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Ciba-Geigy Corporation
520 White Plains Road
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8EHQ-80-340

August 23, 1996

8E. Substantiation

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(Attn: Section 8(e) Coordinator)
Office of Pollution Prevention and Toxics
U. S. Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

RE: **TSCA 8(e) Notice; 8EHQ-0480-0340; CASRN 70624-18-9, Supplemental Submission**

Dear Section 8(e) Coordinator:

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

CASRN 70624-18-9 was the subject of five previous 8(e) submissions as outlined below:

a) **April 21, 1980** - a low acute inhalation LC50 value and irreversible eye irritation were reported for a dusting version of this substance.

b) **July 22, 1987** - toxic effects observed during a 3-month oral administration toxicity study with rats.

c) **March 16, 1989** - toxic effects, similar to b), observed in a six-month oral administration study with rats.

d) **April 10, 1989** - reproductive toxicity effects observed during a range-finding test for a two-generation reproductive study with rats.

e) **November 2, 1993** - results seen in an immunotoxicity screening assay.

In accordance with EPA's March 16, 1978, policy statement on Section 8(e) reporting under the Toxic Substances Control Act, and EPA's June, 1991 TSCA Section 8(e) Reporting Guide, Ciba-Geigy Corporation (Ciba) wishes to bring to the attention of the Environmental Protection Agency results seen in a 6-month toxicity study in rats conducted with TK 12509/LD. Chemically, TK 12509/LD is Poly[[6-[(1,1,3,3-tetramethylbutyl)amino]-s-triazine- 2,4-diyl][(2,2,6,6-tetramethyl-4-piperidyl)imino] hexamethylene [(2,2,6,6-tetramethyl-4-piperidyl)imino]] having a CASRN 70624-18-9.

CONTAINS NO CBI

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4300 6/95

In the attached study, the test article was administered to male and female rats at daily dietary doses of 0, 5, 30 and 200 mg/kg/day for six months. Treatment was not found to cause any effect on the appearance or behavior of the rats and was not found to cause treatment related deaths. No effects on the eyes were recorded following ophthalmological examination.

The primary target organ following administration of the test compound was the mesenteric lymph node. At the 50 and 200 mg/kg dose levels, inflammatory and necrotizing changes were recorded. The 200 mg/kg dosed animals also showed evidence in some rats of effects on the ovary, adrenal, and spleen, characterized by chronic inflammatory and necrotizing changes and foam cells. A secondary effect on the bone marrow, spleen, thymus, and kidney was also recorded in some rats at the 200 mg/kg dose level.

The no-observable effect level (NOEL) was reported as 5.04 and 5.37 mg/kg for male and female rats, respectively.

A copy of the study, entitled "Final Report, TK 12509/LD, 6 Months Toxicity Study in Rats, Test No. 870950," is enclosed.

As a result of these new findings, Ciba will revise its Material Safety Data Sheet. Furthermore, Ciba markets this product as a low dusting material, thus minimizing the potential risk due to inhalation. The product is also a relatively high molecular weight polymer which, when combined with the recommended protective clothing and/or equipment in our Material Safety Data Sheet, minimizes the likelihood of dermal contact.

Please call the undersigned if you have any questions about this submittal.

Very truly yours,

A. Di Battista

Anthony Di Battista

AD08216a.WP/dch
Enclosure

CIBA-GEIGY LIMITED
EXPERIMENTAL TOXICOLOGY
4332 STEIN / SWITZERLAND

January 25, 1989

Rcvd. 8/15/96
K Fassuliotis

FINAL REPORT

TK 12509/LD

6 MONTHS TOXICITY STUDY IN RATS

TEST NO. 870950

P170204 / BAR

QUALITY ASSURANCE STATEMENT

Study Title	: 6 MONTHS TOXICITY STUDY IN RATS
Test Article	: TK 12509/LD
Batch	: EN 36927.62

Test Number : 870950

Study Director : Dr. W. Basler

QA Inspector : R. Murbach

I hereby certify that the following Quality Assurance activities were performed :

Inspections/Audits performed	Inspections/Audits reported to Management
1) 12.03.87	1) 12.03.87
2) 04.05.87	2) 06.05.87
3) 24.06.87	3) 25.06.87
4) 24.08.87	4) 25.08.87
5) 26.10.87	5) 27.10.87
6) 25.11.87	6) 25.11.87
7) 02.12.87	7) 03.12.87
8) 31.08.88	8) 01.09.88
9) 28.09.88	9) 30.09.88
10) 28.11.88 - Final Report Audit	10) 29.11.88

Dr. H.J. Weideli
Quality Assurance Manager
Central Function,
Product Safety

H.J. Weideli
Signature

8.12.88
Date

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1. INTRODUCTION

Purpose

The present study in rats was conducted in order to determine the potential toxicity of the test compound upon continuous administration in the diet for 6 months.

Basis for the study

This study was carried out in accordance with the Principles of Good Laboratory Practice as set forth in the "Verfahren und Grundsaetze der guten Laborpraxis (GLP) in der Schweiz" BUS, BAG, IKS March 1986, which intrinsically are in compliance with the OECD Principles of Good Laboratory Practice (GLP), adopted May 12, 1981 by the OECD Council, and the Good Laboratory Practice Regulations, US-FDA, dated December 22, 1978, and was subjected to periodic quality assurance evaluation.

Sponsor

CIBA-GEIGY Limited

Plastics and Additives Division

Basle, Switzerland

Test number

870950

Testing facility

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

CIBA-GEIGY Limited

Experimental Toxicology

4332 Stein, Switzerland

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Personnel and responsible scientists

The following scientists, professionals and supervisory personnel were involved in the conduct of the study:

Study director:	Dr. phil. II W. Basler Experimental Toxicology
Reporting:	Mr. B. Hunter B.Sc. Consultant
Technical assistant:	T. Wernli Experimental Toxicology
Statistics:	P. Christen dipl. stat. Scientific Computing Center
Laboratory investigations:	Dr. med. vet. P. Gretener Experimental Toxicology
Laboratory assistant:	T. Gersl Experimental Toxicology
Pathology:	Dr. med. E. Froehlich D.A.B.T. Toxicological Pathology
	G. Krinke, MVDr Toxicological Pathology
	Dr. med. M. Germer Toxicological Pathology
Pathology assistant:	Ms. R. Fiechter Toxicological Pathology
Autopsy:	G. Seifert Experimental Toxicology
Analytics:	Dr. H.P. Horntsch Central Analytical Laboratories

The job descriptions and the summaries of training and professional experience of personnel participating in this study are archived with CIBA-GEIGY Limited.

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Archiving and distribution

Archives are located at CIBA-GEIGY Limited, Werk Stein WST 458, CH-4332 Stein, Switzerland. Raw data, protocol and report, specimens and raw data of laboratory investigations will be stored at this location.

Raw data of the analytical determinations are stored in the archives of analytical laboratories of the Plastics and Additives Division.

Raw data of the histopathological examination and specimens (wet tissues, tissue blocks or histological slides) are stored in the Archives of Toxicological Pathology, CIBA-GEIGY Limited, CH-4002 Basel, Switzerland.

This report was distributed to:

Prof. Dr. R. Hess (Summary)

Dr. A. von Schulthess

Archive

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

Study Director Dr. phil. II W. Basler

.....
date: December 13, 1988

Responsible for Reporting Mr. B. Hunter B.Sc.

.....
date: 13 December 1988

Responsible for Laboratory Investigations Dr. med. vet. P. Gretener

.....
date: December 13, 1988

Responsible for Pathology Dr. med. E. Froehlich D.A.B.T.

.....
date: Jan 20, 1989

Senior pathologist G. Krinke, MVDr

.....
date: December 23, 1988

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Pathology staff Dr. med. M. Germer

M. Germer
.....
date: December 21, 1988

Responsible for Statistics P. Christen dipl. stat.

P. Christen
.....
date: January 24, 1989

Responsible for Analytics Dr. H.P. Hornisch

.....
date: January 25, 1989

This report was reviewed and approved by the undersigned.

Facility Management Dr. med. vet. W. Gfeller

W. Gfeller
.....
date: December 13, 1988

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2. SUMMARY AND ASSESSMENT

In the present study a total of 160 albino rats, 20 males and 20 females per dose group was used. The test article TK 12509/LD was administered in the diet at doses of 0, 5, 30, and 200 mg/kg bodyweight per day for 6 months. The results of this study are summarised as follows:

Dosage level

Average achieved intakes corrected for the quantity of TK 12509/LD as determined by frequent chemical analysis of the diets were: 5.04, 29.2 and 196 mg/kg bodyweight per day for males and 5.37, 30.8 and 214 mg/kg bodyweight per day for females of groups 2, 3 and 4 respectively.

Symptoms

Treatment had no effect on the appearance and behaviour of the rats.

Mortality

No treatment related deaths occurred during the study.

Bodyweight

A small reduction of mean bodyweights was recorded for rats of group 4 (200 mg/kg) resulting in a difference from control values of 8.6% and 5.5% for males and females respectively by the end of the study.

Food consumption

The food intake of males of group 4 (200 mg/kg) was slightly lower (6%) than control values during the last 8 weeks of the study. Other groups had intakes similar to those of the control group.

Food consumption ratio

Ratios for treated groups were similar to those of the control group.

Eye examinations

No changes were detected in the animals examined.

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Hematology

Treatment at the high dose level (200 mg/kg) was associated with a hypochromic, microcytic anemia with higher incidences of anisocytosis, poikilocytosis, hypochromasia and polychromasia of the red blood cells. In addition, a marked leucocytosis with neutrophilia and relative lymphopenia occurred, and hypersegmentation of neutrophils was noted in most ot these animals. Furthermore, there was a small increase in the number of thrombocytes and a minor prolongation of prothrombin time.

In rats of group 3 (30 mg/kg) a minimally higher thrombocyte count was recorded.

Blood chemistry

Changes were confined to rats of group 4 (200 mg/kg) and included: increased activities for the aminotransferases and alkaline phosphatase; slightly higher levels of plasma urea, creatinine and phosphate; minimally lower levels of plasma glucose and cholesterol; lower plasma albumin concentration and an increased globulin fraction associated with lower A/G ratios.

Organ weights

Treatment related effects were confined to the high dose group (200 mg/kg) and consisted of significantly heavier relative liver weights in females and significantly heavier relative spleen and kidney weights in males and females.

Pathology

Enlargement of the mesenteric lymph nodes was the major finding at necropsy in the majority of rats of group 4 (200 mg/kg). Microscopic examination revealed slight to marked chronic inflammatory and necrotizing changes, partly with abscess formation, associated with the presence of foam cells in the mesenteric lymph nodes in some rats of group 3 (30 mg/kg) and in all rats of group 4.

In other organs from group 4 rats similar chronic inflammatory and necrotizing changes, with or without foam cells, were seen in the ovaries of all females, in the adrenal cortex of some males and females and in the spleen of two males. In addition, hyperplasia of the splenic white pulp was noted in some males and females.

In the liver of group 4 males there was an increased incidence and intensity of focal intralobular and portal inflammatory cell infiltration. In two of these males a slight to moderate hypertrophy of the periportal hepatocytes was noted. Females of this group showed a slightly increased incidence of inflammatory cell infiltration of the liver.

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In the mucosa of the distal small intestine, small numbers of foam cells were present in males and females of group 4.

The following changes in group 4 rats were considered to result from the inflammatory and necrotizing effects of treatment: hypercellularity of the bone marrow in a few males and females and increased extramedullary hematopoietic activity in the spleen; slight to moderate atrophy of the thymus in a few males and one female; the presence of pus in the renal pelvis of four males and slightly increased incidence and amount of precipitate in the urinary bladder of males.

Conclusion

Treatment with TK 12509/LD at dose levels up to 200 mg/kg had no effect on survival or on the appearance and behaviour of the rats.

Slightly lower bodyweights were recorded for males and females of group 4 (200 mg/kg) and towards the end of the study food intakes of these males were slightly lower.

A dose level of 200 mg/kg resulted in a hypochromic microcytic anemia and a marked leucocytosis with neutrophilia. The latter probably associated with the inflammatory and necrotizing changes identified in these rats.

Blood chemistry changes were confined to rats receiving 200 mg/kg and suggested disturbance to liver and kidney function, subsequently confirmed by organ weight analysis and microscopy.

Pathology identified the primary target organ as the mesenteric lymph node. All rats of group 4 (200 mg/kg) and some rats of group 3 (30 mg/kg) presented with inflammatory and necrotizing changes, progressing to abscess formation in some animals, and with foam cells present in some rats.

At the high dose level, ovary, adrenal and spleen in some rats showed evidence of the chronic inflammatory and necrotizing changes and foam cells. In a few rats inflammatory changes occurred in the liver and in the majority of high dose level rats an increased number of foam cells was present in the small intestine.

As a reaction to the inflammatory and necrotizing changes, indirect effects were seen in the bone marrow, spleen, thymus and kidneys of some rats of group 4 (200 mg/kg).

The performance of group 2 rats was not disturbed by treatment. Therefore, the no observable effect level can be defined as 5.04 and 5.37 mg/kg bodyweight per day for males and females respectively.

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3. MATERIALS AND METHODS

3.1. Test material

Code No:	TK 12509/LD
Batch No:	EN 36927.62
Description:	solid
Purity:	commercial quality
Received:	March 24, 1987
Validity:	10 years
Storage:	room temperature

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3.2. Test system

3.2.1. Experimental animals

Species:	albino rats
Stock:	Tif: RAIf (SPF), hybrids of RII/1 x RII/2
Source:	Animal Production CIBA-GEIGY Limited 4332 Stein / Switzerland
Initial body weight:	range at week -1
	89.19 to 125.1 g in males 81.26 to 115.7 g in females
Initial age:	approx. 4-5 weeks

3.2.2. Husbandry

The experiment was carried out under specified pathogen free (SPF) standard laboratory conditions. The animals were housed in groups of 5 in Macrolon cages type 4 with standardised granulated soft wood bedding (Societe Parisienne des Sciures Pantin).

The animal room was air conditioned:

Temperature:	22 ± 2°C
Relative humidity (%):	55 ± 10
Ventilation:	16-20 air changes/hour
Light cycle:	12 hours light per day

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

3.2.3. Identification

The animals were identified by the number of the cage and individual identification (1-5) by tatoo of the ear auricle.

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3.3. Procedure

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

Number of animals: 160 (total)

20 males, 20 females (per group)

Delivery of animals: April 23, 1987

3.3.1. Acclimatisation period

An acclimatisation period of eleven days 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 Limited, Basle, Switzerland. Furthermore, they were weighed and the first eye examination was performed.

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

Start of acclimatisation: April 23, 1987

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3.3.2. Animal distribution

The general outline of the experiment is presented in the following animal distribution table.

DOSE	Group 1 Control	Group 2 5 mg/kg	Group 3 30 mg/kg	Group 4 200 mg/kg
MALE animal no. (cage no.)	1 - 20 (1 - 4)	21 - 40 (5 - 8)	41 - 60 (9 - 12)	61 - 80 (13 - 16)
FEMALE animal no. (cage no.)	81 -100 (17 - 20)	101-120 (21 - 24)	121-140 (25 - 28)	141-160 (29 - 32)

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3.3.3. Treatment period

Start of administration: May 4, 1987

Completion date: October 27 to 29, 1987

Duration of dosing: 177 to 179 days

3.3.4. Treatment levels

Dose levels:

0, 5, 30, 200 mg/kg bodyweight per day

Rationale:

The dosage levels selected were based on the results of the following previously conducted studies:

Project no. 801458

Acute, oral toxicity study in rats with TK 12509:
LD50 above 7000 mg/kg

and

Project no. 801624

Acute, oral toxicity study in rats with TK 12509/d:
LD50 above 5000 mg/kg

and

Project no. 801625

28-day range finding study in rats with TK 12509/LD
TK 12509/LD was administered at dosage levels of 1'000, 3'000 and 10'000 ppm, corresponding to 80-83, 206-211 and 788-838 mg/kg

A positive trend in the aspartate aminotransferase and in the alanine aminotransferase activities was detected in females at the upper dosage levels. Further, a slight increase in the liver weight of male group 4 (10'000 ppm) with concomitant hypertrophy of the hepatocytes in some animals.

The "no-effect-level" was 3000 ppm in males and 1000 ppm in females, corresponding to a mean daily intake of 211 mg/kg in males and 81 mg/kg in females.

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and

Project No. B78/0848

Centraal Instituut voor Voedingsonderzoek, Zeist, February 1980.
Subchronic (13 week) oral toxicity study with Chimassorb 944 in rats
The test article was administered at dosage levels of 1'000, 3'000 and 10'000'ppm.

The level of alkaline phosphatase and aspartate aminotransferase was relatively high in males and females at 10'000 ppm. Changes were noted in the white cells line in animals of both sexes given 3'000 and 10'000 ppm. The relative weight of spleen was increased at the upper dosage levels. Upon microscopical examination, treatment- and dose-related changes were observed in mesenteric lymph node and in spleen even at the lowest dose level in males.

and

Project No. B80-1927

Centraal Instituut voor Voedingsonderzoek, Zeist, June 1982.
Restricted subchronic (13 week) oral toxicity study with Chimassorb 944 in rats
The administered doses were 0, 100, 300, 1'000 and 3'000 ppm.

Similar changes as in the preceding study were detected: Increased activity of aspartate aminotransferase in both sexes at 3'000 ppm, increased number of neutrophils in females of the same group, histopathological changes in mesenteric lymph node and partly in spleen of males treated at 300, 1'000 and 3'000 ppm and in females and in females at 1'000 and 3'000 ppm. The no-observable effect level in this study was 100 ppm.

and

Project no. 840701

3-month toxicity study in rats
(Draft Report)
The dietary administration of the test article at 100, 500, 2000 and 10 000 ppm resulted in a daily intake of about 7, 33-35, 140-150 and 660-780 mg/kg

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The most remarkable alterations were observed at the two upper dosage levels. They included hypochromic microcytic anemia and leucocytosis, increased activity of aspartate aminotransferase, gamma-glutamyl transpeptidase and alkaline phosphatase and decrease of the albumin fraction. Further, increased spleen weight and enlarged mesenteric lymph nodes with histopathological change of these organs and of the liver, too.

The "no observable effect level" was 100 ppm, corresponding to a mean daily intake of 6.7 mg/kg bodyweight in males and 7.0 mg/kg in females.

The following doses were selected:

200 mg/kg was expected to result in observable toxicological effects.

30 mg/kg was expected to result in minimal toxicological effects.

5 mg/kg was expected to be a no observable effect level.

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3.4. Compound administration and diet

3.4.1. Route of administration

The test substance was administered orally in the diet (admixed to pelleted food).

3.4.2. Diet

Pelleted, certified standard diet Nafag No. 890 Tox containing the appropriate concentration of the test compound was provided 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 Limited, Pharmaceuticals Division PH 2.162).

3.4.3. Preparation of the diet

TK 12509/LD was weighed on a calibrated Mettler balance. The pulverised food was then homogenously mixed with the appropriate concentrations of the compound and about 25% water was added before pelleting to ensure the necessary pellet quality. The pellets were subsequently airdried.

The animals in the control group (group 1) were fed with similarly pelleted food without the test compound.

3.4.4. Chemical analysis of diet concentrations

Food samples were analysed for concentration, homogeneity and stability. No degradation could be demonstrated in pellets after 35 days of storage at room temperature (Analysis: Ciba-Geigy Analytical Services Rosental, Report S-3/87 dated August 20, 1987).

Analyses of the diet concentration were undertaken periodically with the diet batches applied during the study. These analyses were carried out by Analytical Services R of CIBA-GEIGY Limited, Basle/Switzerland.

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3.4.5. Water

Tap water was given ad libitum. The drinking water quality fulfilled the critical parameters in the specifications of the "Schweizerisches Lebensmittelbuch" (Ed. 1972). The 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 Limited, as well as the results of in house chemical analysis by the analytical laboratories of the Pharmaceuticals Division, CIBA-GEIGY Limited.

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3.5. Observations and records

Symptoms:	daily
Mortality:	daily (a.m. and p.m. on working days)
Bodyweight:	weekly (midweek)
Food consumption:	weekly
Food consumption ratio:	mean of individual calculations according to the following formula: $\frac{\text{weekly food consumption (g)}}{\text{midweek bodyweight (g)}} \times \frac{1000}{7}$ Unit: g food/kg bodyweight/day
Eye examination:	male and female animals of the highest dose level and of the control group were examined before the beginning (day -6) and towards the end (day 177) of the treatment period.

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3.6. Laboratory investigations

To reduce the biological variability due to circadian rhythms, blood sampling for hematology and blood chemistry was in the morning. Food was withheld overnight prior to blood removal. Ether anesthesia was used to restrain the animals. Blood was removed from the orbital sinus using microhematocrit glass capillary tubes.

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 collected into individual vials.

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

Laboratory investigations were carried out in all animals at the end of the testing period (October 27 / 28, 1987).

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3.6.1. Quality control systems

Hematology	4 C-PLUS LOW	Coulter Electronics[1]
	4 C-PLUS NORMAL	Coulter Electronics
	4 C-PLUS HIGH	Coulter Electronics
Coagulation	CI-TROL 1	Merz + Dade[2]
	CI-TROL 2	Merz + Dade
	CI-TROL 3	Merz + Dade
Blood chemistry	PRECINORM U	Boehringer Mannheim[3]
	PRECIPATH U	Boehringer Mannheim
	ISE CONTROL LOW	Boehringer Mannheim
	ISE CONTROL HIGH	Boehringer Mannheim

1. Coulter Electronics LTD, IG Instrumenten Gesellschaft, 8045 Zuerich, Switzerland
2. Merz + Dade LTD, 3186 Duedingen, Switzerland
3. Boehringer Mannheim (Schweiz) AG, 6343 Rotkreuz, Switzerland

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3.6.2. Methods used in hematology

Parameter	Method of analysis Instrument	Abbre-viation	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	1
Mean corpuscular volume	Coulter Counter S-Plus	MCV	fl
Mean corpuscular hemoglobin	Coulter Counter S-Plus	MCH	fmol
Reticulocytes	Staining with New methylene blue N; Counting by means of the Hematrak 590	Reti	1
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	1
	Band neutrophils	Band N	1
	Segmented neutrophils	Seg N	1
	Eosinophils	Eos	1
	Basophils	Baso	1
	Lymphocytes	Lympho	1
	Monocytes	Mono	1
	Plasma cells	Plasma C	1
	Atypical lymphocytes	Atyp Ly	1
	Blast cells	Blast C	1
	Other cells	Other C	1
	Normoblasts	N-RBC	%WBC

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Parameter	Method of analysis Instrument	Abbre-viation	Units
Thrombocyte Count	Coulter Counter S-Plus	Plt	G/l
Prothrombin Time	Quick's one-stage method Microcoagulometer Greiner	PT	sec

Abbreviations used in hematology

+	slight, few
++	moderate, some
+++	marked, many
Tox-Gr	Toxic granula in neutrophils
Hypseg N	Hypersegmented neutrophils
Azgr Ly	Azure granules in lymphocytes
Atyp Plt	Atypical platelets

Red cell morphology

Aniso	Anisocytosis
Micro	Microcytes
Macro	Macrocytes
Hypochro	Hypochromasia
Polychro	Polychromasia
Poik	Poikilocytosis
Sphero	Spherocytes
Target C	Target cells
Sickle C	Sickle cells
Baso st	Basophilic stippling
H-J B	Howell-Jolly bodies
Heinz B	Heinz bodies
RBC-Roul	Erythrocytes rouleaux
Ovalo	Ovalocytes

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3.6.3. Methods used in blood chemistry

Parameter	Method of analysis Instrument	Abbre-viation	Units
Glucose	Hexokinase/G6P-DH HITACHI 737	Gluc	mmol/l
Urea	Urease/GLDH HITACHI 737	Urea	mmol/l
Creatinine	Enzymatic colorimetric test HITACHI 737	Creat-e	umol/l
Total bilirubin	Reaction with 2,5-Di-chlorophenyldiazonium salt HITACHI 737	Bili-tot	umol/l
Total protein	Biuret reaction HITACHI 737	Prot	g/l
Albumin	Bromcresol green method HITACHI 737	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 HITACHI 737	Chol	mmol/l
Sodium	Ion selective electrode HITACHI 737	Na+	mmol/l
Potassium	Ion selective electrode HITACHI 737	K+	mmol/l
Calcium	o-Cresolphthalein complexone method HITACHI 737	Ca++	mmol/l

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Parameter	Method of analysis Instrument	Abbre-viation	Units
Chloride	Ion selective electrode HITACHI 737	Cl-	mmol/l
Phosphorus inorganic	Phosphomolybdate reaction HITACHI 737	PO4-in	mmol/l
Aspartate amino-transferase EC 2.6.1.1	MDH/NADH coupled reaction method HITACHI 737	ASAT (GOT)	U/l
Alanine amino-transferase EC 2.6.1.2	LDH/NADH coupled reaction method HITACHI 737	ALAT (GPT)	U/l
Alkaline phosphatase EC 3.1.3.1	p-Nitrophenyl-phosphate as substrate HITACHI 737	AlP	U/l

In the tables showing the blood chemistry data the parameters estimated by the Hitachi 737 analyser are marked with an asterisk to facilitate computer capture of data for historical records.

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3.7. Pathology

3.7.1. Macroscopical examination

At the end of the test period all control and treated rats were bled under ether anesthesia and subjected to detailed autopsy.

Besides the weight of the exsanguinated body the following organs were weighed except for female rat no.102 from group 2 (5 mg/kg), which died after blood collection.

brain
liver
kidneys
adrenals
gonads
spleen

The following organs and tissues were preserved in neutral buffered 4% formalin:

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
epididymis

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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
Zymbal's gland
muzzle
organs and tissues showing macroscopical changes

3.7.2. Microscopical examination

After fixation, samples from the organs listed below were embedded in paraplast, sectioned at 3-5 microns, stained with hematoxylin and eosin, and subjected to microscopical examination.

skin
mammary area
spleen
mesenteric lymph node
axillary 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

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epididymis
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
organs and tissues showing macroscopical changes

In selected male and female rats from the control group and the high dose group (200 mg/kg) additional sections from mesenteric lymph nodes, ovaries, adrenals and intestine were stained by the PAS-method, Prussian Blue, Ziehl-Neelsen or silver impregnation and examined microscopically.

Abbreviations used in pathology tables

S1, S2....	scheduled sacrifice(s)
MS	moribund sacrifice
FD	found dead
AD	accidental death

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3.8. Statistical analysis

General

For each time point and parameter a univariate statistical analysis was conducted. Due to the routine manner of the analysis system, parameter free (nonparametric) methods were applied. Each treated group was compared to the control group in respect of dispersion and displacement<1>. This test is a combination of Wilcoxon's and Ansari-Bradley's statistics, i.e. a combined test for location and variation. In addition a trend test<2> was applied considering all groups.

Statistical analysis is performed to draw attention to distinct values. Mean values differing from the control by combined location and/or variation are marked by an asterisk. A statistically significant difference between two values does not necessarily imply biological 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> A. R. Jonckheere, Biometrika (1954) 41: pp. 133-145

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Explanation of signs and remarks

*	sign. difference at the 5% level (two-sided)	
**	sign. difference at the 1% level (two-sided)	
+	sign. positive trend at the 1% level (two-sided)	
-	sign. negative trend at the 1% level (two-sided)	
N	number of samples or number of animals	
ns	no statistical tests performed	
n.d.	not determined	

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3.9. Deviations

Personnel

During the study Dr. F. Zak, Head of Pathology, retired and was succeeded by Dr. E. Froehlich.

Mr. A.P. Grieve, responsible for statistics, left the company and was replaced by Mr. P. Christen.

Starting date of acclimatisation

The rats were delivered four days earlier than was foreseen when the protocol was finalised. Hence the start of acclimatisation was April 23, 1987, and continued for eleven days.

Relative humidity

On 5 days during the study the relative humidity (%) deviated from the limits defined in the protocol (55±10) to a maximum value of 70%.

Pathology

In addition to the tissues listed in the protocol, Zymbal's gland, popliteal lymph node and muzzle were preserved from all animals but not processed.

The preservative for tissues was a 10-fold dilution of concentrated formalin (approximately 40%). Therefore, tissues were preserved in 4% formalin and n 10% as stated in the protocol.

The above mentioned deviations are considered to have no impact on the validity of the study. There were no known circumstances that could have affected the quality and/or integrity of the data.

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