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DATA EVALUATION REPORT

Reg. No. 83 258 - VINCLOZOLIN

STUDY TYPE: CHRONIC FEEDING - RAT (83-1a)

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Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by

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[VINCLOZOLIN]

Chronic Oral Study (83-1a)

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Review Section II, Toxicology Branch I (7509C)

DATA EVALUATION REPORT

STUDY TYPE: Chronic Feeding - Rat (83-1b)

TOX. CHEM. NO: 323C

P.C. CODE: 113201

MRID NO.: 43254702

TEST MATERIAL: Reg. No. 83-258 (Vinclozolin), >99% a.i.

SYNONYMS: 3-(3,5-Dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione; 3-(3,5-dichlorophenyl)-5-methyl-5-vinyloxazolidine-2,4-dione; BAS 353F; Ronilan (Merck Index)

STUDY NUMBER: 71S0375/88109

SPONSOR: BASF Corporation, Agricultural Products, Research Triangle Park, NC

TESTING FACILITY: BASF Aktiengesellschaft, Department of Toxicology, Ludwigshafen/Rhein, FRG

TITLE OF REPORT: Second Chronic Toxicity Study with Reg. No. 83 258 - Vinclozolin in Rats Administration in the Diet for 24 months.

AUTHOR: W. Mellert

REPORT ISSUED: May 4, 1994 (study completion date)

EXECUTIVE SUMMARY: In a chronic toxicity study, 20 male and 20 female Wistar rats were administered 0, 25, or 50 ppm (0, 1.2, or 2.4 mg/kg/day, respectively, for males and 0, 1.6, or 3.2 mg/kg/day, respectively, for females) of vinclozolin in their diets for 104 weeks.

No treatment-related systemic effects were observed in either sex at the dietary concentrations used in this study. **Therefore, this study established a NOEL of 50 ppm for both male and female rats (2.4 and 3.2 mg/kg/day, respectively).**

This study showed no evidence of carcinogenicity. The increased incidence of thymomas in male rats and benign thyroid C-cell tumors in female rats are not considered to be treatment-related.

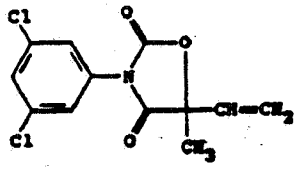
This study and the first chronic study (MRID No. 432547-01) combined receive a classification of core - **minimum**, and satisfy the guideline requirements for a chronic feeding study in rodents (83-1). The classification of this study alone is supplementary upgradable. This deficiency was corrected by the first chronic study that used higher doses and established a LOEL at 150 mg/kg/day. All pertinent endpoints were evaluated; however, the study author did not calculate average severity ratings for nonneoplastic lesions or present results of statistical analysis of incidence data.

Special Review Criteria (40 CFR 154.7) None

A. MATERIAL

1. Test material: Reg. No. 83 258 (Vinclozolin)

Description: solid crystal (Merck Index)
Lot/Batch No.: N 183
Purity: 99.2% a.i.
Stability of compound: at least 2 years
CAS #: 50471-44-8 (Merck Index)
Structure: Merck Index



2. Vehicle and/or positive control

The test material was mixed directly with food; no other vehicle was used. A positive control was not included in this study.

3. Test animals

012053

Species: rat
Strain: Wistar (Chbb:THOM (SPF))
Age and weight at the start of study: 42 days; males weighed 173 to 197 g (mean, 185 g); females weighed 136 to 162 g (mean, 149 g)
Source: Dr. Karl Thomae GmbH, Biberach/Riss, FRG
Housing: single housing in stainless steel wire mesh cages
Environmental conditions:
Temperature: 20 to 24°C
Humidity: 30 to 70%
Air Changes: not reported
Photoperiod: 12 h day/12 h dark
Acclimation period: 9 days

B. STUDY DESIGN

1. Animal assignment

Animals were assigned randomly by weight to the test groups in Table 1. No scheduled interim sacrifice was included in this study.

TABLE 1. STUDY DESIGN

Dose Group	Conc. in diet (ppm)	Dose (mg/kg/day)[a]		No. of Animals	
		Male	Female	Male	Female
0	0	0	0	20	20
1	25	1.2	1.6	20	20
2	50	2.4	3.2	20	20

Data taken from MRID No. 432547-02, page 20.
[a]Time-weighted average daily compound intake reported by the authors using body weight and food consumption measurements from days 14, 42, 70, 98, and 126 to 714.

Dose selection rationale: This study was conducted as a supplement to the first chronic toxicity study (MRID No. 432547-01), which did not establish a no-observed-effect level (NOEL). The lowest dose (150 ppm) in the first study caused effects in

the testes, prostate, and eyes of male rats and effects in the ovaries of female rats. The high dose selected for this supplementary study is threefold lower than the lowest dose used in the first chronic toxicity study.

2. Diet preparation and analysis

The diet was prepared at about 4-week intervals by mixing the test material with a small amount of food in a Bosch household mixer followed by adding an appropriate amount of food to the mixture (to obtain the target concentration) and mixing the preparation in a GEBR. LÖDIGE laboratory mixer for about 10 min. The conditions of storage were not reported. Concentrations of the test material (triplicate samples per concentration) in the diet were verified at the start of the study and at 3-month intervals thereafter. The stability and homogeneity of the test material in the diet was reported in MRID No. 432547-01.

Results -

a. Homogeneity analysis - Reported in MRID No. 432547-01 (page 1748); all samples were within 10% of the target concentrations.

b. Stability analysis - Reported in MRID No. 432547-01 (page1749); the test material is stable up to 32 days.

c. Concentration analysis - There were variations in the concentrations of the dietary preparations: **25 ppm**: At the beginning of the study, one sample averaged 154% and another averaged 147.6% of the target; the next two measurements taken at 3 months averaged -13 and -15% of the target; all other 25-ppm samples were within ±10% of the target. **50 ppm**: At the beginning of the study one sample averaged -17% of the target; the next two samples taken at 3 months averaged -15 and -17% of the target; all other samples were within ±10% of the target.

3. Diet

Animals received food (Kliba 343 Mehl) and water *ad libitum*.

4. Statistics

The statistical tests used to analyze these data are presented in the Appendix of this Data Evaluation Report (DER) (taken from MRID No. 432547-02, pages 36, 37, and 730).

5. Signed and dated quality assurance and GLP statements were present.

C. METHODS AND RESULTS

1. Observations

Animals were inspected twice daily on week days and once a day on weekends and holidays for signs of toxicity and mortality. Comprehensive clinical examinations were conducted once a week.

Results - No noteworthy clinical signs of toxicity were observed in either male or female rats receiving vinclozolin. There were no treatment-related effects on the survival rates. At the end of the study, 80, 75, and 75% of the control, 25-, and 50-ppm males were alive and 75, 80, and 75% of the control, 25-, and 50-ppm females were alive.

2. Body weight

Individual animals were weighed once weekly up to week 14 and once every 4 weeks thereafter.

Results - No treatment-related effects were observed on body weight or body weight gain in either male or female rats during any time period of the study. At 50 weeks, control, 25- and 50-ppm male rats weighed 646.6, 655.9, and 648.4 g; at termination they weighed 648.9, 687.5, and 648.7 g, respectively. At 50 weeks, control, 25-, and 50-ppm female rats weighed 349.9, 340.5, and 342.7 g, respectively; at the end of the study, they weighed 382.7, 397.8, and 388.6 g, respectively, . Male rats gained 464.8, 503.4, and 462.2 g during the course of the study and female rats gained 233.7, 250.3, and 241.1 g, respectively.

3. Food consumption and compound intake

Food consumption for each cage was recorded once a week for 1 week during the first 14 weeks of the study and at 4-week intervals thereafter. Food consumption was reported as g/animal/day. Food efficiency [(body weight gain in g/food consumption in g per unit time) × 100] was calculated. Compound intake (mg/kg/day) was calculated based on concentration of the compound in the diet, food consumption, and body weight data recorded on days 14, 42, 70, 98, and 126 to 714.

Results -

a. Food consumption - Food consumption was similar in animals receiving the test material and their respective controls throughout the study. Therefore, no treatment-related effect was noted. The authors did not calculate the total food consumed per animal during the study.

b. Compound consumption (time-weighted average) - The average compound intake calculated by the study authors was about 1.2 and 2.4 mg/kg/day for male rats and about 1.6 and 3.2 mg/kg/day for female rats receiving 25 and 50 ppm,

respectively, of test material in the diet. Compound consumption was slightly higher during the first 14 weeks of the study, 1.8 and 3.5 mg/kg/day in male rats receiving 25 and 50 ppm, respectively; and 2.1 and 4.1 mg/kg/day, respectively in females.

c. Food efficiency – Food efficiency did not differ significantly between controls and treated animals of either sex and showed no treatment-related patterns. The study author did not present data on cumulative food consumption; therefore, a net food efficiency for the entire study could not be calculated.

4. Ophthalmoscopic examination

The eyes of all animals were examined 1 day before treatment started and at approximately 3-month intervals until the end of the study.

Results – No abnormalities attributable to treatment were observed. Cataracts were detected bilaterally in only one 50-ppm male and unilaterally in one control, one 25-ppm, and one 50-ppm male. Cataracts were not detected in female rats.

5. Blood was collected from the retroorbital venous plexus of nonfasted nonanesthesized animals for hematology and clinical analysis on days 92, 184, 365, 548, and 726. All surviving animals were bled. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
|X|	Hematocrit (HCT)*	|X|	Leukocyte differential count*
|X|	Hemoglobin (HGB)*	|X|	Mean corpuscular HGB (MCH)
|X|	Leukocyte count (WBC)*	|X|	Mean corpusc. HGB conc. (MCHC)
|X|	Erythrocyte count (RBC)*	|X|	Mean corpusc. volume (MCV)
|X|	Platelet count*	|X|	Reticulocyte count
|X|	Blood clotting measurements		
|X|	(Thromboplastin time)		
| |	(Clotting time)		
| |	(Prothrombin time)		

* Required for chronic studies

Results – There were no treatment-related effects on hematologic parameters.

b. Clinical chemistry

X
Electrolytes:
|X| Calcium*
|X| Chloride*
|X| Magnesium
|X| Phosphorus*
|X| Potassium*
|X| Sodium*
Enzymes:
|X| Alkaline phosphatase (ALK)
| | Cholinesterase (ChE)
| | Creatinine phosphokinase*
| | Lactic acid dehydrogenase (LDH)
|X| Serum alanine aminotransferase (also SGPT)*
|X| Serum aspartate aminotransferase (also SGOT)*
|X| Gamma glutamyl transferase
| | Glutamate dehydrogenase

X
Other
|X| Albumin*
|X| Blood creatinine*
|X| Blood urea nitrogen*
|X| Cholesterol*
|X| Globulins
|X| Glucose*
|X| Total bilirubin
|X| Total serum protein (TP)*
| | Albumin/globulin ratio
|X| Triglycerides
| | Serum protein electrophoresis

* Required for chronic studies

Results – Clinical chemistry values were similar in treated animals and their corresponding controls.

6. Urinalysis*

Urine was collected overnight from male and female rats on days 86 175, 359, 539, and 716. Food and water were withdrawn during collection. The CHECKED (X) parameters were examined.

X
|X| Appearance*
|X| Volume*
|X| Specific gravity*
|X| Turbidity
|X| pH
|X| Sediment (microscopic)*
|X| Protein*

X
|X| Glucose*
|X| Ketones*
|X| Bilirubin*
|X| Blood pigments
|X| Nitrite
|X| Urobilinogen

* Required for chronic studies

Results – There were no treatment-related effects on urinalysis parameters.

7. Sacrifice and pathology

All animals dying before termination, sacrificed due to moribundity, or killed on schedule (day 729 and 730) were subject to gross pathological examination. The animals were killed by decapitation under carbon dioxide anesthesia after a 16- to 20-h fasting period. The CHECKED (X) tissues were collected for histological examination. All gross lesions from all groups, all tissues from control and 50-ppm

groups, and lungs, liver, adrenal glands, kidneys, uterus, testes/ovaries and accessory organs, skeletal muscle, and eyes from the 25-ppm groups were examined histopathologically. The (XX) organs were weighed (organs containing large masses were not included in the mean weights).

X		X		X	
Digestive system		Cardiovasc./Hemat.		Neurologic	
| |	Tongue	| X|	Aorta*	|XX|	Brain*+
| X|	Salivary glands*	| X|	Heart*	| X|	Periph. nerve*
| X|	Esophagus*	| X|	Bone marrow*	| X|	Spinal cord (3 levels)*
| X|	Stomach*	| X|	Lymph nodes*	| X|	Pituitary*
| X|	Duodenum*	| X|	Spleen	| X|	Eyes (optic n.)*
| X|	Jejunum*	| X|	Thymus*	Glandular	
| X|	Ileum*	Urogenital		|XX|	Adrenal gland*
| X|	Cecum	|XX|	Kidneys*+	| |	Lacrimal gland
| X|	Colon*	| X|	Urinary bladder*	| X|	Mammary gland*
| X|	Rectum*	|XX|	Testes*+	| X|	Parathyroids*
|XX|	Liver*+	|XX|	Epididymides	| X|	Thyroids*
| X|	Pancreas*	| X|	Prostate	Other	
Respiratory		| X|	Seminal vesicle	| X|	Bone*
| X|	Trachea*	|XX|	Ovaries*	| X|	Skeletal muscle*
| X|	Lung*	| X|	Uterus*	| X|	Skin*
| |	Nose	| X|	Vagina	| X|	All gross lesions and masses*
| |	Pharynx				
| |	Larynx				

* Required for chronic studies.
+ Organ weight required for chronic studies.

Results –

a. Organ weight – Absolute and relative organ weights were similar between treated groups and their corresponding controls, with one exception. The relative weight of the kidney (0.621 ± 0.051) in the 25-ppm male group was significantly ($p<0.05$) decreased compared with the control (0.689 ± 0.076). The lack of a decrease (0.696 ± 0.089) at the 50-ppm level suggests that this effect is not treatment related.

b. Gross pathology – The liver, lungs, adrenal glands, pancreas, eyes, testes and accessory organs, and ovaries were identified as targets in rats fed vinclozolin at doses ranging from 150 to 4500 ppm for 2 years (first chronic toxicity study, MRID No. 432547-01). Significant increases in the number of animals with gross lesions were limited to thymus masses in male rats (0, 1, 4 [$p=0.05$] at 0, 25, and 50 ppm, respectively). The thymus was not identified as a target in the first chronic study. Lesions having incidences that were marginally significant or lacked a clear dose-response relationship were liver foci (1, 3, and 5 [$p<0.09$]), liver cysts (3, 9 [$p=0.04$], 7) and adrenal cortical cysts (2, 7 [$p=0.06$], 1) in female rats receiving 0, 25, and 50 ppm; and unilateral masses in the testes (3, 5, 8 [$p=0.07$]) of male rats. Cataracts (a characteristic lesion in the first chronic study) were seen in a few male rats (1, 1, and 2 at 0, 25, and 50 ppm) but in none of the female rats.

c. Microscopic pathology -

1) Non-neoplastic - The incidences of nonneoplastic lesions in the liver, lungs, pancreas, skeletal muscle, eyes, and adrenal gland were elevated in male and female rats fed 150 to 4500 ppm of vinclozolin in the first chronic study (MRID No. 432547-01). The first chronic study also showed increased incidences of lesions in the kidney, testes, and accessory sex organs of male rats and ovaries of female rats.

Treatment with 25 or 50 ppm of the test material caused no notable effects in the seminal vesicle, coagulation gland, prostate, skeletal muscle, lungs, kidneys, pancreas, or adrenal gland. The incidence of unilateral Leydig cell hyperplasia in the testes was increased, but not significantly, in male rats receiving 50 ppm (10/20 vs 5/20 in controls; $p>0.05$) of the test material. The combined incidences of unilateral and bilateral Leydig cell hyperplasia (16/20, 17/20, 18/20 for 0, 25, and 50 ppm, respectively) was comparable between the treatment and control groups as was the severity of the lesions. The incidence of oligospermia was also increased in male rats fed 50 ppm (9/20 vs 4/20 in controls; $p=0.09$) of the test material, but the combined incidences of azoospermia and oligospermia were comparable between the treatment groups and controls. There were no statistically significant increases in the incidence of liver lesions. A significantly decreased incidence of basophilic foci was observed in male rats receiving 25 ppm (4/20 vs 13/20 in controls; $p<0.01$), but not at 50 ppm, suggesting that the effect is not treatment-related. Focal hyperplasia of the pituitary (pars distalis) was observed in six female rats receiving 50 ppm compared with only two controls ($p=$N.S.), and pituitary cysts (pars intermedia) were observed in six males receiving 50 ppm compared with only two controls ($p>0.05$). Female rats did not develop ovarian lesions at significantly increased incidences, but stromal hyperplasia of the ovary occurred with a slightly increased severity at 50 ppm (2.79 compared with 2.44 for controls). Lesions (lenticular degeneration) developed in the eyes of four control, eight 25-ppm, and five 50-ppm male rats and in two female rats of each treatment and control group. The severity of the lesion in male rats was slightly increased in treated animals compared with controls (2.25, 2.88, 2.80, respectively). All other lesions occurred with comparable incidences in treatment and control groups.

2) Neoplastic - In the first chronic study (MRID No. 432547-01), the testes and liver in male rats and the adrenal cortex and ovary in females showed increased incidences of neoplastic lesions in animals fed vinclozolin. In rats fed 25 or 50 ppm of the test material, there was a slight nonsignificant increase in the incidence of Leydig cell tumors in male rats receiving 50 ppm compared with the controls and a statistically significant increase in the incidence of thymomas (Table 2 and page 0779 from the submitted report). Females fed 50 ppm of the test material had a significantly increased incidence

of thyroid C-cell tumors. The incidence of pituitary adenomas was decreased in female rats fed 50 ppm, but the incidence of adenomas/carcinomas combined was similar to that of controls. The number of animals developing at least one neoplasm of any type, at least one benign neoplasm, or at least one malignant neoplasm was similar in control and treated groups of both sexes.

TABLE 2. NEOPLASTIC LESIONS IN MALE AND FEMALE RATS FED VINCLOZOLIN

Organ/Lesions	Dietary concentration (ppm)		
	0	25	50
Males			
Testes Leydig cell tumors	9/20[a]	9/20	12/20
Thymus Thymoma	0/20	2/7	4/20*
Total animals with tumors Total animals with benign tumors Total animals with malignant tumors	17/20 16/20 2/20	15/20 15/20 3/20	19/20 19/20 4/20
Females			
Thyroid gland C-cell tumor, benign C-cell tumor, benign/malignant	 1/20 1/20	 4/20 4/20	 6/20* 7/20*
Pituitary Pars distalis, adenoma Pars distalis, carcinoma	 11/20 0/20	 10/20 1/20	 4/20 2/20
Total animals with tumors Total animals with benign tumors Total animals with malignant tumors	17/20 14/20 7/20	18/20 17/20 5/20	16/20 13/20 6/20

Data taken from Text Tables 9, 25, 26, 27, 28, 29 (pages 739-756) and the pathology summary tables, pages 774-780 and 795.
[a]Number of animals showing a lesion/number of animals examined.
*$p \leq 0.05$, ** $p \leq 0.01$ [Fisher exact tests, calculated by the reviewer (Number Cruncher Statistical System, Version 5.03)]

D. DISCUSSION

Groups of 20 male and 20 female Wistar rats were given vinclozolin in their diet at concentration 0, 25, or 50 ppm continuously for 2 years. Calculated doses reported by the study author were 0, 1.2 and 2.4 mg/kg/day, respectively, for male rats and 0, 1.6, and 3.2 mg/kg/day, respectively, for female rats. Vinclozolin caused no noteworthy clinical signs of toxicity, and it had no statistically significant effect on survival, body weight gain, food consumption, or food efficiency in either male or female rats. There were no treatment-related effects on hematologic, clinical chemistry, or urinalysis parameters.

Cataracts as detected by ophthalmoscopic examination conducted at about 3-month intervals during the study, gross examination at termination, or microscopic examination of the eyes (lenticular degeneration) occurred at similar incidences in treated animals and controls. The increased severity of lens degeneration in 25- and 50-ppm males (2.88 and 2.80, respectively) compared with controls (2.25) suggest a mild effect on the eyes. However, in the carcinogenicity study (MRID No. 432547-03), the severity of lenticular degeneration in male rats receiving 50 ppm of vinclozolin for 2 years was not increased, suggesting that the lesion is not treatment related. [There were other differences between the current study and the carcinogenicity study that will be discussed in greater detail in the DER for the carcinogenicity study.] The study author noted that vinclozolin has antiandrogenic activity. However, the testes and sex accessory organs (epididymides, seminal vesicle, prostate, and coagulation gland), identified as targets in the first chronic study, showed no statistically or biologically significant effects from feeding vinclozolin to male rats at concentrations of 25 or 50 ppm. No treatment-related gross or microscopic lesions were observed in the adrenal gland, ovaries, liver, lungs, kidneys, pancreas, or skeletal muscle.

The thymus of male rats and thyroid gland of female rats showed statistically significant increased incidences of neoplasms at 50 ppm. Neither of these organs were targets for neoplastic responses in the first chronic study using higher doses and no evidence of neoplastic responses was observed in the carcinogenicity study using the same dose and a larger number of animals. Therefore, it appears that the increased incidence of thymomas in male rats and thyroid C-cell tumors in female rats are not treatment-related. The total number of treated animals developing neoplasms was not different from that of controls. This study was conducted as a supplement to the first chronic study to establish a no-effect level; therefore, the doses were, by necessity, well below the MTD.

In conclusion, treatment-related effects were not observed in this study of male and female rats fed vinclozolin at doses of 25 and 50 ppm for 2 years. Therefore, a no-observed-effect level (NOEL) is established at 50 ppm (2.4 and 3.2 mg/kg/day for male and female rats, respectively).

E. STUDY DEFICIENCIES

- Incidence data for gross and microscopic lesions were not analyzed statistically and average severity ratings were not calculated.

- The 25-ppm dietary preparations greatly exceeded the target concentration by about 50% at the beginning of the study. No effects were noted, because the concentration was still below the NOEL (50 ppm). Dietary concentrations from 3 months to termination were within an acceptable range of the target concentration.

- This study was conducted as a supplement to the first chronic study, which established a lowest-observed-effect level (LOEL) at 150 ppm but not an NOEL. Consequently, the lack of effects at the highest dose is not considered a deficiency.

3.9. PATHOLOGY

For description of the methods used see separate PATHOLOGY REPORT.

3.10. STATISTICAL EVALUATION

The statistical evaluation of the data was carried out on the computing systems of the Department of Toxicology (Dr. H.D. Hoffmann responsible).

3.10.1. Clinical examinations

Means and standard deviation for the variables food consumption, water consumption, body weight, food efficiency and test substance intake (except control group) were calculated for the animals of each test group. They were printed out in the summary and individual value tables, with the exception that for food efficiency and test substance intake only summary tables were prepared.

For the parameter body weight a parametric one-way analysis of variance was done via the F-test (ANOVA) (2). If the resulting p-value was equal or less than 0.05, a comparison of each dose group with the control group was carried out. These comparisons were performed simultaneously via Dunnett's test (3, 4) for the hypothesis of equal means. If the results of this test were significant, labels (* for $p \leq 0.05$, ** for $p \leq 0.01$) were printed together with the group mean in the tables. Both tests were performed two-sided.

3.10.2. Clinical chemistry and hematology

For various parameters mean and standard deviation were calculated for each group and tabulated together with the individual values.

For all parameters, excepting the differential blood count, a parametric one-way analysis of variance was done via the F-test (ANOVA) [2]. If the resulting p-value was equal or less than 0.05, a comparison of each dose group with the control group was carried out. These comparisons were performed simultaneously via Dunnett's test [3, 4] for the hypothesis of equal means. If the results of this test were significant, labels (* for $p \leq 0.05$, ** for $p \leq 0.01$) were printed together with the group means in the tables.

Both tests were performed two-sided.

3.10.3. Urinalyses

With the exception of volume, color and turbidity the scale for the urine parameters is divided into 4 sections (0, 1, 2, 3).
For the parameter "Nitrite" only a division in two sections (0, 1) is made.

The parameters, which were recorded in 4 sections, were sorted into 2 classes. This was done for the statistical analysis.

A pairwise comparison of each dose group with the control was carried out using Fisher's exact test [6] for the hypothesis of equal proportions. If the results of this test are significant, labels (* for $p \leq 0.05$, ** for $p \leq 0.01$) were printed in the tables.

3.11. RETENTION OF RECORDS

The study protocol, the raw data, the reserve sample and the specimens, as well as the original of this report, will be stored at BASF Aktiengesellschaft for at least the period of time specified in the GLP regulations. The specimens will be retained for only as long as the quality of the material allows evaluation.

Details concerning responsibilities or locations of archiving can be seen from the respective SOPs and from the raw data.

MATERIALS AND METHODS

Statistical Evaluation

The calculations were carried out on the computer systems of the Department of Toxicology (Dr. H.D. Hoffmann responsible).

Mean and standard deviation were calculated for the statistical evaluation of the study for the variables of terminal body weight and of absolute and relative organ weights (related to terminal body weight) of the animals in each test group and tabulated together with the individual values (absolute and relative organ weights).

The statistical evaluation was carried out using the DUNNETT test (1, 2) for a simultaneous comparison of several dose groups with a control group.

If the results of this test are significant, p-markers (* for $p \leq 0.05$, ** for $p \leq 0.01$) were printed together with the group mean in the tables.

(1) DUNNETT, C.W. (1955):
A multiple comparison procedure for comparing several treatments with a control
J. Amer. Statist. Assoc. 50, 1096-1121

(2) DUNNETT, C.W. (1964):
New tables for multiple comparisons with a control
Biometrics 20, 482-491

INCIDENCE OF MICROSCOPIC FINDINGS - ALL ANIMALS

Sacrifice group	F1					
Sex	M			F		
Dose group	0	1	2	0	1	2
Animals in selected Group	20	20	20	20	20	20
Cervix cont.						
- Fibroplasia	.	.	.	2	5	2
- Fibrosis	.	.	.	2	1	1
- Inflammation	.	.	.	2	1	3
- Leiomyosarcoma	.	.	.	1	.	.
- Schwannoma,malignant	.	.	.	2	1	2
- Carcinoma, squam. c.	.	.	.	.	1	.
Vagina	.	.	.	20	20	20
- Cyclic changes	.	.	.	.	1	.
- Vaginitis	.	.	.	3	2	3
Mammary gland	11	5	11	20	6	20
- Glandular cyst(s)	1	2	3	17	3	17
- Hyperplasia, glands	.	.	1	7	.	2
- Fibroadenoma	.	.	.	5	3	2
- Adenoma	.	.	.	.	.	1
- Adenocarcinoma	.	.	.	1	1	.
Heart	20	6	20	20	5	20
- Atrial thrombus	1	.	.	1	.	1
- Focal necrosis	1	.	2	2	2	3
- Myocardial fibrosis	17	6	16	15	3	14
- Calcification, endoc	2	1	1	5	3	2
- Encocardial hyperpl.	.	.	2	.	.	.
Aorta	20	5	20	20	4	20
Bone marrow (femur)	20	5	20	20	4	20
- Hypercellularity	1	.	1	2	1	.
Spleen	20	5	20	20	5	20
- Congestion	.	2	1	.	1	.
- Pigment storage	3	1	2	13	1	13
- Hematopoiesis	14	.	13	16	2	15
- Lymphoid hyperplasia	.	.	.	1	.	.
- Lymphocyte depletion	.	.	.	.	.	2
- Thrombus	.	1	.	.	.	.
- Hemangiosarcoma	.	1	2	.	.	.
Thymus	20	7	20	20	5	20
- Congestion	1	.	.	.	1	.
- Cyst(s)	.	.	1	7	2	5
- Hyperplasia, epithel	.	.	1	1	1	2
- Hyperplasia	.	.	.	.	.	1
- Thymoma	.	2	4	2	1	1
Liver lymph node	3	1	1	.	1	1
- Blood resorption	2	.	.	.	1	1
- Pigment storage	2	1	1	.	.	1
- Hyperplasia plasma c	2	.	.	.	1	.
Mesenteric lymph n.	20	5	20	20	5	20
- Blood resorption	.	.	1	.	.	1
- Pigment storage	.	.	.	3	.	2
- Lymphoid hyperplasia	1	.	1	1	.	2

17

p=0.02

p=0.05

INCIDENCE OF MICROSCOPIC FINDINGS - ALL ANIMALS

Sacrifice group	F1					
Sex	M			F		
Dose group	0	1	2	0	1	2
Animals in selected Group	20	20	20	20	20	20
Mesenteric lymph n. cont.						
- Sinus histiocytosis	20	4	19	20	4	20
- Lymphocyte depletion	1	4	.	1	1	1
- Cyst(s)	.	.	.	1	1	.
- Hyperplasia, anigiom	2	1	1	1	.	1
- Hemangioma	2	.	.	.	.	.
- Hemangiosarcoma	.	.	.	.	.	1
- Metastasis tumor	.	.	1	.	.	.
Mediastinal lymph n.	1	2	1	.	1	1
- Blood resorption	1	2	.	.	1	.
- Pigment storage	.	1	.	.	.	.
- Lymphoid hyperplasia	.	1	.	.	.	.
- Hyperplasia plasma c	.	.	.	.	1	.
- Sinushistiocytosis	1	.	.	.	.	.
- Hyperplasia, angiom.	1	1	.	.	.	.
- Metastasis tumor	.	.	.	.	.	1
Axillary lymph nodes	.	2	2	3	5	1
- Blood resorption	.	.	.	.	3	.
- Pigment storage	.	.	1	2	3	1
- Lymphoid hyperplasia	.	.	2	1	3	1
- Hyperplasia plasma c	.	1	.	1	1	.
- Inflammation, granul	.	1	.	.	.	.
Iliac lymph nodes	17	15	16	6	5	8
- Blood resorption	6	5	3	.	2	1
- Pigment storage	8	7	7	2	3	7
- Lymphoid hyperplasia	1	.	3	2	1	5
- Hyperplasia plasma c	8	8	9	1	.	1
- Sinus histiocytosis	.	.	.	.	1	.
- Cyst(s)	2	5	4	.	.	.
- Periarteritis	.	.	.	.	1	.
- Abscess	.	1	.	.	.	.
Renal lymph nodes	5	2	1	.	1	2
- Blood resorption	4	1	.	.	1	1
- Pigment storage	5	2	.	.	1	1
- Lymphoid hyperplasia	2	1	.	.	.	.
- Hyperplasia plasma c	.	1	1	.	.	.
- Cyst(s)	1	.	.	.	.	1
Mandibular lymph n.	20	5	20	20	4	20
- Blood resorption	.	1	.	.	.	1
- Pigment storage	2	.	1	6	.	7
- Lymphoid hyperplasia	3	.	2	.	.	.
- Hyperplasia plasma c	13	1	16	18	4	18
- Cyst(s)	1	.	.	.	.	.
- Metastasis tumor	.	.	.	.	.	1
Popliteal lymph node	2	2	.	2	1	1
- Lymphoid hyperplasia	.	.	.	.	.	1
- Hyperplasia plasma c	2	2	.	.	1	.
- Cyst(s)	1	1	.	.	.	.

Project No. 71S0375/88109

Summary of the ophthalmologic findings

Males

Days			-1	89	180	272	363	448	537	631	722
Finding	Group										
Remainders of the pupillary membrane	0 0 ppm	o.s.[1] b.s.	3/20[2] 14/20	3/20 14/20	2/20 14/20	2/20 14/20	3 /20 14/20	2/20 14/20	3/20 14/20	3/19 14/19	4/17 12/17
	1 25 ppm	o.s. b.s.	7/20 8/20	5/20 10/20	6/20 9/20	6/20 9/20	6/20 9/20	6/20 9/20	6/20 9/20	6/19 8/19	4/15 6/15
	2 50 ppm	o.s. b.s.	4/20 7/20	1/20 10/20	1/20 10/20	1/20 10/20	1/20 10/20	1/20 10/20	1/19 9/19	1/19 9/19	1/17 8/17
Corneal stipplings	0 0 ppm	o.s. b.s.	3/20 0/20	4/20 1/20	2/20 2/20	4/20 0/20	4/20 0/20	5/20 0/20	5/20 0/20	5/19 0/19	5/17 1/17
	1 25 ppm	o.s. b.s.	0/20 0/20	3/20 2/20	2/20 2/20	2/20 2/20	2/20 2/20	2/20 2/20	2/20 2/20	2/19 2/19	2/15 2/15
	2 50 ppm	o.s. b.s.	0/20 0/20	2/20 2/20	5/20 2/20	6/20 2/20	6/20 3/20	4/20 5/20	3/19 5/19	3/19 5/19	2/17 5/17
Striations	0 0 ppm	o.s. b.s.	3/20 3/20	4/20 9/20	3/20 14/20	2/20 16/20	2/20 16/20	1/20 16/20	1/20 16/20	1/19 15/19	1/17 13/17
	1 25 ppm	o.s. b.s.	0/20 5/20	0/20 10/20	1/20 15/20	0/20 17/20	0/20 17/20	0/20 17/20	0/20 17/20	0/19 17/19	0/15 13/15
	2 50 ppm	o.s. b.s.	1/20 3/20	2/20 6/20	3/20 15/20	3/20 15/20	3/20 16/20	2/20 16/20	2/19 16/19	1/19 16/19	1/17 14/17
Opacities	0 0 ppm	o.s. b.s.	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	2/20 0/20	1/20 1/20	1/20 1/20	2/19 4/19	6/17 4/17
	1 25 ppm	o.s. b.s.	0/20 0/20	0/20 0/20	0/20 0/20	1/20 0/20	3/20 0/20	2/20 1/20	3/20 1/20	2/19 3/19	3/15 2/15
	2 50 ppm	o.s. b.s.	0/20 0/20	0/20 0/20	1/20 0/20	2/20 0/20	3/20 0/20	4/20 1/20	4/19 2/19	4/19 4/19	6/17 4/17
Cataracts	0 0 ppm	o.s. b.s.	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/19 0/19	1/17 0/17
	1 25 ppm	o.s. b.s.	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/19 0/19	1/15 0/15
	2 50 ppm	o.s. b.s.	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	1/20 0/20	0/20 1/20	0/19 1/19	0/19 1/19	1/17 1/17

1 one sided
both sided

2 No. of animals with findings/No. of alive animals examined

Project No. 71S0375/88109

Summary of the ophthalmologic findings

Females

Days			-1	89	180	272	363	448	537	624	722
Finding	Group										
Remainders of the pupillary membrane	0 0 ppm	o.s.[1] b.s.	2/20[2] 6/20	1/20 7/20	2/20 7/20	2/20 7/20	2/20 7/20	1/20 7/20	2/20 7/20	1/18 7/18	0/15 5/15
	1 25 ppm	o.s. b.s.	4/20 5/20	4/20 5/20	5/20 5/20	5/20 5/20	5/20 5/20	4/20 6/20	6/20 5/20	5/20 5/20	2/16 4/16
	2 50 ppm	o.s. b.s.	7/20 6/20	6/20 7/20	7/20 6/20	7/20 6/20	7/20 6/20	7/20 6/20	6/19 6/19	6/19 6/19	5/16 6/16
Corneal stipplings	0 0 ppm	o.s. b.s.	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	1/20 0/20	4/20 1/20	8/20 1/20	6/18 0/18	4/15 0/15
	1 25 ppm	o.s. b.s.	1/20 0/20	0/20 1/20	0/20 1/20	0/20 1/20	0/20 1/20	2/20 3/20	2/20 3/20	2/20 3/20	2/16 2/16
	2 50 ppm	o.s. b.s.	1/20 0/20	1/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	1/19 0/19	1/19 0/19	1/16 0/16
Striations	0 0 ppm	o.s. b.s.	2/20 1/20	3/20 8/20	0/20 14/20	0/20 18/20	0/20 19/20	0/20 20/20	0/20 20/20	1/18 1718	1/15 14/15
	1 25 ppm	o.s. b.s.	3/20 1/20	1/20 10/20	2/20 16/20	1/20 19/20	0/20 20/20	0/20 20/20	1/20 19/20	0/20 20/20	0/16 16/16
	2 50 ppm	o.s. b.s.	2/20 3/20	1/20 13/20	1/20 17/20	0/20 20/20	0/20 20/20	0/20 20/20	0/19 19/19	0/19 19/19	0/16 16/16
Bosselated structure of lens	0 0 ppm	o.s. b.s.	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/18 0/18	0/15 0/15
	1 25 ppm	o.s. b.s.	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	1/20 0/20	1/16 0/16
	2 50 ppm	o.s. b.s.	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/19 0/19	0/19 0/19	0/16 0/16
Bulbiform thickenings	0 0 ppm	o.s. b.s.	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/18 0/18	0/15 0/15
	1 25 ppm	o.s. b.s.	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	2/20 0/20	0/20 0/20	0/20 0/20	0/16 0/16
	2 50 ppm	o.s. b.s.	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 1/20	0/20 1/20	0/19 1/19	0/19 1/19	0/16 0/16
Opacities	0 0 ppm	o.s. b.s.	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	2/20 0/20	2/20 0/20	3/18 1/18	6/15 2/15
	1 25 ppm	o.s. b.s.	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	0/20 0/20	2/20 1/20	2/20 1/20	2/20 1/20	3/16 3/16
	2 50 ppm	o.s. b.s.	0/20 0/20	0/20 0/20	0/20 0/20	1/20 0/20	3/20 0/20	3/20 2/20	2/19 2/19	3/19 3/19	5/16 3/16

[1] one sided
both sided

[2] No. of animals with findings/No. of alive animals examined

20