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CIBA-GEIGY CORP

Contractor

Document Title

SUPPORT: LIFESPAN FEEDING STUDY IN RATS WITH COVER LETTER
DATED 081992

Chemical Category

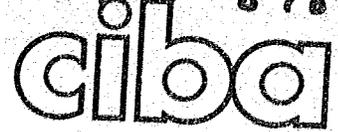
CGA-72651 TECHNICAL

8EHQ-92-8784
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Ciba Plant Protection

Contains No CBI



Ciba-Geigy Corporation
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CORRECTED LETTER

CERTIFIED MAIL/RETURN RECEIPT REQUESTED

August 19, 1992

207

93 SEP 22 PM 12:15

Document Processing Center (TS-790)
Office of Toxic Substances
Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

Attention: Section 8(e) Coordinator
(CAP Agreement)

SUBJECT: 8E CAP - 0024

Dear Section 8(e) Coordinator:

Enclosed are the original and two copies of a study Ciba-Geigy Corporation is submitting pursuant to the TSCA Section 8(e) Compliance Audit Program and CAP Agreement number 8E CAP-0024. The information being submitted is not considered Confidential Business Information. We are submitting the following information, as required by the CAP Agreement:

Company Name, Address and Telephone No.: Ciba-Geigy Corporation
Attn.: Mr. Anthony Di Battista
Toxicology, Regulatory Auditing
and Compliance Department
444 Saw Mill River Road
Ardsley, New York 10502-2699
Tel. No. 914-479-2776

Tested Chemical: CGA-72651 Technical;
N-formyl-4-chloro-o-toluidine
(Currently a manufacturing intermediate
no longer in use)

CAS Registry No.: 21787-81-5

Report Title: Lifespan (Chronic Toxicity and Carcinogenicity) Feeding Study in Rats (Study Number 05688/1, June 16, 1980)



8EHQ-92-8784
SP001 09/22/93



89930000207

Sectio. 9/ , Coordinator
August 19, 1992
Page 2

Summary:

Albino rats were fed 0, 2, 20, 100, or 500 ppm N-formyl-4-chloro-o-toluidine in the diet for 105 weeks. Liver weights were increased at the high dose. There were increased incidences of hyperplasia of small biliary ducts and multiloculated cholangiogenic biliary cysts in high-dose animals.

Category:

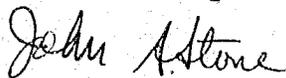
Unit II.B.2.b

Prior Reporting:

Not Applicable

Please call the undersigned at telephone number 919-632-2179 if you have any questions about this submittal.

Very truly yours,



John A. Stone
Manager, Environmental Issues

0907F2JG:/RD17:(crm)

Enclosures (Two additional copies of this letter and three copies of the submitted study)

cc: Mr. A. Di Battista

Contains NO CBP

CIBA - GEIGY LIMITED
BASLE / SWITZERLAND

TOXICOLOGY
GU 2

4-CHLORO-O-TOLUIDINE·HCL
LIFESPAN (CHRONIC TOXICITY AND CARCINOGENICITY) FEEDING STUDY
IN RATS

FINAL REPORT

PROJECT NO.: SISS R 05687/1

JULY 1, 1980

CIBA-GEIGY LIMITED
Basel/Switzerland
Toxicology
GU 2

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Toxicology
GU 2

LIFESPAN (CHRONIC TOXICITY AND CARCINOGENICITY) FEEDING STUDY
IN RATS WITH 4-CHLORO-O-TOLUIDINE·HCL - FINAL REPORT

Material: 4-Chloro-o-toluidine·HCl
Subject: Lifespan feeding study - Rats
Project No.: Siss R 05687/1
Sponsor: CIBA-GEIGY LTD., Agricultural Division, Basle and
SCHERING AG., Berlin

Study Director

Dr. med. vet. K. Sachsse
.....
date: 1. 7. 1980

Responsible for the animal experimentation

Dr. phil. II P. Suter
.....
date: 3. 7. 1980

Responsible for the laboratory investigations

H. Luetkenmeier, B.Sc.
.....
date: 3. 7. 1980

Responsible for the pathology

F. Zak, M.D., Ph.D.
.....
date: 23. 7. 1980

Reviewed and approved by

Prof. Dr. med. R. Hess
.....
date: 23 - 7 - 80

July 1, 1980/stu

CIBA-GEIGY LIMITED
Basel/Switzerland

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Toxicology
GU 2

Reported: July 1, 1980

Archives: Sisseln WST 452

Basle, Rosental 1040

GIBA-GEIGY LIMITED
Basle/Switzerland

Toxicology
GU 2

SUMMARY AND ASSESSMENT

4-Chloro-o-toluidine-HCl was administered to rats continuously in the feed over a total period of 103 weeks. Following this period all rats were fed with untreated food up to a survival rate of 20 % per sex and per group. At this time the remaining animals were killed. Groups of 180 rats (90 males, 90 females) received concentrations of 2 ppm (group 2), 20 ppm (group 3), 100 ppm (group 4) and 500 ppm (group 5) in the diet - estimated to be 0.1, 1, 4.6 and 24.6 mg/kg/day for the males and 0.1, 1, 5 and 28 mg/kg/day for the females.

An additional group of 180 rats served as the control (group 1) receiving ground diet without substance. Twenty rats (10 males and 10 females) of each group were killed and autopsied after 27 and 54 weeks. After 106 weeks 20 males and 20 females (satellite group for laboratory investigations) of the control and the treated groups were killed and autopsied.

The experiment was carried out under specified pathogen free (SPF) standard laboratory conditions.

The food intake and body weight gain of all treated and control rats were comparable during the whole experimental period with the exception of a lower body weight gain of the animals of group 5 until weeks 27 for males and 78 for females.

The mean food conversion of all treated rats was generally comparable to the controls.

No clinical symptoms were observed. Eye examinations and hearing tests did not reveal changes which were related to the administration of the substance.

The death rate was comparable between the treated and control rats (survival rate of females was longer than that of the males).

The results of the haematological investigation, blood chemistry data and the urinalysis were generally unremarkable for both treated rats and controls.

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Toxicology
GU 2

At week 4 the haemoglobin concentration was slightly but significantly below that of the controls in the female rats of group 5. A similar change was observed in the females of groups 4 and 5 at week 13, and in both sexes of group 5 at week 26.

Slight but significant decreases were observed in the erythrocyte count and packed cell volume in the female rats of group 5 at weeks 4 and 26.

Marginal reticulocytosis was also found to occur in the female rats of group 5 at week 13 and in both sexes at week 26.

Furthermore, in both male and female rats of group 5 the methaemoglobin level was found to be slightly, though significantly above that of the controls at weeks 4, 13, 26, 52, 78 and 104. At weeks 13, 52 and 78 this change was also observed in the females of group 4.

Heinz bodies were not observed at weeks 4, 13, 26 and 104, however, at week 52 in 15/19 female and week 78 in 5/12 female rats Heinz bodies were seen in group 5.

In addition, at week 4 the urine volume was found to be somewhat above that of the controls in the animals of groups 4 and 5 with a concomitant decrease in specific gravity.

Organ weights, organ to body weight and organ to brain weight ratios revealed some statistically significant differences between treated and control animals sacrificed after 27, 54 and 106 experimental weeks. With the exception of the absolute and relative liver weights after 106 weeks, these findings were not dose-related.

In rats from the highest (500 ppm) concentration group a slightly but significantly increased incidence of multilocular cholangiogenic cysts was observed in the liver. These biliary cysts were seen in 10/85 female and in 3/90 male rats from the 500 ppm group, but only in 4/89 female and in none of the male control animals (0/90). The incidence of cholangiogenic cysts in rats from the 100, 20 and 2 ppm concentration group was not higher than in the controls. In accordance with Burek (J.D. Burek, Pathology of Aging Rats, CRC Press, West Palm Beach 1978) the multilocular biliary cysts, occurring also spontaneously in aging rats are considered as hyperplasiogenic in nature and not as genuine tumours.

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Toxicology
GU 2

Numerous benign and malignant tumours were observed in both control and treated rats. Frequency and type of the neoplasms occurring in these animals were not influenced by the treatment. Also all other gross and histopathological lesions and changes seen in both control and test animals and described as congenital, degenerative or inflammatory in origin are attributed to the naturally occurring diseases which are common in aged rats of this breeding colony.

It can be inferred from the observations made during the above study that 20 ppm (approximately 1 mg/kg/day) represents a "no observable effect level" for male and female rats. There was no evidence of carcinogenic potential.

July 1, 1980/stu

Compound: 4-Chloro-o-toluidine.HCl

Purpose to determine the chronic toxicity and oncogenicity of 4-chloro-o-toluidine.HCl in rats.

Procedure According to protocol of 2.9.76 and the amendments of 24.4.77 and 24.7.78.

METHOD

Material 4-chloro-o-toluidine.HCl
Batch No.: AG 17/145 (1-3)
Purity: 99.8 %
Description: white powder
Received: September 15, 1976

Project No. Siss R 05687/1

Species RAIf SPF rats (RA 25) bred on the premises.
Equal numbers of males and females.

Mean initial body weight (week -1) 78 - 80 g, males
73 - 74 g, females

Initial age approx. 4 weeks

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 standardized granulated soft wood bedding (Société Parisienne des Sciures Pantin)

The animal room was air conditioned:
temperature: 22 ± 1°C
relative humidity: 55 ± 10 %
15 - 17 air changes/h
10 hours light/day

Neither insecticides nor chemicals were applied in the animal room with the exception of a disinfectant: Fungitex SB (Prod. Nr. 30071, CIBA-GEIGY).

No. of animals/
experimental group 90 males, 90 females
whereas 40 ♂ and 40 ♀ including the animals for laboratory investigations were for interim sacrifices.

Number of Animals / Experimental Group

	MALES					FEMALES				
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 1	Group 2	Group 3	Group 4	Group 5
a)	1- 5	91- 95	181-185	271-275	361-365	451-455	541-545	631-635	721-725	811-815
	6- 10	96-100	186-190	276-280	366-370	456-460	546-550	636-640	726-730	816-820
	11- 15	101-105	191-195	281-285	371-375	461-465	551-555	641-645	731-735	821-825
	16- 20	106-110	196-200	286-290	376-380	466-470	556-560	646-650	736-740	826-830
	21- 25	111-115	201-205	291-295	381-385	471-475	561-565	651-655	741-745	831-835
	26- 30	116-120	206-210	296-300	386-390	476-480	566-570	656-660	746-750	836-840
b)	31- 35	121-125	211-215	301-305	391-395	481-485	571-575	661-665	751-755	841-845
	36- 40	126-130	216-220	306-310	396-400	486-490	576-580	666-670	756-760	846-850
	41- 45	131-135	221-225	311-315	401-405	491-495	581-585	671-675	761-765	851-855
	46- 50	136-140	226-230	316-320	406-410	496-500	586-590	676-680	766-770	856-860
a)	51- 55	141-145	231-235	321-325	411-415	501-505	591-595	681-685	771-775	861-865
	56- 60	146-150	236-240	326-330	416-420	506-510	596-600	686-690	776-780	866-870
d)	61- 65	151-155	241-245	331-335	421-425	511-515	601-605	691-695	781-785	871-875
	66- 70	156-160	246-250	336-340	426-430	516-520	606-610	696-700	786-790	876-880
a)	71- 75	161-165	251-255	341-345	431-435	521-525	611-615	701-705	791-795	881-885
	76- 80	166-170	256-260	346-350	436-440	526-530	616-620	706-710	796-800	886-890
c)	81- 86	171-175	261-265	351-355	441-445	531-535	621-625	711-715	801-805	891-895
	86- 90	176-180	266-270	356-360	446-450	536-540	626-630	716-720	806-810	896-900

a) Animals for oncogenicity

b) Animals for laboratory investigations and interim sacrifice after 24 months

c) Animals for interim sacrifice after 6 months

d) Animals for interim sacrifice after 10 months

Compound: 4-Chloro-o-toluidine·HCl

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Route of Administration	oral in the diet
Food	pelleted standard diet (Nafag No. 890) ad libitum
Water	available at all times
Treatment Mixture	<p>to avoid rapid dissipation of the compound the test substance was mixed with the feed, pelleted and immediately deepfrozen. The concentration of the test agent in the food was regularly checked in food samples by chemical analysis*. Food pellets were prepared fresh every two weeks. Aliquots of the pelleted treatment mixture were removed from the freezer every day to be offered to the test animals.</p> <p>The animals in the control group (group 1) were fed with similarly pelleted food without compound.</p>
Duration of Acclimatisation	7 days
Duration of Administration	24 months
Duration of the Study	Life time or until 80 % of the animals per group and sex died (the latter refers to the period following the 24 months treatment).
Starting date of Treatment	September 20, 1976
Termination date of Treatment	September 19, 1978
Termination date of Study	December 14, 1978, ♂ of group 2 January 2, 1979, ♂ of group 1 January 10, 1979, ♂ of group 4 January 17, 1979, ♂ of group 3 February 7, 1979, ♂ of group 5 February 28, 1979, ♀ of group 3 March 5, 1979, ♀ of group 2 March 12, 1979, ♀ of group 4 March 20, 1979, ♀ of group 1 April 30, 1979, ♀ of group 5

*) Analysis carried out in the Residue Laboratories of the Agricultural Division of CIBA-GEIGY LTD., Basle/Switzerland

Compound: 4-Chloro-o-toluidine-HCl

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Concentration of active ingredient (nominal) 2, 20, 100, 500 ppm

Observations and Records

Mortality	daily
Symptoms	signs of local and/or systemic toxicity - daily
Tumour Incidence	external signs of tumour formation - weekly
Eye Examination	as well as hearing tests were performed monthly
Body Weight	weekly (first 3 months), monthly thereafter
Food Consumption	once weekly (first 7 months and monthly thereafter) calculated for individual animals
Mean Food Conversion (g food/kg body weight/day)	was calculated according to the following formular: $MFC = \frac{\text{weekly food consumption (g)}}{\text{midweek body weight (g)}} \times \frac{1000}{7}$

Statistical Analysis for others than Laboratory Investigations

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*. In addition a trend test** was applied considering all groups.

*) Y. Lepage, Biometrika (1971) 58: pp. 213-217

**) H.R. Jonckheere, Biometrika (1954) 41: pp.133-145

Compound: 4-Chloro-o-toluidine.HCl

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Statistical Analysis for Laboratory Investigations

Student's "t" test and the analysis of variance were employed to assess the significance of difference between concentration groups and controls whenever indicated.

Clinical Laboratory Investigations

Haematologic, blood chemistry and urinalysis measurements were carried out by standard methods on 200 randomised rats (20 males, 20 females per group) from the control and four concentration groups at 4, 13, 26, 52, 78 and 104 experimental weeks.

To reduce the biologic variability due to circadian rhythms, blood sampling for haematology and blood chemistry was between the hours of 8.00 and 9.00 a.m. For blood chemistry measurements food was withheld for 18 hrs prior to blood removal.

The site of blood removal was the orbital sinus and a micro-haematocrit glass capillary tube was 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.

No anaesthesia was used to restrain the animals. All blood collection was by manual restraint only.

Urine for analysis was collected overnight. The individual rats were housed in special metabolism cages. Food and water was withheld during the time of urine collection.

The quantitative assay of all blood parameters was completed within an 8 hr. period under "Quality Control" conditions.

The quality control systems used in haematology, blood chemistry and urinalysis were as follows:

Compound: 4-Chloro-o-toluidine·HCl

Haematology Reference Control:	CH-60 Normal	(Merz + Dade)
	CH-60 Abnormal	" "
	4C Normal	(Coulter)
	4C Abnormal	" "
Coagulation Reference Control:	PLACHECK-100	(TOA Medical)
	CI-TROL-1	(Merz + Dade)
	CI-TROL-2	" "
	CI-TROL-3	" "
	CI-TROL-PTT	" "
	HYLAND 100 %	(Hyland)
	HYLAND 20 %	" "
	CONTROL PLASMA	(Behringwerke)
Blood Chemistry Reference Control:	PATHOPLASMA-1	" "
	PATHOPLASMA-2	" "
	MONI-TROL I	(Merz + Dade)
	MONI-TROL II	" "
	ENZA-TROL	" "
	SERONORM	(Nyegaard)
Urinalysis Reference Control:	LEDER-NORM	(Cyanamid)
	LEDER-TROL	" "
	TEK-CHEK	(Ames)

Parameter used in Haematologic, Blood Chemistry and Urinalysis Studies

<u>HAEMATOLOGY</u>	<u>METHOD</u>	<u>UNIT</u>
<input checked="" type="checkbox"/> Haemoglobin (Hb)	Cyanmethaemoglobin. Coulter Haemoglobinometer	g/100 ml
<input checked="" type="checkbox"/> Methaemoglobin (MHb)	IL 282 CO-OXIMETER	%
<input type="checkbox"/> Carboxyhaemoglobin (COHb)	IL 282 CO-OXIMETER	%
<input checked="" type="checkbox"/> Erythrocytes (RBC)	Coulter Counter Model 2F	X 10 ⁶ Cells/cmm
<input checked="" type="checkbox"/> Packed Cell Volume (PCV)	Clay-Adams Microhaematocrit Centrifuge. Centrifugation by 12,500 r.p.m./3 min.	Vol.-%
<input checked="" type="checkbox"/> Mean Corpuscular Volume (MCV)	Calculated value - $\frac{PCV (\%) \times 10}{RBC (X10^6/mm^3)}$	cu
<input checked="" type="checkbox"/> Mean Corpuscular Haemoglobin (MCH)	Calculated value - $\frac{Hb (g) \times 10}{RBC (X10^6/mm^3)}$	mu

Reticulocytes:

Supravital staining with brilliant cresyl blue. % Retics/1000 RBC

Inclusion Bodies (I.B.):
(Heinz Bodies)

Supravital staining with neutral red and brilliant green. Examination of 8-10 fields if only a few Heinz Bodies were seen. When numerous 100 cells were counted and the rating made in accordance with the following scheme:

% RBC showing Heinz Bodies

Negative
Occasional
1 - 10 %
11 - 40 %
41 - 75 %
76 - 100 %

Evaluation

0
1
2
3
4
5

Thrombocytes:

Electronic counting with TOA Platelet Counter (Model PL-100)

x 10³ cells/cmm

Prothrombin Time (P):

Quick's one-stage method using Coagulometer of Schnitger and Gross with plasma and Thromboplastin (Merz & Dade). secs.

Activated Partial Thromboplastin Time (APTT)

Dade's activated Cephaloplastin method using Coagulometer of Schnitger and Gross: The partial thromboplastin time with kaolin, Proctor, R.R. & Rapaport, S.I., Amer. J. Clin. Path. 36:212 (1961). secs.

Plasma Viscosity:

Harkness Viscometer. Readings made at 25°C to 0.02 sec. and compared with distilled water (relative viscosity = 1.0). "Rate of Shear" was 660 sec.⁻¹. cp.

Leucocytes:

Total Count:

Differential Count:

Coulter Counter Model ZF

X 10³ cells/cmm

Blood smear stained with the "Ames Hema-Tek Slide Stainer" using "Hema-Tek" Stain-Pak. Meta-Myl.
Band = Metamylocytes
Seg = Band Cells
Ly = Segmented Neutrophils
Mo = Lymphocytes
Eo = Monocytes
Ba = Eosinophils
NBL/100 WBC = Normoblast

expressed as a % of total count

Blood Chemistry
=====

Glucose:

LKB Ultralab System. UV-Glucose dehydrogenase method. Banauch, D. et al., 2. klin. Chem. Klin. Biochem. 13, 101-107 (1975). mg/100 ml

Urea (Urea-N):

LKB Ultralab System. Urease method (Berthelot-Reaction). Fawcett, J.K. & Scott, J.E., J. Clin. Path. 13, 156 (1960). mg/100 ml

Total Bilirubin: mg/100 ml
LKB Ultralab System. Diazotization reaction in the presence of accelerator caffeine-sodium benzoate. Jedrassik, L. & Grof, P., Biochem. Ztschr. 297, 81 (1938).

Total Protein: g/100 ml
LKB Ultralab System. Biuret reaction. Henry, R.J., Clinical Chemistry: Principles and Techniques, Harper and Row Publishers, New York, (1964).

Protein Electrophoresis:
Agarose gel electrophoresis utilizing Bloware's Cool Pak electrophoretic cell. Evaluation with the Helena Quick Scan and Quick Quant.

Electrolytes:
 Sodium (Na⁺): mEq/l
 Potassium (K⁺): mEq/l
 Chloride (Cl⁻): mEq/l
Flame Photometer (EEL 450)
Flame Photometer (EEL 450)
Coulometric-Amperometric titration (Buchier-Cotlove Chloridometer).

<input checked="" type="checkbox"/>	Glutamate-Oxalacetate Transaminase (GOT):	Eppendorf KEA-5080. UV-absorption with NADH	mU/ml
<input checked="" type="checkbox"/>	Glutamate-Pyruvate Transaminase (GPT):	Eppendorf KEA-5080. UV-absorption with NADH	mU/ml
<input checked="" type="checkbox"/>	Lactate Dehydrogenase (LDH):	Eppendorf KEA-5080. UV-absorption with NADH	mU/ml
<input checked="" type="checkbox"/>	Alkaline Phosphatase (AP):	Eppendorf KEA-5080. Enzymatic hydrolysis of p-nitro-phenylphosphate. O.A. Bersey et al., J. Biol. Chem. 164, 321 (1946).	mU/ml
<input checked="" type="checkbox"/>	γ -Glutamyl Transpeptidase (γ -GT):	Eppendorf KEA-5080. γ -glutamyl-p- nitroanilide + glycylglycine. Szasz, G.: Clin. Chem., 15, 124 (1969).	mU/ml
<input type="checkbox"/>	Acetyl Cholinesterase (Erythrocyte, Plasma and Brain):	Colorimetric: Modified acetylthiocholine DTNB method. Voss, G. and Sachsse, K. Toxicol. Appl. Pharmacol. 15, 764 (1970).	Klett Units

Urinalysis
=====

Urine Volume:

Specific Gravity:

pH:

Protein:

Glucose:

Ketones:

Direct measurement.

ml/18 hr.

TS Refractometer. (American Optical).

Multistix reagent strips. (Ames Co.).

Multistix reagent strips. (Ames Co.).

- 0 = negative
- 1 = trace to 30 mg %
- 2 = 30 to 100 mg %
- 3 = 100 to 300 mg %
- 4 = >300 mg %

Multistix reagent strips (Ames Co.).

- 0 = negative
- 1 = trace
- 2 = moderate amount
- 3 = large amount

Multistix reagent strips. (Ames Co.).

- 0 = negative
- 1 = trace
- 2 = moderate amount
- 3 = large amount

Blood: Multistix reagent strips. (Ames Co.).
 0 = negative
 1 = trace
 2 = moderate amount
 3 = large amount

Bilirubin: Ictotest and Multistix reagent strips.
 (Ames Co.).
 0 = negative
 1 = positive

Urobilinogen: Multistix reagent strips (Ames Co.).
 0 = negative
 1 = positive

Eile Salts: Pettenkofer and Hay's method.
 0 = negative
 1 = positive

Urine Sediment: Specimen centrifugation at 1000 to 1500 r.p.m. for 5 minutes.
 Supravital cytodagnostic staining of sediment employing a modified Sternheimer-Malbin stain.
 Microscopy of sediment by 500 x for:
 Erythrocytes
 Leucocytes
 Epithelial cells (Squamous & Round)
 Casts
 Crystals (Normal & Abnormal)
 Abnormal constituents
 0 = negative
 1 = occasional
 2 = few
 3 = moderate amount
 4 = large amount

=====

Compound: 4-Chloro-o-toluidine·HCl

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RESULTS

Observations and Records

The food intake and body weight gain of all treated and control rats were comparable during the whole experimental period with the exception of a lower body weight gain of the animals of group 5 (500 ppm) until week 27 for males and week 78 for females.

The mean food conversion of all treated and control rats was generally comparable.

No clinical symptoms related to treatment were observed during the study.

The death rate was comparable between the treated and control rats. The survival rate of females was longer than that of the males.

Eye Examination

Eye examination did not reveal any ocular changes which were related to treatment.

Auditory Perception

No change of hearing ability which was related to treatment was registered.

Clinical Laboratory Investigations

Haematology:

At 4, 13, 26, 52, 78 and 104 experimental weeks, haematological changes apart from the spontaneous age related occurrences were generally unremarkable.

Compound: 4-Chloro-o-toluidine·HCl

There was a slight but statistically significant ($p < 0.01$) decrease in the haemoglobin concentration in the female rats of group 5 at week 4 and in groups 4 and 5 at week 13. A similar change occurred in both sexes of group 5 at week 26.

Furthermore, at weeks 4 and 26 slight but significant ($p < 0.01$) decrease in the erythrocyte count and packed cell volume were seen in the female rats of group 5.

Marginal but significant ($p < 0.01$) reticulocytosis was also found to occur in the female rats of group 5 at week 13 and in both sexes at week 25.

In both male and female rats of group 5 the methaemoglobin level was found slightly, though significantly ($p < 0.01$) above that of the controls at weeks 4, 13, 26, 52, 78 and 104. At weeks 13, 52 and 78 this change was also observed in the females of group 4.

Heinz bodies generally associated with methaemoglobin formation were not observed at weeks 4, 13, 26 and 104. However, at week 52 in 15/19 female and week 78 in 5/12 female rats of group 5 Heinz bodies were seen (size approx. $1 \mu \emptyset$). No Heinz bodies were observed in the male rats.

Blood Chemistry:

By the assessment of blood chemistry values at 4, 13, 26, 52, 78 and 104 weeks no changes were observed which could be related to the treatment.

Urinalysis:

The findings in the urine apart from spontaneous age related occurrences were generally unremarkable.

At week 4 the overnight urine volume was found to be slightly but significantly ($p < 0.01$) above that of the controls in both sexes of groups 4 and 5 with a concomitant decrease in specific gravity. In contrast there was no significant change in the animals at weeks 13, 26, 52, 78 and 104.

Most rats revealed some degree of physiological proteinuria including those of the control group. This is considered normal in laboratory rats.

D O S A G E L E V E L S
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EXP. NO. : R05687/1
COMPOUND : 4-CHLOR-0-TCL-P-CL

SPECIES : RAT
SEX : MALE

WEEK	2.000		20.000		100.000		500.000	
	%	MEAN	%	MEAN	%	MEAN	%	MEAN
1	125.0	0.60	78.0	3.72	68.0	16.59	75.0	89.07
9	93.0	0.15	78.0	1.21	76.0	3.94	74.0	29.31
23	90.0	0.09	61.0	0.62	61.0	3.12	62.5	16.75
39	40.0	0.04	72.0	0.64	64.0	2.96	75.0	15.84
56	73.0	0.08	85.0	0.93	67.0	3.64	66.8	17.04
65	42.0	0.03	94.0	0.71	78.0	3.14	74.0	15.94
70	42.0	0.04	94.0	0.82	78.0	3.59	74.0	16.48
74	42.0	0.04	94.0	0.81	78.0	3.52	74.0	17.08
87	71.0	0.06	69.0	0.57	71.0	3.04	84.0	19.33
92	71.0	0.05	69.0	0.55	71.0	2.85	84.0	17.40
101	81.0	0.07	82.0	0.69	73.0	3.34	73.0	17.49
105	85.0	0.07	89.0	0.67	79.0	3.16	79.0	19.59
MEAN	71.3	0.11	80.4	0.99	72.0	4.57	74.6	24.61

% = PERCENTAGE OF ACTUAL AMOUNT OF SUBSTANCE
(FOUND BY CHEMICAL ANALYSIS)

EXP. NO. : R05687/1
 COMPOUND : 4-CHLOR-0-TOL-SHCL

D O S A G E L E V E L S
 =====
 MG SUBST./KG BW/DAY

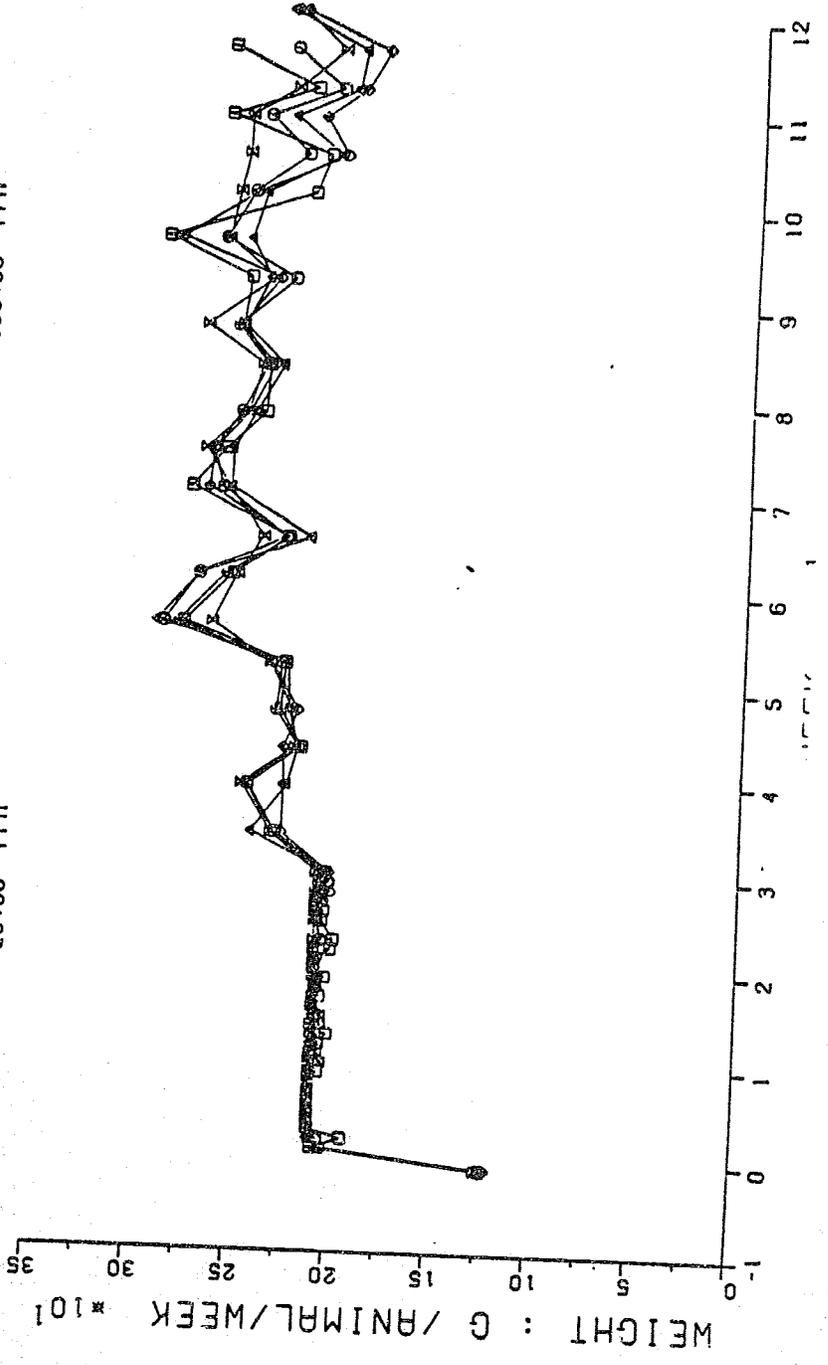
SPECIES : RAT
 SEX : FEMALE

WEEK	2.000		20.000		100.000		500.000	
	%	MEAN	%	MEAN	%	MEAN	%	MEAN
1	125.0	0.55	78.0	3.36	68.0	14.93	75.0	82.10
9	93.0	0.18	78.0	1.41	76.0	7.12	74.0	36.39
23	90.0	0.11	61.0	0.77	61.0	3.99	62.5	21.57
39	40.0	0.05	72.0	0.73	64.0	3.54	75.0	25.29
56	73.0	0.08	85.0	0.96	67.0	3.88	66.8	20.40
65	42.0	0.04	94.0	0.97	78.0	3.90	74.0	20.62
70	42.0	0.04	94.0	0.95	78.0	3.95	74.0	20.22
74	42.0	0.04	94.0	1.00	78.0	4.00	74.0	21.97
87	71.0	0.08	69.0	0.70	71.0	3.42	84.0	23.75
92	71.0	0.07	69.0	0.70	71.0	3.59	84.0	21.76
101	81.0	0.08	82.0	0.81	73.0	3.38	73.0	19.47
105	85.0	0.09	89.0	0.95	79.0	4.01	79.0	22.36
MEAN	71.3	0.12	80.4	1.11	72.0	4.98	74.6	27.99

% = PERCENTAGE OF ACTUAL AMOUNT OF SUBSTANCE
 (FOUND BY CHEMICAL ANALYSIS)

MEAN FOOD CONSUMPTION

SPEC.: RAT
 SEX.: MALE
 EXP.: R05687/1
 COMP.: 4-CHLOR-0-TOL.*HCL
 GROUP 1 = 0.00 PPM
 GROUP 2 = 2.00 PPM
 GROUP 3 = 20.00 PPM
 GROUP 4 = 100.00 PPM
 GROUP 5 = 500.00 PPM



WEIGHT : G /ANIMAL/WEEK * 101

MEAN FOOD CONSUMPTION
 SPEC.: RAT
 SEX.: FEMALE
 EXP.: R05687/1
 COMP.: 4-CHLOR-0-TOL.*HCL

□	GROUP 1 =	0.00	PPH
○	GROUP 2 =	2.00	PPH
△	GROUP 3 =	20.00	PPH
◇	GROUP 4 =	100.00	PPH
⊗	GROUP 5 =	500.00	PPH

