groups 1 and 4 rats. For groups 2 and 3, only the lungs, skin, and organs with abnormal macroscopic appearance were examined histologically. Tissues Checked (xx) were also weighed.

E. Statistical Analysis of the Data:

Intergroup differences in body weight, food consumption, organ weights and clinical laboratory data were assessed by the univariate one-way analysis of variance and either a Dunnett-test (variables assumed to follow a normal distribution) or a Steel-test (variables not assumed to follow a normal distribution. The overall spontaneous mortality data was analysed using the Fisher's exact test for 2x2 tables. Test statistics were calculated on the basis of exact values for means and pooled variances and then rounded off to two decimal places.

F. Results:

The mean concentrations of the a.i. found in the test material samples collected on treatment weeks 2, 5, 9, and 13 were acceptable (range = 94.1 to 121.9% of the respective nominal concentrations). The range of homogeneity was 83.5 to 129.1% of the respective target concentrations. The test material was found to be stable in the vehicle at room temperature over a period of eight days. Batches of test material suspensions in the vehicle were made fresh every week.

No treatment-related deaths were observed during the study. The report unspecifically stated that "no signs of systemic

toxicity were detected during the course of the study". Other signs included transient incidences of sore spots on the flank, hind thigh, thoraco-dorsal region, or the tail which were recorded in all groups. These were stated to be scratching lesions, caused by the rats' attempts to free themselves from their dressings. Two group 4 females had transient swelling, one of the chest wall and the other of the head.

There were no treatment-related dermal adverse effects in either groups 2 or 3. Erythema was observed in both male and female groups 4. In the male group, erythema was slight to well defined (mean group score= 0.1 to 0.9), with the first incidence occuring on treatment day 4, and with maximal effect lasting from week 3 to week 6. The maximum number of rats affected at one time was 10 (days 40-41). Erythema subsequently improved and became barely perceptible at the end of the treatment period. In the female group, erythema was more severe (mean group score=0.1 to 1.9), with the first incidence also occuring on day 4, but with an earlier maximal effect period (week 2 and 3), and a greater number of animals being affected (13-14 rats on days 7-15). The time course of the effect was, however, comparable to that of the male group. There were occasional incidences of eschar, crust and/or scabs formation accompanying erythema in both male and female of groups 4. No edema was observed in any group.

There were no significant intergroup differences in body weights. From week 3 to the end of the treatment peroid, the mean body weight gain in males of group 4 was marginally lower than that of the respective control group. The biological significance of this difference is doubtful. Food consumption was comparable in every group.

No treatment-related opthalmologic adverse effects were observed. The report stated that "the assesment of hematological, biochemical, and urinalysis data indicated no changes of toxicological significance at the end of the treatment nor at the end of the recovery period". These were corroborated by the data. There was no significant intergroup differences in either absolute or relative organ weights.

No macroscopic/microscopic abnormalities were observed except for the presence of necrotic foci at the treated skin site in two group 4 females, which translated histologically into one moderate case of subacute dermatitis and one slight case of ulcerative dermatitis.

G. Compliance:

Signed statements of Confidentiality Claim, compliance with EPA GLP's, and Quality Assurance were provided.

H. Conclusions:

Based on the results of this study, the dermal NOEL for Acarosan moist powder is 100 mg/kg/day in rats of both sexes. The dermal LEL is 1000 mg/kg/day based on incidences of slight to well defined erythema in males and of slight to transiently severe erythema in females. The systemic NOEL is greater than 1000 mg/kg/day.

It is noted that this study was conducted on the end-product Acarosan moist powder (5% benzyl benzoate as the a.i.), and not on technical grade benzyl benzoate.

I. Core Classification: MINIMUM

Two deviations from guideline 82-3 acceptance criteria were noted:

1. Initial body weights of the female rats (166-242g) were below those required (200-300g).

2. The report was unspecific about the clinical signs, only stating that "observation for clinical signs was performed at least once daily". The only recorded signs (individual daily clinical signs, pp 149-180) were of eyes injury and skin sores. According to guideline 82-3 "cageside observations should include, but not limited to, changes in skin and fur, eyes and mucus membranes, respiratory, circulatory, autonomic, and central nervous system, somatomotor activity and behavior pattern". While these deviations are not expected to affect the results of the study, the report should be more detailed in the descriptions of clinical signs, as such data may be useful for judging the overall toxicity of the end-product.