UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Acarosan Moist Powder (containing 5% Benzyl Benzoate as the a.i.): 13-week Dermal Study in rats and Immediate Type Hypersensitivity Study in Guinea Pigs.

EPA MRID No. 416145-02, 03
EPA ID No. 059820-00001
EPA record No. S383994
EPA Pesticide Chem. Code 009501
Project Nos. 0-2005 and 0-1843
Caswell No. 082

TO: P. Hutton, PM #17
Registration Division (H7505C)

THRU: Roger Gardner, Section Head
Review Section 1 Roger Gardner 12-11-90
Toxicology Branch I
Health Effects Division (H7509C) KB 12/12/90

FROM: Nguyen Bich Thoa, Ph.D.
Review Section 1
Toxicology Branch I
Health Effects Division (H7509C)

Registrant: Werner & Mertz, gmbh, Rheinallee 96, D-6500 Mainz 1, Germany

ACTIONS REQUESTED: Review of a subchronic 13-week dermal study in rats and an immediate type hypersensitivity study in guinea pigs with Acarosan Moist Powder.

CONCLUSIONS:

Based on the results of the 13-week dermal toxicity study conducted with Acarosan Moist Powder in rats, the dermal NOEL in both sexes was 100 mg/kg/day (mid dose). The dermal LEL in both sexes was 1000 mg/kg/day (highest dose tested) based on the presence of erythema. The systemic NOEL was greater than 1000 mg/kg/day. The study is classified CORE MINIMUM.

The immediate type hypersensitivity study, which was submitted under guideline 81-6 (dermal sensitization in guinea pigs) is classified as SUPPLEMENTARY. The study reported that following 2 consecutive IP and SC sensitizations, respiratory function of female guinea pigs was unaffected by inhalation

challenge with Acarosan Moist Powder. In contrast, the positive control material ovalbumin induced allergic bronchospasms, as evidenced by a significant decrease in tidal volume and by significant compensatory increases in respiratory rate and minute volume. Both the study design and the reported conclusions do not apply to guideline 81-6. Consequently the study is SUPPLEMENTARY and cannot be upgraded for this purpose.

An updated Toxicology Profile and Data Evaluation Records for the submitted studies on Acarosan Moist Powder are appended.

Toxicology Profile

Updated 12/10/90

Acarosan Moist Powder Formulation (5% a.i)

Guide-line	Study Identification and Classification	Results
81-6	Dermal Sensitization in guinea pigs MRID: 416145-02 RCC Res & Consult. Co # 099538 04/12/88 Supplementary	Following 2 consecutive IP and SC sensitizations, one week apart, respiratory rate tidal volume, and minute volume of female guinea pigs were unaffected by an inhalation challenge with Acarosan Moist powder. Positive control: ovalbumin. Both the design and conclusions of the study do not apply to guideline 81-6.
82-3	Subchronic Dermal (90-day) in rats MRID: 416145-03 RCC Res & Consult. Co. # 099540 03/30/88 Minimum	Dermal NOEL = 100 mg/kg/day. Dermal LEL = 1000 mg/kg/day based on incidences of slight to well defined erythema in males and of slight to transiently severe erythema in females. Erythema was reversible. Systemic NOEL > 1000 mg/kg/day. Doses used: 0, 10, 100, or 1000 mg/kg/day.
84-2(a)	Mutagenicity - Gene Mutations MRID: 408453-11 Cytotest Cell Res. # 118405 11/26/87 Acceptable	Acarosan Moist Powder (10, 100, 333, 1000, or 5000 ug/plate was negative in the Ames test with or without metabolic activation. S. typhimurium strains: TA-1535, 1537, 1538, 98, and 100. The HD was weakly cytotoxic in strain TA100, without metabolic activation.
84-2	Mutagenicity - Gene Mutations (e. coli) MRID: 408471-10 Cytotest Cell Res. # 118506 11/26/87 Acceptable	Acarosan Moist Powder (10, 100, 333, 1000, and 5000 ug/plate was neither cytotoxic nor mutagenic in e. coli wp2 uvra, with or without metabolic activation. The HD was not completely soluble.

Reviewed by Nguyen B. Thoa, Ph.D. 12-11-90
Section 1, Toxicology Branch 1
Secondary Reviewer: Roger Gardner 12-11-90
Section 1, Toxicology Branch 1

DATA EVALUATION RECORD

STUDY TYPE: Dermal sensitization in the guinea pig. (Guideline 81-6)

IDENTIFICATION NO.s: EPA MRID No 416145-02
EPA ID No 059820-00001
EPA Record No S383994
EPA Pesticide Chemical Code 009501
Caswell No 082
HED Project No 02005

TEST MATERIAL: Acarosan Moist Powder (5% Benzyl Benzoate as the a.i.), Batch no. Prod. Sept. 1987.

SYNONYMS: Acarosan Feuchtpulver

REPORT NUMBER(S): 099538

SPONSOR: Werner & Mertz, GMBH Rheinallee 96, D-6500 Mainz 1, Germany

TESTING FACILITY: RCC Research and Consulting Co. AG
1, rte de Troinex, CHH 1227, Carouge, Switzerland

TITLE OF REPORT: Acarosan Moist Powder: Examination of the Immediate Type Hypersensitivity of Acarosan Moist Powder in Guinea Pigs.

AUTHOR(S): T. Imamura, and Ph. Thevenaz

STUDY COMPLETION DATE: 04-12-88

CONCLUSIONS: Following 2 consecutive IP and SC sensitizations, respiratory function of female guinea pigs was unaffected by inhalation challenge with Acarosan moist powder. In contrast, the positive control material ovalbumin induced allergic bronchospasms, as evidenced by a significant decrease in tidal volume and by significant compensatory increases in respiratory rate and minute volume. Based on these results, Acarosan moist powder does not appear to induce any pulmonary hypersensitivity allergic reaction under the experimental conditions of the study.

CORE CLASSIFICATION: Supplementary

The data were adequate to support the reported conclusions. This study is a valid scientific study. Both the study design and the reported conclusions do not, however, apply to guideline 81-6 (dermal sensitization study in guinea pigs). The study cannot

fulfill the data requirements set forth by guideline 81-6 and is therefore supplementary and cannot be upgraded for this purpose.

A. MATERIALS:

Test animals were 48 female Dunkin-Hartley guinea pigs, weighing from 250.1 to 342.2g.

The test material was Acarosan Moist Powder, a white solid end-product, containing 5% Benzyl Benzoate as the active ingredient. The vehicles were 0.9% NaCl containing $Al(OH)_3$, 100 mg/ml, for the IP and SC sensitizing injections and 0.9% NaCl for the inhalation challenge. The positive control material was ovalbumin.

B. METHODS:

The animals were alloted into 6 experimental groups (8/group). An unspecified area of skin on their backs was shaved for the SC injections.

The sensitizing phase consisted of 2 injections, one by the IP route on day 1 and the other by the SC route, on 3 different sites on the shaved skin of the backs, on day 8, as follows:

GROUP	SENSITIZING INJECTIONS IP	SC
1 (negative control)	1 ml vehicle	1 ml vehicle
2 (positive control)	1 ml vehicle with 5 mg ovalbumin	1 ml vehicle with 5 mg ovalbumin
3 (challenge control)	0	0
4, 5 and 6 (test)	1 ml vehicle with 5 mg test material	1 ml vehicle with 5 mg test material

The challenge phase consisted of exposing the sensitized animals to an atmosphere containing either ovalbumin or the test material at a final concentration of 1%. The schedule of exposure was as follows:

GROUP	CHALLENGING MATERIAL	DAY OF CHALLENGE
1	Ovalbumin	22
2	Ovalbumin	22
3	Test material	29
4	Test material	15
5	Test material	22
6	Test material	29

The guinea pigs were dynamically exposed nose-only for 5 minutes to an aerosol with a target MMAD of $\leq$ 3 um. It was stated that "the time for the analytical concentration at an animal port to reach 99% of its ultimate value is 34 seconds".

C. OBSERVATIONS:

All guinea pigs were checked for mortality twice daily. Clinical signs were observed at least once daily. These included general behavior, motor activity, body position, mobility, respiration, skin/fur appearance, eye and nose effects, salivation, crying, diarrhea, emaciation, and poor condition. Individual body weights were recorded on day 1 and before termination. The respiratory flow was recorded prior to the challenge (10 minutes), during the 5-minute challenge, and during the recovery period (15 minutes). It was integrated to give the tidal volume. The respiratory rate was also measured. The minute volume was calculated from these 2 values.

D. PATHOLOGY:

All animals were necropsied. Surviving animals were killed by an IP injection of sodium pentobarbital. The incidences of intraperitoneal granulomas and the presence of SC injection sites were recorded. The lungs and tracheas of all animals showing intraperitoneal granulomas were fixed in formaldehide. No histology was performed.

E. STATISTICAL ANALYSIS OF THE DATA:

The 3 respiratory parameters (respiratory rate, tidal volume, and minute volume) were reported as the percent change over the prechallenge control values.

Intergroup differences in body weights, body weight gains, and respiratory parameters were assessed by the univariate one-way analysis of variance and either a Dunnett-test (variables are assumed to be normally distributed) or a Steel-test (variables are

not assumed to be normally distributed). Mortality was analyzed 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.

E. REPORTED RESULTS:

Two animals died, one group 5 animal on day 22, before the challenge, and one group 6 animal, on day 4. A slight mass at the thoraco-dorsal region was observed in the former animal. The latter suffered a slight body weight loss, prior to death. These deaths were considered accidental. Another group 5 animal was observed with alopecia of the head. No other clinical signs were recorded. Body weights and body weight gains were comparable in all groups.

The respiratory parameters were comparable in the negative control group, the challenge group, and the 3 test groups. In contrast, there was a significant decrease (30%) in the tidal volume, indicative of bronchial constriction, and significant increases in both the respiratory rate (127%) and the minute volume (53%) in the positive control group, compared to the negative control group.

Except for group 3, which was not sensitized, all other groups presented peritoneal granulomas and 3 sites of SC injections on the skin of the back. The report considered these as signs of a sucessful sensitization program. One group 2 and one group 5 animals presented red foci on the lungs. No other macroscopic findings were recorded.

F. COMPLIANCE:

A signed Quality Assurance Statement was provided.

Reviewed by: Nguyen Bich Thoa, Ph.D 12-11-90
Section 1, Toxicology Branch I
Secondary Reviewer: Roger Gardner 12-11-90
Section Head, Section 1, Toxicology Branch I

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

C. 019-

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.