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CIBA-GEIGY

EPA-OTS



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CONTAINS NO CBI

February 3, 1987

CERTIFIED MAIL
RETURN RECEIPT REQUESTED

Document Control Officer
Chemical Information Division
Office of Toxic Substances (WH-557)
Environmental Protection Agency
401 M Street, SW
Washington, D.C. 20460



Re: TSCA Section 8(e) Notice; 28-Day Study in the Rat,
Phenidone Derivative

Dear Madam/Sir:

This letter and the attached toxicity study contain no Confidential Business Information.

On December 19, 1986, CIBA-GEIGY Corporation submitted a "For Your Information" notice based on preliminary findings in a "FLASH REPORT" of a study entitled "TK 12850, 28-Day Study in the Rat". TK 12850 is a test code designation for IRGAFORM 1266, also known commercially as Dimezone S, which is 4-(hydroxymethyl)-4-methyl-1-phenyl-3-pyrazolidinone, CAS. No. 13047-13-7.

Irgaform 1266 is a phenidone derivative used as a photographic developing agent. We have imported approximately 1,200 lbs. over the past two years, 880 lbs. of which is still in inventory. Three to five major potential customers in the U.S. are already using this chemical substance commercially. Additionally, there are many smaller photographic developing companies which would purchase developer chemicals containing this substance in solution. The total U.S. market for the substance, which has been in use commercially for about 20 years, is about 10,000 lbs. The vast majority of this chemical substance used in the U. S. is produced or imported by other companies. CIBA-GEIGY is a very minor supplier to this market.

We have now received and evaluated a copy of the aforementioned final report. In accordance with EPA's March 16, 1978 policy statement on Section 8(e) reporting under the Toxic Substances Control Act, CIBA-GEIGY Corporation herewith submits a copy of the subject 28-day study which indicates that anemia and testicular lesions were the primary effects induced in animals which received the high doses (40 and 150 mg/kg/day) by gastric intubation. The NOEL was determined to be 10 mg/kg/day.

2

-2-

Although we are reporting this information under TSCA Section 8(e), CIBA-GEIGY Corporation does not believe that the product constitutes a substantial risk of injury to human health or the environment for the following reasons:

- a) The primary effects, while significant, were produced after administration by gastric intubation and mainly had a minimal grade of severity. In addition, the effects may be reversible.
- b) Because the product is a skin sensitizer, the recommended handling precautions on our label and Material Safety Data Sheet would result in minimal exposure. These precautions are designed to avoid eye, skin, and inhalation exposure through engineering controls or the wearing of personal protective equipment; i.e. chemical goggles, impervious gloves, and a NIOSH approved respirator, if necessary.
- c) Professional X-ray developing is the major use. Developer personnel wear impervious gloves, to the best of our knowledge, when handling the product either neat (powder form) or in solution.
- d) The level of this product in commercial developer solutions is only 1 to 2 gms/Liter, i.e. 0.1 to 0.2%.

CIBA-GEIGY Corporation has already revised its MSDS and product label to incorporate the information obtained in this new study. Copies of each are enclosed.

If you have any questions regarding this submission, please contact me.

Sincerely yours,

A. Di Battista

Anthony Di Battista
Manager, Toxic Substances Compliance
Safety, Health & Ecology

ADTB4/03/bt
Attachments

3

CIBA-GEIGY LTD.
BASEL / SWITZERLAND
GU? TOXICOLOGY

December 19, 1986

FINAL REPORT

TK 12850

28-DAY SUBACUTE, ORAL TOXICITY STUDY IN RATS (GAVAGE)

GU PROJECT NO. 850737

IRGAFORM 1266

170214 / The

TABLE OF CONTENTS

1	INTRODUCTION.....	1
	Purpose.....	1
	Basis for the study.....	1
	Sponsor.....	1
	GU Project No.....	1
	Testing facility.....	1
	Personnel and responsible scientists.....	1
	Archivation and distribution.....	2
2	SUMMARY AND ASSESSMENT.....	5
	MATERIALS AND METHODS.....	8
3.1	Test article.....	8
	Identity.....	8
	Stability.....	8
	Stability in the application form.....	8
3.2	Experimental animals.....	9
	Stock.....	9
	Source.....	9
	Initial body weights.....	9
	Initial age.....	9
	Individual identification.....	9
	Husbandry conditions.....	9
	Diet.....	9
	Water.....	10
3.3	Pretreatment period and procedure.....	11
	Delivery of the animals.....	11
	Acclimatisation period.....	11
	Number of animals.....	11
	Animal distribution.....	11
3.4	Treatment.....	12
	Dose levels.....	12
	Starting date of administration.....	12
	Date of completion.....	12
	Duration of treatment.....	12
	Route of administration.....	12
	Frequency of administration.....	12
	Form of administration.....	12
	Vehicle.....	12
	Volume of suspension applied.....	12
	Control analyses.....	12
	Control animals.....	13
3.5	Observations and records.....	13
3.5.1	Observations during life.....	13
	Clinical signs.....	13
	Mortality.....	13
	Eye examination.....	13

TABLE OF CONTENTS (cont'd)

	Body weight.....	13
	Feed consumption.....	13
	Feed conversion.....	14
	Water consumption.....	14
3.5.2	Hematology and blood chemistry.....	14
	Quality control systems.....	15
	Methods used in hematology.....	16
	Abbreviations used in hematology (remarks)..	17
	Methods used in blood chemistry.....	13
3.5.3	Necropsies.....	20
	Organ weights.....	20
	Organ and tissue sampling.....	20
3.5.4	Histopathological evaluation.....	21
3.6	Statistical Analysis.....	21
	General.....	21
	References.....	22
	Graphs.....	22
	Explanation of signs and remarks used in tables.....	22
3.7	Deviations.....	23
	Deviations from protocol.....	23
	Amendment to protocol.....	23
4	RESULTS.....	24
4.1	Dose levels.....	24
4.2	Mortality.....	25
4.3	Antemortem findings.....	25
	Clinical signs.....	25
	Eye examination.....	25
4.4	Body weight.....	26
4.5	Feed consumption.....	31
4.6	Feed conversion.....	36
4.7	Water consumption.....	41
4.8	Laboratory investigations.....	46
	Hematology.....	46
	Blood chemistry.....	46
4.9	Organ weights and ratios.....	51
4.10	Pathology.....	56
	Macroscopical findings.....	56
	Microscopical findings.....	56

7

TABLE OF CONTENTS (cont'd)

5 APPENDICES..... 64

 List of appendices..... 64

 Appendix 1.

 Body weights: statistics and individual values..... 65

 Appendix 2.

 Feed consumption: statistics and individual values..... 73

 Appendix 3.

 Water consumption: statistics and individual values..... 81

 Appendix 4.

 Laboratory data: statistics and individual values..... 89

 Appendix 5.

 Organ weights: statistics and individual values..... 123

 Appendix 6.

 Macroscopical and microscopical findings in individual animals.. 147

 Appendix 7.

 Analytical reports of test article suspensions in vehicle..... 173

1. INTRODUCTION

Purpose

This study was conducted in order to determine the eventual oral toxicity of the test article upon continuous daily administration by gavage for 4 weeks, and estimate a no-observable effect level of exposure.

Basis for the study

This study was carried out based on:

The OECD Guideline for testing of chemicals Nr 407: "Repeated Dose Oral Toxicity - Rodent : 28-day or 14-day Study" (adopted May 12, 1981), conforming with EC Commission Directive of April 25, 1984 (84/449 / EEC, Part B.7).

The OECD Principles of Good Laboratory Practice (GLP), adopted May 12, 1981, by the OECD council.

It was subjected to periodic quality assurance evaluation.

Sponsor

CIBA-GEIGY LTD.,
Plastics & Additives Division
Basle/Switzerland

GU Project No.

850737

Testing facility

All in-life testing was done at the Sisseln facility:

CIBA-GEIGY LTD.
Experimental Toxicology
4332 Stein / Switzerland

Personnel and responsible scientists

Study director	Dr. phil. II Ph. Thevenaz Experimental Toxicology
Technical assistants	T. Wernli and J. Matt Experimental Toxicology
Responsible for laboratory investigations	Dr. med. vet. P. Gretener Experimental Toxicology

Assistant laboratory investigations	T. Gersl Experimental Toxicology
Responsible for autopsies	G. Seifert Experimental Toxicology
Responsible for histopathology	F. Zak MD PhD Toxicological Pathology
Senior pathologist	W. Malinowski PhD. DVM Dipl. CVMA Toxicological Pathology
Staff pathologist	Dr. med. vet. K. Heider Toxicological Pathology
Laboratory assistant histopathology	Mrs. R. Fiechter Toxicological Pathology
Responsible for statistics	A. P. Grieve, M. Sc. Scientific Computing Center
Responsible for analytics	Mr. R.F. Wurster Analytical laboratories of CIBA-GEIGY AG Basle

Archivation and distribution

The relevant documents, specimens, tissues, slides etc. of this study are stored in the archives of CIBA-GEIGY Ltd. / Switzerland according to the standard operating procedure.

This report was distributed to:

Prof. Dr. R. Hess (Summary)
Dr. A. von Schulthess (Sponsor)
Archive

10

This report presents the results of the investigations as compiled by the undersigned:

Study Director

Dr. phil. II Ph. Theveraz

Ph. Theveraz

.....
date: December 11, 1986

Responsible for

Laboratory Investigations Dr. med. vet. P. Gretener

.....
date: *P. Gretener*
December 12, 1986

Responsible for
Pathology

F. Zak MD. PhD.
Head of Pathology

F. Zak

.....
date: December 17, 1986

Senior pathologist

W. Malinowski PhD. DVM Dipl. CVMA

W. Malinowski

.....
date: December 17, 1986

Pathology staff

Dr. med. vet. K. Heider

K. Heider

.....
date: December 17, 1986

This report was reviewed and approved by:

Dr. phil. II W. Basler
Head Rodent Toxicology

..... *W. Basler*
date: *November 19, 1986*

Dr. med. vet. W. Gfeller
Facility Management

..... *W. Gfeller*
date: *December 15, 1986*

12

2. SUMMARY AND ASSESSMENT

In the present study a total of 40 RAIF (SPF) rats, 5 males and 5 females per dosage group, were used. The test article TK 12850 was administered daily by gavage for 4 weeks at doses of 0, 10, 40, 150 mg/kg bw. Administered quantities of the test article were adjusted daily to individual animal body weight.

The results of the study are summarized as follows:

Mortality

No death occurred in the course of the study.

Antemortem findings

No treatment-related clinical symptoms and no signs of systemic toxicity were observed during the study.

Eye examination

Eye examination performed before and towards the end of the study revealed no evidence of a reaction to the treatment.

Body weight

The mean body weight of all treated male and female groups was similar to that of the respective control groups.

Feed consumption

The mean feed consumption in all male and female treatment groups was comparable to that of the respective control groups.

Feed conversion

The mean feed conversion in all male and female treatment groups was comparable to that of the respective control groups.

Water consumption

The mean water consumption in all male and female treatment groups was comparable to that of the respective control groups.

Hematology

Analysis of the hematological data revealed a mild, slightly macrocytic and hyperchromic anemia with Heinz bodies in the females of group 4 (150 mg/kg bw.), substantiated by a decrease in red blood cell count,

hemoglobin concentration and packed cell volume and increases in mean corpuscular volume and mean corpuscular hemoglobin. In the males of group 4 (150 mg/kg bw.) trends in the same direction were observed (especially increased corpuscular volume and Heinz bodies in one male animal).

The higher number of reticulocytes noted in males and females of the high dose group (150 mg/kg bw.) is a sign of increased hematopoiesis to compensate the decline in red cells.

Blood chemistry

Alanine aminotransferase activity and plasma glucose level showed a slight, statistically not significant increase in female group 4 (150 mg/kg bw.).

Organ weights

A slight increase in mean spleen weight and spleen to body and to brain ratios was observed in male groups 3 (40 mg/kg bw.) and 4 (150 mg/kg bw.), and in female group 4 (150 mg/kg bw.).

Mean kidneys weight and kidneys to brain ratios were slightly increased in male groups 3 (40 mg/kg bw.) and 4 (150 mg/kg bw.).

Macroscopical findings

No macroscopical changes of toxicological relevance were found at terminal necropsy.

Microscopical findings

Treatment related changes were observed in the following organs in animals from the top and intermediate dosage groups 3 (40 mg/kg bw.) and 4 (150 mg/kg bw.):

Spleen: congestion, hemosiderosis, and extramedullary hematopoiesis in male and female groups 3 (40 mg/kg bw.) and 4 (150 mg/kg bw.).

Liver: hepatocellular hemosiderosis in female group 4 (150 mg/kg bw.), and Kupffer cell hemosiderosis in female groups 3 (40 mg/kg bw.) and 4 (150 mg/kg bw.).

Kidney: proximal convoluted tubule: partially PAS - positive eosinophilic bodies in male groups 3 (40 mg/kg bw.) and 4 (150 mg/kg bw.).

Epididymis: cellular debris in male groups 3 (40 mg/kg bw.) and 4 (150 mg/kg bw.), and spermatoc granuleomas in male group 4 (150 mg/kg bw.).

14

Testis, spermatogenic epithelium: atrophy and a few sper-
matic giant cells in 1/5 rats from male group 4
(150 mg/kg bw.).

Testis, interstitial cells of Leydig: hyperplasia in male
group 4 (150 mg/kg bw.).

Conclusion

It can be inferred from the observations made during the
above study that a "no observable effect level" for
TK 12850 when administered by gavage over a period of 28
days is 10 mg/kg bw. per day in rats of both sexes.

3. MATERIALS AND METHODS

3.1 Test article

Identity

Company code No.: TK 12850
Trade name: IRGAFORM 1266
Batch No.: EN 17017.52
Purity: commercial grade
Description: solid
Date of receipt: July 23, 1985
Storage conditions: room temperature

Stability

Guaranteed by the sponsor until the end of the experiment.

Stability in the application form

Prior to the start of the study, a stability analysis of the test article in the selected vehicle, at nominal concentrations of 1 and 50 mg of test article per ml of vehicle, was requested by the analytical laboratories of CIBA-GEIGY Ltd, Basel/Switzerland.

The results of this analysis (Report No. S-14/85) are filed in the appendix section of this report.

According to these, no significant change of the high concentration (50 mg/ml) of the test article in the vehicle could be observed over a 4-hour period. However the lower concentration (1 mg/ml) was only about 91% of the nominal concentration over the same period.

Further information on the content and stability of the test article collected during the test is presented in the chapter "Dose levels" in section "Results" of this report.

16

3.2 Experimental animals

Albino rats.

Stock

Tif: RAIf (SPF), hybrid of RII 1/Tif x RII 2/ Tif

Source

Animal Production
CIBA-GEIGY LTD.
4332 Stein / Switzerland

Initial body weights

males: 171-195 g
females: 160-182 g
at the first weighing session during the acclimatisation period.

Initial age

approximately 6-7 weeks at delivery.

Individual identification

By number of cage for individual identification.
By tattoo of ear auricle for group identification.

Husbandry conditions

The experiment was carried out under specified pathogen free (SPF) standard laboratory conditions. The animals were housed individually in macrolon cages type 3 with standardised granulated soft wood bedding (Societe Parisienne des sciures Pantin).

An air-conditioned room with 16-20 air-changes per hour, maintained at a temperature of 22 ± 2 °C, relative humidity of 55 ± 10 and 12 hours light per day was used.

Neither insecticides nor chemicals were applied in the animal room with the exception of disinfectant: BRADOPHEN™.

Diet

Pelleted, certified standard diet Nafag No. 890 Tox, ad libitum (except as noted under Laboratory Investigations). All batches of diet were assayed for composition and contaminant levels by the manufacturer. Analytical results are available at the animal supply office (CIBA-GEIGY LTD., Pharmaceuticals Division PH 2.162).

17

Water

Tap water ad libitum, drinking water quality according to the specifications of the "Schweizerisches Lebensmittelbuch" (Ed. 1972) Results of the routine chemical examination of water at source (Grundwasserfassung Stein) as conducted periodically by the water authority (Baudepartement des Kantons Aargau, Abteilung Gewaesserschutz) are available to CIBA-GEIGY LTD., as well as the results of inhouse chemical analysis by the analytical laboratories of the Pharmaceuticals Division, CIBA-GEIGY LTD.

3.3 Pretreatment period and procedure

A written protocol was prepared prior to the initiation of this study.

Delivery of the animals

February 5, 1986

Acclimatisation period

A 8-day acclimatisation period was allowed between delivery and the start of treatment.

Immediately after delivery, the animals were distributed into groups. In order to set up a fully randomised experiment, they were assigned to those groups by means of random numbers generated by the IBM computer of CIBA-GEIGY LTD., Basle/Switzerland.

Further, during this period, they were weighed, and the first eye examination was performed (see "Observations and records").

From the same batch of animals a small number were retained for possible replacement during the acclimatisation period. These animals were subjected to identical conditions during this period, and those not used were discarded at the start of the experiment.

Number of animals

40 (total)

5 males, 5 females per dosage group.

Animal distribution

Animal No. (=cage No.)	Group 1 Control	Group 2 10 mg/kg bw.	Group 3 40 mg/kg bw.	Group 4 150 mg/kg bw.
MALES	1- 5	6-10	11-15	16-20
FEMALES	21-25	26-30	31-35	36-40

3.4 Treatment

Dose levels

0, 10, 40, 150 mg/kg bw.

Administered quantities were adjusted daily to individual animal body weight.

Starting date of administration

February 13, 1986

Date of completion

March 13, 1986

Duration of treatment

28 days.

Route of administration

Orally by gavage.

Frequency of administration

1 dose per day, 7 times per week.

Form of administration

Suspensions of the test article in the selected vehicle at the appropriate concentrations were freshly prepared every day immediately prior to the dosing of the animals and administered within about 2 hours.

Vehicle

Distilled water containing 0.5% carboxymethylcellulose and 0.1% Tween 80 (prepared by Pharmaceuticals Division, Ciba-Geigy Ltd.).

Volume of suspension applied

10 ml/kg bw.

Control analyses

Control analyses of the test article concentration in the vehicle were carried out at all dose levels on samples collected on experimental days 1, 19, 23 and 28. The samples were immediately dispatched to the analytical laboratories of CIBA-GEIGY Ltd., Basel / Switzerland.

20

The results of the analyses (Report no. F-3/86) are filed in the appendix section of this report, and annotated in the section "Dose levels".

Control animals

The control animals were treated in the same way as the dosed rats with the vehicle, without the test article.

3.5 Observations and records

3.5.1 Observations during life

Clinical signs

In order to detect changes in state of health or behaviour, or, in the case of dosed animals, any reaction to treatment examination was carried out daily, and observations were recorded at least weekly.

Mortality

All animals were checked daily (a.m. and p.m. on working days, a.m. on weekends and holidays), in order to record mortality, and to allow dead or moribund animals to be submitted to necropsy as soon as possible during working time.

Eye examination

Animals of the highest dosage group and of the control group were examined prior to treatment (day -7) and towards the end (day 27) of the treatment. Examination included observation of eye appearance and of periorcular region, and functional examination using an ophthalmoscope.

Body weight

The weight of all animals were recorded individually at weekly (midweek) weighing sessions. The first weighing occurred during the acclimatisation period.

Feed consumption

The individual feed consumption was recorded weekly.

Feed conversion

Feed conversion ratio was calculated according to the following formula:

$$\frac{\text{weekly feed consumption (g)}}{\text{midweek body weight (g)}} \times \frac{1000}{7}$$

Unit: g feed/kg body weight/day

Water consumption

The individual water consumption was recorded weekly.

3.5.2 Hematology and blood chemistry

Laboratory investigations were carried out in all animals of each sex and group at the end of the treatment period.

To reduce the biological variability due to circadian rhythms, blood sampling for hematology and blood chemistry was performed between the hours of 0700 and 0900 a.m. For blood chemistry measurements, feed was withheld for about 18 hours prior to blood removal. Ether anesthesia was used to restrain the animals. The site of blood removal was the orbital sinus and microhematocrit glass capillary tubes were used.

Blood samples from each animal with the respective anticoagulant (EDTA for performing the complete blood count, 3.8 % Sodium citrate for coagulation testing and Heparin for blood chemistry measurements) were aliquoted into individual vials.

The parameters and the quality control systems used are listed in the following tables.

22

Quality control systems

HEMATOLOGY	4 C-PLUS M+D LOW M+D NORMAL M+D HIGH	Coulter Electronics' Merz + Dade' Merz + Dade Merz + Dade
COAGULATION	CI-TROL 1 CI-TROL 2 CI-TROL 3	Merz + Dade Merz + Dade Merz + Dade
BLOOD CHEMISTRY	"N" Roche "P" Roche MONI-TROL I -E MONI-TROL II-E	Roche Diagnostica' Roche Diagnostica Merz + Dade Merz + Dade

-
1. Coulter Electronics LTD, IG Instrumenten Gesellschaft,
8045 Zuerich, Switzerland
 2. Merz + Dade LTD, 3186 Duedingen, Switzerland
 3. Hoffmann La Roche & Co., 4002 Basel, Switzerland

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23

Methods used in hematology

Parameter	Method of analysis Instrument	Abbreviation	Units
Erythrocyte Count	Coulter Counter S-Plus	RBC	T/l
Hemoglobin	Cyanmethemoglobin method Coulter Counter S-Plus	HB	mmol/l
Hematocrit	Coulter Counter S-Plus	HCT	l
Mean corpuscular volume	Coulter Counter S-Plus	MCV	fl
Mean corpuscular hemoglobin	Coulter Counter S-Plus	MCH	fmol
Mean corpuscular hemoglobin concentration	Coulter Counter S-Plus	MCHC	mmol/l
Reticulocytes	Staining with New methylene blue N Counting by means of the Hematrak 590	Reti	l
Leucocyte Count	Coulter Counter S-Plus	WBC	G/l
Differential Leucocyte Count	Blood smear stained with a Wright stain Counting by means of the Hematrak 590		
	Metamyelocytes	Metamyel	l
	Band neutrophils	Band	l
	Segmented neutrophils	Seg	l
	Eosinophils	Eo	l
	Basophils	Ba	l
	Lymphocytes	Ly	l
	Monocytes	Mo	l
	Plasma cells	Pl	l
	Other cells (= atypical lymphocytes)	Other	l
	Normoblasts	N-RBC	WBC

24

Parameter	Method of analysis Instrument	Abbre- viation	Units
Heinz Bodies	Staining with New methylene blue N Examination by means of a light microscope	Heinz Bodies	score
Thrombocyte Count	Coulter Counter S-Plus	Plt	G/l
Prothrombin Time	Quick's one-stage method Microcoagulometer Greiner	PT	sec

Abbreviations used in hematology (remarks)

- * slight, few
- ** moderate, some
- *** marked, many

Red cell morphology

- ANC Anisocytosis
- HOC Hypochromasia
- HRC Hyperchromasia
- MAC Macrocytosis
- MIC Microcytosis
- POC Polychromasia
- POI Poikilocytosis
- SPH Spherocytosis

Methods used in blood chemistry

Parameter	Method of analysis Instrument	Abbreviation	Units
Glucose	Hexokinase/G6P-DH COBAS-BIO	Gluc	mmol/l
Urea	Urease/GLDH COBAS-BIO	Urea	mmol/l
Total protein	Biuret reaction COBAS-BIO	Prot	g/l
Albumin	Bromocresol green method COBAS-BIO	Alb	g/l
Globulins	Calculated value (Total Protein minus Albumin)	Glob	g/l
A/G Ratio	Calculated value (Albumin/Globulins)	A/G	1
Cholesterol	Enzymatic, CHOD/PAP COBAS-BIO	Chol	mmol/l
Aspartate amino- transferase EC 2.6.1.1	MDH/NADH coupled reaction method COBAS-BIO	AST (GOT)	U/l
Alanine amino- transferase EC 2.6.1.2	LDH/NADH coupled reaction method COBAS-BIO	ALT (GPT)	U/l
Alkaline phosphatase EC 3.1.3.1	p-Nitrophenyl- phosphate as substrate COBAS-BIO	Alk.-P	U/l
Sodium	Flame photometry Corning-EEL 450	Na+	mmol/l
Potassium	Flame photometry Corning-EEL 450	K+	mmol/l

24

Parameter	Method of analysis Instrument	Abbreviation	Units
Calcium	Methylthymol blue COBAS-BIO	Ca++	mmol/l
Phosphate inorganic	Phosphomolybdate reaction COBAS-BIO	PO4-in	mmol/l

3.5.3 Necropsies

At the end of the experiment, all animals were exsanguinated under ether anesthesia, and subjected to detailed autopsy, in order to detect and record gross lesions as to location and type, and whenever possible number and size.

Organ weights

Besides the weight of the exsanguinated body, the following organs were weighed:

brain
heart
liver
kidneys
adrenals
thymus
ovaries / testes
spleen

Organ and tissue sampling

The following organs and tissues were preserved in 10% neutral formalin for possible histopathological examination:

skin
mammary area
spleen
mesenteric lymph node
axillary lymph node
popliteal lymph node*
sternum with bone marrow
femur with joint
skeletal muscle
trachea
lung
heart
aorta
submandibular salivary gland
liver
pancreas
oesophagus
stomach
small intestine
large intestine
kidney
urinary bladder
prostate
seminal vesicle
testis

28

epididymis
vagina*
uterus
ovary
pituitary gland
adrenal gland
thyroid with parathyroid gland
thymus
peripheral nerve
brain
spinal cord
eye with optic nerve
orbital gland
extraorbital lacrimal gland
zygomatic gland*
muzzle with tongue*

(*organs/tissues not submitted to histopathological evaluation)

3.5.4 Histopathological evaluation

Microscopical examination was performed on the organs/tissues listed above (except for those marked with an asterisk) in all animals from the control group and group 4 (150 mg/kg bw.). Additionally liver, spleen, kidney, testicle and epididymis were examined in all animals (respectively all males) from groups 2 (10 mg/kg bw.) and 3 (40 mg/kg bw.).

The fixed tissue samples of all these organs and tissues were embedded in paraplast, cut at 3-5 microns, stained with hemotoxylin and eosin and microscopically examined. On the request of the pathologist an iron stain (Perl's method) was performed on all spleen and liver sections in both males and females from the control and all test groups. Additionally, a PAS stain was requested for all kidney sections in both male and female rats from the control and all test groups.

3.6 Statistical Analysis

General

For each time point and parameter a uni-variate statistical analysis was conducted. Due to the routine manner of the analysis system, parameter free methods were applied. Each treated group was compared to the control group in respect of dispersion and displacement <1>. In addition a trend test <2> was applied considering all groups.

Statistical analysis is performed to draw attention to distinct values. A statistically significant difference between two values does not necessarily imply biological

29

relevance of that deviation and is not conclusive for a treatment related effect.

Hence, the responsible scientist may not comment on statistically significant values lying within the physiological range and on the other hand may comment on statistically not significant values, which differ substantially from the expected normal values.

References

- <1> Y. Lepage, Biometrika (1971) 58: pp. 213-217
- <2> H. R. Jonckheere, Biometrika (1954) 41: pp. 133-145

Graphs

Body weights and feed consumption were measured midweek. The seemingly two weeks interval between week -1 and week 1 of the study, as shown in the graphs, is merely a consequence of display and does not represent the real time elapse, i.e. one week measuring intervals.

Explanation of signs and remarks used in tables

- * = SIGN. DIFFERENCE (LOCATION AND/OR DISPERSION) BETWEEN CONTROL GROUP AND TREATED GROUP
- = NO STATISTICAL TEST PERFORMED
- @ = SAMPLE TOO SMALL FOR STATISTICAL TEST
- <-- = SIGN. NEG. TREND FROM CONTROL TO HIGHEST DOSAGE GROUP
- > = SIGN. POS. TREND FROM CONTROL TO HIGHEST DOSAGE GROUP

- N = NUMBER OF LIVING ANIMALS / GROUP
- *** = STATISTICAL TEST PERFORMED AT SIGNIFICANCE LEVEL
0.050 COMPARISON BETWEEN CONTROL AND TREATED GROUP
0.010 TREND (CONTROL TO HIGHEST DOSAGE GROUP)

30

3.7 Deviations

Deviations from protocol

No deviations from the protocol were detected.

There were no known circumstances that could have affected the quality and/or integrity of the data.

Amendment to protocol

After consultation with the responsible pathologist, and according to proviso in protocol, additional histological examination was performed in intermediate dosage groups 2 (10 mg/kg bw.) and 3 (40 mg/kg bw.) on testicle and epididymis in males, and liver, spleen and kidney in animals of both sexes, where histopathological changes were seen at the highest dose level.

4. RESULTS

4.1 Dose levels

Following dosages were used:

Group	Dose level (mg/kg bw.)	nominal concentration of test article in vehicle (mg/ml)
1	0	0
2	10	1
3	40	4
4	150	15

Samples of the suspensions were collected after administration at all dose levels on experimental days 1, 19, 23 and 28. The samples were dispatched to the analytical laboratories of CIBA-GEIGY AG, Basle / Switzerland, for content analysis of the test article concentration in vehicle.

Results of the analyses (Report no. F-3/86) are filed in the appendix section of this report.

The results of the first two analyses (samples taken on days 1 and 19 of the test) showed instability of the test article suspension in vehicle over one day or more.

The dispatching procedure was improved for the last 2 analyses (samples taken on days 23 and 28 of the test). The samples were immediately deep frozen after the end of administration, and analysis was performed on day of sampling.

The results of these analyses showed test article recovery from the suspensions over 90% at the 40 and 150 mg/kg bw. dose levels, and over 84% at the 10 mg/kg bw. dose level.

It is assumed that the mean effective daily administration of the test article in all experimental animals was over 85% of the nominal concentration in dosage group 2 (10 mg/kg bw.), and over 90% of the nominal concentration in dosage groups 3 (40 mg/kg bw.) and 4 (150 mg/kg bw.).

52
4.2 Mortality

No death occurred in the course of the study.

4.3 Antemortem findings

Records of the clinical observations in individual animals during the experiment, as well as records of eye examination, are part of the raw data.

The results are summarized below.

Clinical signs

No clinical symptoms related to the administration of the test article and no signs of systemic toxicity were observed during the study.

About one third of the males distributed among controls and treated groups, showed small wounds consecutive to excessive scratching, which is a behaviour common in caged rats of our strain, and unrelated to the treatment.

Eye examination

Eye examination performed on control animals and animal of group 4 (150 mg/kg bw.) before (day -7) and towards (day 27) the end of the study revealed no evidence of a reaction to the treatment.

4.4 Body weight

Mean body weight values are presented in the following tables and plots. The individual body weight values collected during the investigations and the results of the statistical analysis of these data are filed in the appendix section of this report.

The mean body weight of all treated male and female groups was similar to that of the respective controls.

34

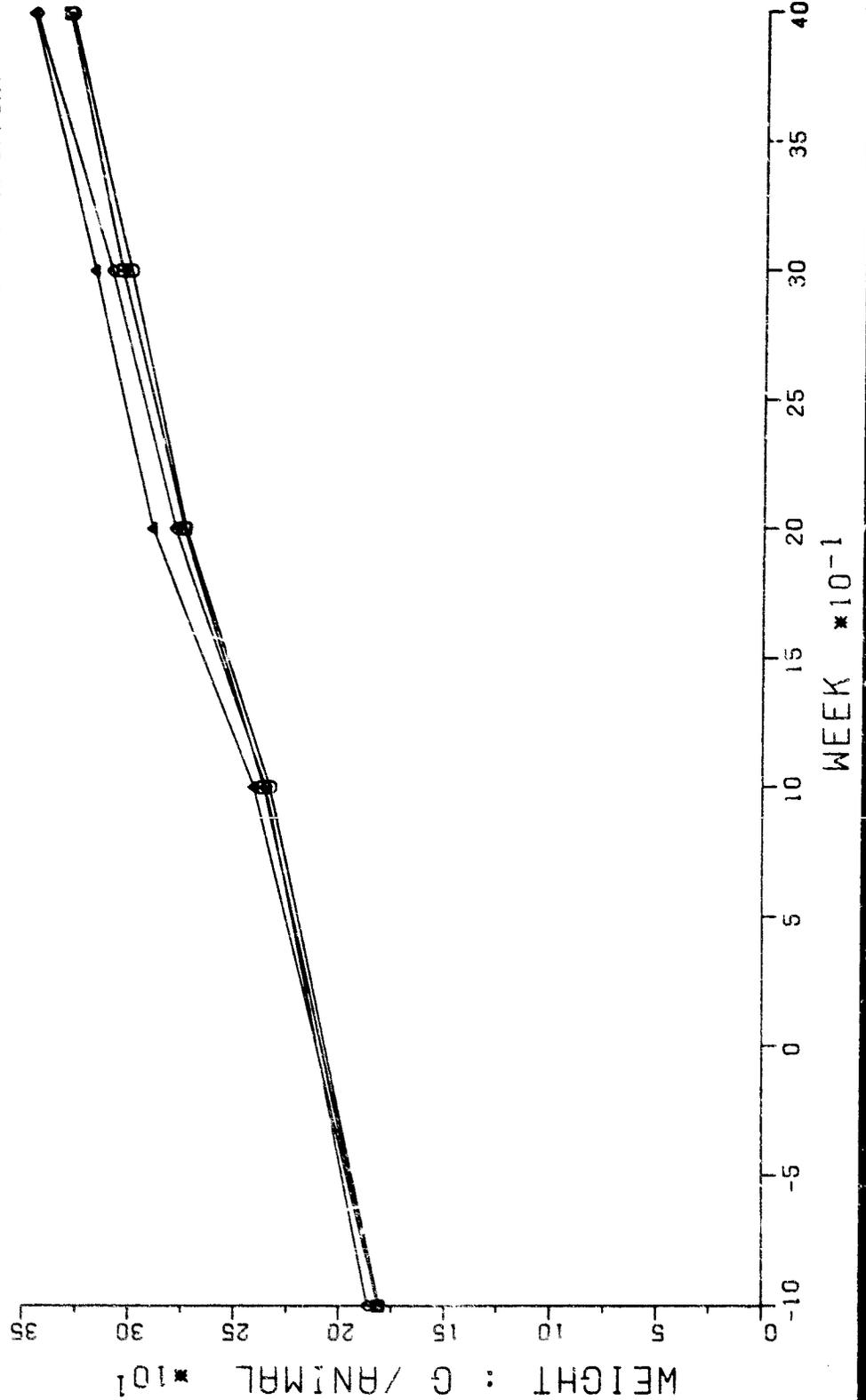
27

MEAN BODY WEIGHT

SPEC.:RAT
SEX.:MALE

GROUP 1 =	0.00	MG/KG	BW/DAY	GROUP 3 =	40.00	MG/KG	BW/DAY
GROUP 2 =	10.00	MG/KG	BW/DAY	GROUP 4 =	150.00	MG/KG	BW/DAY

EXP.:R*850737
COMP.:TK-12850

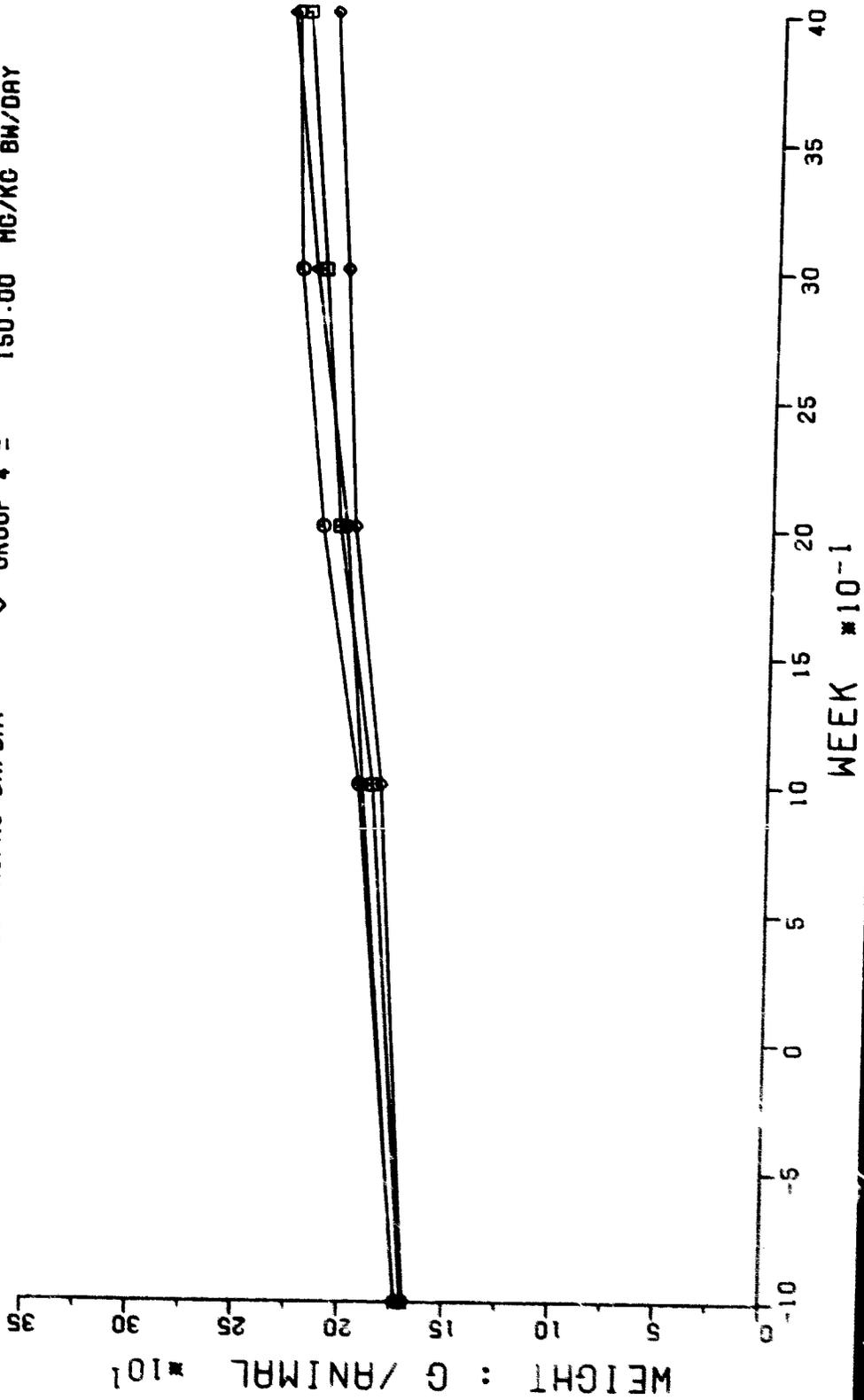


35

MEAN BODY WEIGHT

SPEC.: RAT
SEX.: FEMALE
EXP.: R*850737
COMP.: TK-12850

GROUP 1 =	0.00	MG/KG	BW/DAY	GROUP 3 =	40.00	MG/KG	BW/DAY
GROUP 2 =	10.00	MG/KG	BW/DAY	GROUP 4 =	150.00	MG/KG	BW/DAY



36

EXP. NO. : R*850737
 COMPOUND : TK-12850

MEAN BODY WEIGHT
 G / ANIMAL

SPECIES : RAT
 SEX : MALE

WEEK	DOSE IN 0.000			DOSE IN 10.000			DOSE IN 40.000			DOSE IN 150.000			TREND
	NO	MEAN		NO	MEAN		NO	MEAN		NO	MEAN		
-1	5	181		5	181		5	181		5	186		
1	5	236		5	233		5	240		5	235		
2	5	274		5	273		5	289		5	278		
3	5	303		5	299		5	316		5	308		
4	5	322		5	328		5	346		5	345		

NO = NO. OF LIVING ANIMALS / GROUP

*** NO STATISTICALLY SIGN. DIFFERENCE BETWEEN CONTROL AND TREATED GROUPS (SIGN.L.L.= 0.010)

*** NO STATISTICALLY SIGN. TRENDS (SIGN.L.L.= 0.010)

37

EXP. NO. : R#850737
 COMPOUND : TK-12850

MEAN BODY WEIGHT
 =====
 G / ANIMAL

SPECIES : RAT
 SEX : FEMALE

WEEK	DOSE IN MG/KG BW/DAY			TREND		
	0.000	10.000	150.000			
	NO	MEAN	NO	MEAN	NO	MEAN
-1	5	170	5	173	5	169
1	5	188	5	193	5	183
2	5	205	5	202	5	198
3	5	214	5	218	5	204
4	5	224	5	231	5	211

NO = NO. OF LIVING ANIMALS / GROUP
 *** NO STATISTICALLY SIGN. DIFFERENCE BETWEEN CONTROL AND TREATED GROUPS (SIGN.L. = 0.010)
 *** NO STATISTICALLY SIGN. TRENDS (SIGN.L. = 0.010)

30

4.5 Feed consumption

Mean feed consumption values are presented in the following tables and plots. The individual feed consumption values collected during the investigations are filed in the appendix section of this report.

The mean feed consumption in all male and female treatment groups was comparable to that of the respective control groups.