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Document Title		INITIAL SUBMISSION: LETTER FROM CIBA-GEIGY CORP TO USEPA REGARDING N-FORMYL-4-CHLORO-O-TOLUIDINE: LIFESPAN FEEDING STUDY IN MICE WITH COVER LETTER DATED 081492	
Chemical Category		N-FORMYL-CHLORO-TOLUIDINE	

Agricultural Division  
CIBA-GEIGY Corporation  
P.O. Box 18300  
Greensboro, North Carolina 27419  
Telephone 919 632 6000

CIBA-GEIGY

8EHQ-0992-11512

"Contains NO OIL"

CERTIFIED MAIL/RETURN RECEIPT REQUESTED

August 14, 1992

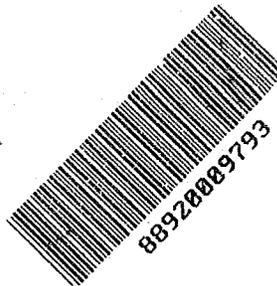


8EHQ-92-11512  
INIT 09/08/92

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



92 SEP -8 PM 8:22

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-chloro-toluidine  
(Currently a manufacturing intermediate  
no longer in use)

CAS Registry No.: 87999-30-2 - see follow-up for  
(81787-81-5) corrected CAS #  
Report Title: Lifespan Feeding Study in Mice (Study  
Number 4762/1, June 7, 1978)

Summary: Albino mice were fed 0, 20, 100, or  
500 ppm N-formyl-4-chloro-o-toluidine in  
the diet for 24 months. There was a

Section 8(e) Coordinator  
August 14, 1992  
Page 2

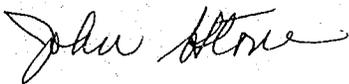
dose-related increased incidence of malignant haemangioendotheliomas in 100- and 500-ppm animals, and there was an early onset time at the high dose.

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

L205CCG0804JAS/RD17

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

cc: Mr. A. Di Battista

CGB-9-79

CIBA - GEIGY LIMITED  
BASLE / SWITZERLAND

TOXICOLOGY  
GU 2

"CONTAINS NO DATA"

N-FORMYL-4-CHLORO-O-TOLUIDINE  
LIFESPAN FEEDING STUDY IN MICE

FINAL REPORT

CGB-9-79

JUNE 7, 1978

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Toxicology

GU 2

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Toxicology

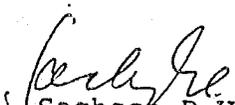
GU 2

LIFESPAN FEEDING STUDY IN MICE WITH N-FORMYL-4-CHLORO-O-TOLUIDINE  
FINAL REPORT

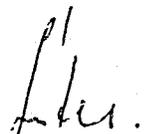
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Material: N-formyl-4-chloro-o-toluidine  
Subject: Lifespan feeding study - Mice  
Project No.: Siss M 4762/1

Study Director

  
K. Sachsse D.V.M.

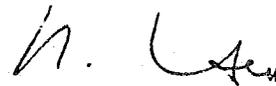
Responsible for the  
animal experimentation

  
P. Suter Ph.D.

Responsible for the  
pathology

  
F. Zak M.D.

Reviewed and approved by

  
R. Hess M.D.

June 7, 1978/stu

SUMMARY AND ASSESSMENT

N-formyl-4-chloro-o-toluidine was administered to mice continuously in their feed for a period of 24 months in a life-time carcinogenicity study.

Groups of 100 mice (50 males and 50 females) received 20 ppm (group 2), 100 ppm (group 3) and 500 ppm (group 4) - estimated to be for males 2.2, 11.1 and 57 mg/kg/day and for females 2.3, 10.8 and 62.1 mg/kg/day. An additional group of 100 mice served as control (group 1) receiving ground diet without test substance.

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

No toxic effects were observed during the 24-month application period.

Due to the occurrence of malignant tumours (haemangioendotheliomas) the survival rate of males and females of group 3 (100 ppm) and 4 (500 ppm) was impaired.

Food intake and body weight gain of the male and female mice of all groups were comparable.

The mean lifespan (in days of experiment) with standard deviation was calculated for the males and females of the different groups.

Group 1 0 ppm				Group 2 20 ppm				Group 3 100 ppm				Group 4 500 ppm			
m		f		m		f		m		f		m		f	
$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
649	180	647	221	635	211	589	219	544	160	531	145	404	72	401	82
								*		*		*		*	

\*) Statistically significant when compared to control group at a level of significance of 1 %.

Lit.: D. R. Cox. J. Roy. Stat. Soc. (Ser. B) 1972, p. 187-220  
J. D. Kalbfleisch and R. L. Prentice. Biometrika 1973, 60,  
p. 267-278

Toxicology  
GU 2

After the 2 years feeding period the surviving mice of each group and sex were killed after about 90 % of the total number of animals had succumbed. This occurred

in the males of group 1 at day 849  
in the females of group 1 at day 927  
in the males of group 2 at day 898  
in the females of group 3 at day 900 and  
in the males of group 3 at day 793.

At the end of their lifespan, mice from group 3 (100 ppm) and group 4 (500 ppm) revealed an increased incidence of haemorrhagic tissue masses upon gross examination in the subcutaneous tissue, the retroperitoneum and in some internal organs. These masses, histologically classified as malignant haemangioendotheliomas, were found in 41 mice (17 males and 24 females) of the 100 ppm group and in 78 mice (40 males and 38 females) of the 500 ppm group. In some animals the tumours were of multiple origin and in the occasional animals metastases of the neoplasms were found.

Furthermore, haemangioendotheliomas occurred also in 6 males and in one female of the 20 ppm group. One malignant haemangioendothelioma occurred also in one male control animal sacrificed after 753 days of experiment. The primary tumour was in the seminal vesicle and produced metastasis into the liver.

Apart from the dose dependent increase in the incidence of malignant haemangioendotheliomas in the treated mice there was an earlier appearance in the animals of the highest concentration level group. In this 500 ppm group the majority of mice bearing malignant haemangiomas died earlier than 450 days of treatment (30/40 males and 26/38 females). In the 100 ppm group only 3 out of the 41 mice (2/17 males and 1/24 females) bearing malignant haemangiomas died prior to the 500th day of the experiment. In the 20 ppm group and in the control all mice with this type of tumour were 2 years of age.

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CIBA-GEIGY LIMITED

Toxicology

GU 2

The incidence and the type of neoplasms other than malignant tumours of vascular origin noted in this study was not influenced by the treatment.

June 7, 1978/stu

Compound: N-formyl-4-chloro-o-toluidine

METHOD

Material N-formyl-4-chloro-o-toluidine  
Batch No. p 1  
Purity 99.7 %  
Description: white powder  
Received: April 75

Project No. Siss M 04762/1

Species Mice (Tif: MAG, SPF-bred)

Initial body weight 20 - 23 g

Initial age approx. 4 weeks

Husbandry air conditioned rooms behind barrier-system  
under SPF standard conditions  
temperature:  $22 \pm 1^{\circ}\text{C}$   
relative humidity:  $55 \pm 5 \%$   
10 hours light/day  
groups of 5 animals in Macrolon cages  
(size 3)

No. of animals/  
experimental group 50 ♂, 50 ♀

Route of  
Administration oral in the diet

Food pelleted standard diet (Nafag No. 890)  
ad libitum but not in excess of 10 g/day/  
animal

Water available at all times

Treatment Mixture 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 pre-  
pared fresh every two weeks. Aliquots of the  
pelleted treatment mixture were removed from  
the freezer every day to be offered to the  
test animals.

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

Compound: N-formyl-4-chloro-o-toluidine

Treatment Mixture	The animals in the control group (group 1) were fed with similarly pelleted food without compound.
Duration of Administration	24 months
Concentration of active ingredient (nominal)	20, 100, 500 ppm
Duration of the study	Life time or until 90 % of the animals per group and sex died (the latter refers to the period following the 24 months treatment)
<u>Observations and Records</u>	
Mortality	daily
Symptoms	signs of local and/or systemic toxicity - daily
Tumour incidence	external signs of tumour formation - weekly
body weight	weekly (first 3 months) monthly thereafter
Food consumption	daily, calculated weekly for individual animals.

Mean Food Conversion  
(g food/kg body weight/  
day)

was calculated according to the following formula:

$$MFC = \frac{\text{weekly food consumption (g)}}{\text{midweek body weight (g)}} \times \frac{100}{7}$$

### Statistical Analysis

For each time point and parameter a uni-variate statistical analysis was conducted. Due to the routine manner of the analysis system parameter free method 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: N-formyl-4-chloro-o-toluidine

## RESULTS

### Observations and Records

The food intake and the body weight gain of all treated mice was comparable to that of the controls. Some values showed statistical significant differences which we do not consider to be of any biological relevance. No clinical signs of toxicity were observed in the treated mice during the test period.

Mean substance intake on the basis of the analytical results per mouse and day (mg/kg) is given on pages 10 and 11.

Mortality rates for the different groups are presented graphically for males and females on pages 104 and 105. With the exception of the 100 ppm and the 500 ppm group there was no significant difference ( $p < 0.01$ ) in the mortality rate between the treated animals and the controls.

D O S A G E L E V E L S  
=====

MG SUBST./KG BW/DAY

SPECIES : MOUSE MAG(=NMRI)  
SEX : MALE

EXP. NO. : M04762/1  
COMPOUND : N-FORM.-4-CHL.-0-

WEEK	DOSE IN PPM			500.000		
	20.000	100.000	500.000	20.000	100.000	500.000
	%	MEAN	%	MEAN	%	MEAN
3	82.0	2.83	63.0	10.82	109.0	90.83
14	61.8	1.82	65.7	9.84	81.0	58.94
25	76.5	2.31	75.3	12.24	72.6	61.44
29	76.3	2.22	77.0	12.81	80.0	65.33
41	92.5	2.38	59.3	7.62	71.0	48.04
57	70.0	1.74	75.0	9.96	75.0	47.86
65	95.0	2.08	80.0	10.81	74.0	43.33
73	73.0	1.74	75.0	10.36	65.0	40.62
85	103.0	2.35	89.0	14.72		
97	85.5	2.33	74.7	11.82		
MEAN	81.6	2.18	73.4	11.10	78.4	57.05

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

EXP. NO. : M04762/1  
 COMPOUND : N-FORM.-4-CHL.-0-10

MG SUBST./KG BW/DAY

SPECIES : MOUSE MAG(=NMRI)  
 SEX : FEMALE

WEEK	DOSE IN PPM			500.000			
	20.000	100.000	500.000	%	MEAN	%	MEAN
3	82.0	2.91	63.0	11.71	109.0	94.15	
14	61.8	1.78	65.7	9.79	81.0	58.15	
25	76.5	2.03	75.3	10.34	72.6	60.47	
29	76.3	1.91	77.0	11.42	80.0	62.91	
41	92.5	2.52	59.3	7.81	71.0	45.96	
57	70.0	1.89	75.0	9.07	75.0	59.47	
65	95.0	2.38	80.0	9.68	74.0	68.12	
73	73.0	2.31	75.0	10.83	65.0	47.67	
85	103.0	2.44	89.0	13.41			
97	85.5	2.55	74.7	13.73			
MEAN	81.6	2.27	73.4	10.78	78.4	62.11	

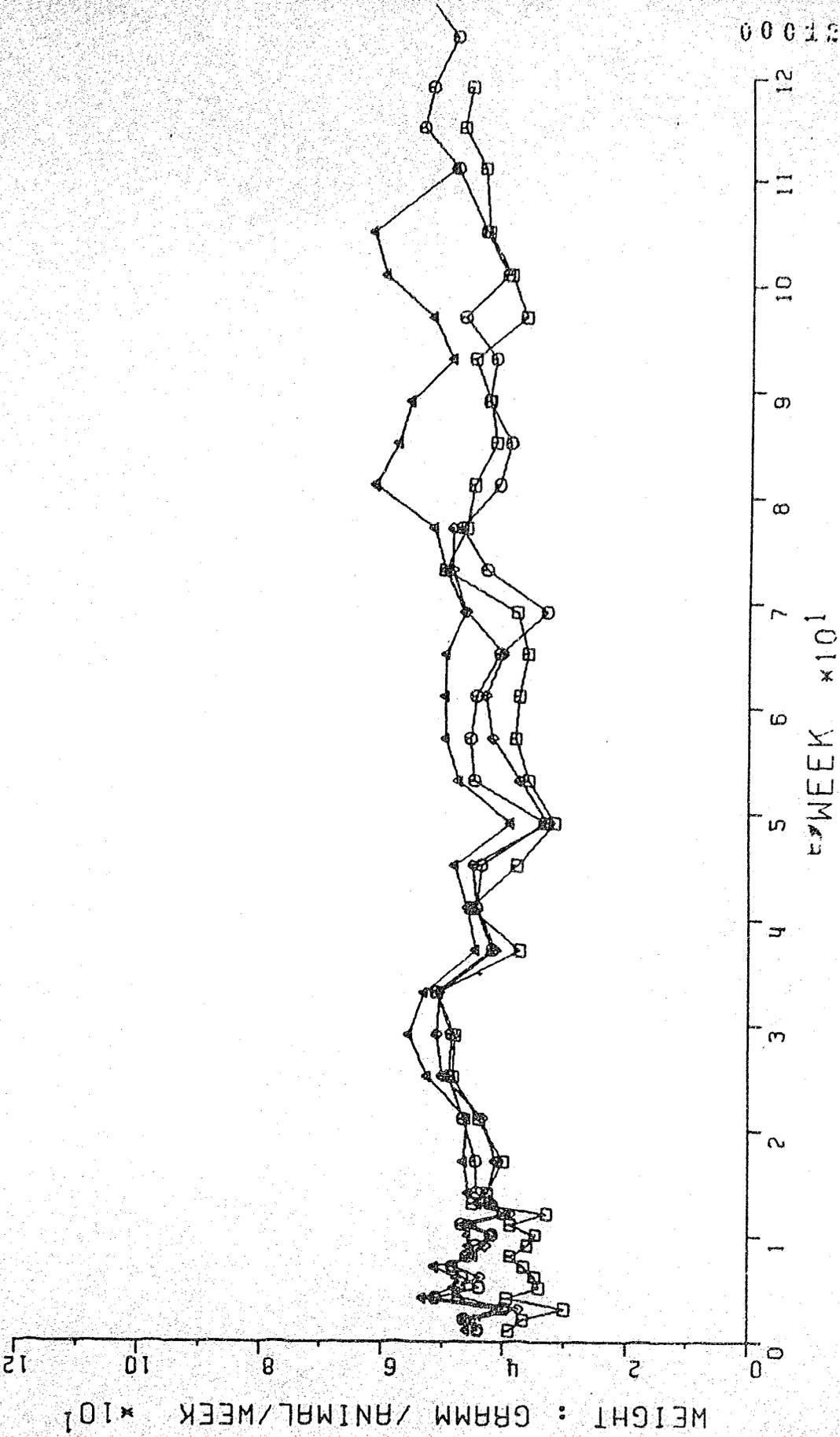
% = PERCENTAGE OF ACTUAL AMOUNT OF SUBSTANCE  
 (FOUND BY CHEMICAL ANALYSIS)

M E A N F O O D C O N S U M P T I O N

=====  
 SPEC.: MOUSE MAG (= EXP.: M04762/1  
 SEX.: MALE  
 COMP.: N-FORM.-4-CHL.-0-TOL

□ GROUP 1 = 0.00 PPM  
 ○ GROUP 2 = 20.00 PPM

▲ GROUP 3 = 100.00 PPM  
 ◇ GROUP 4 = 500.00 PPM

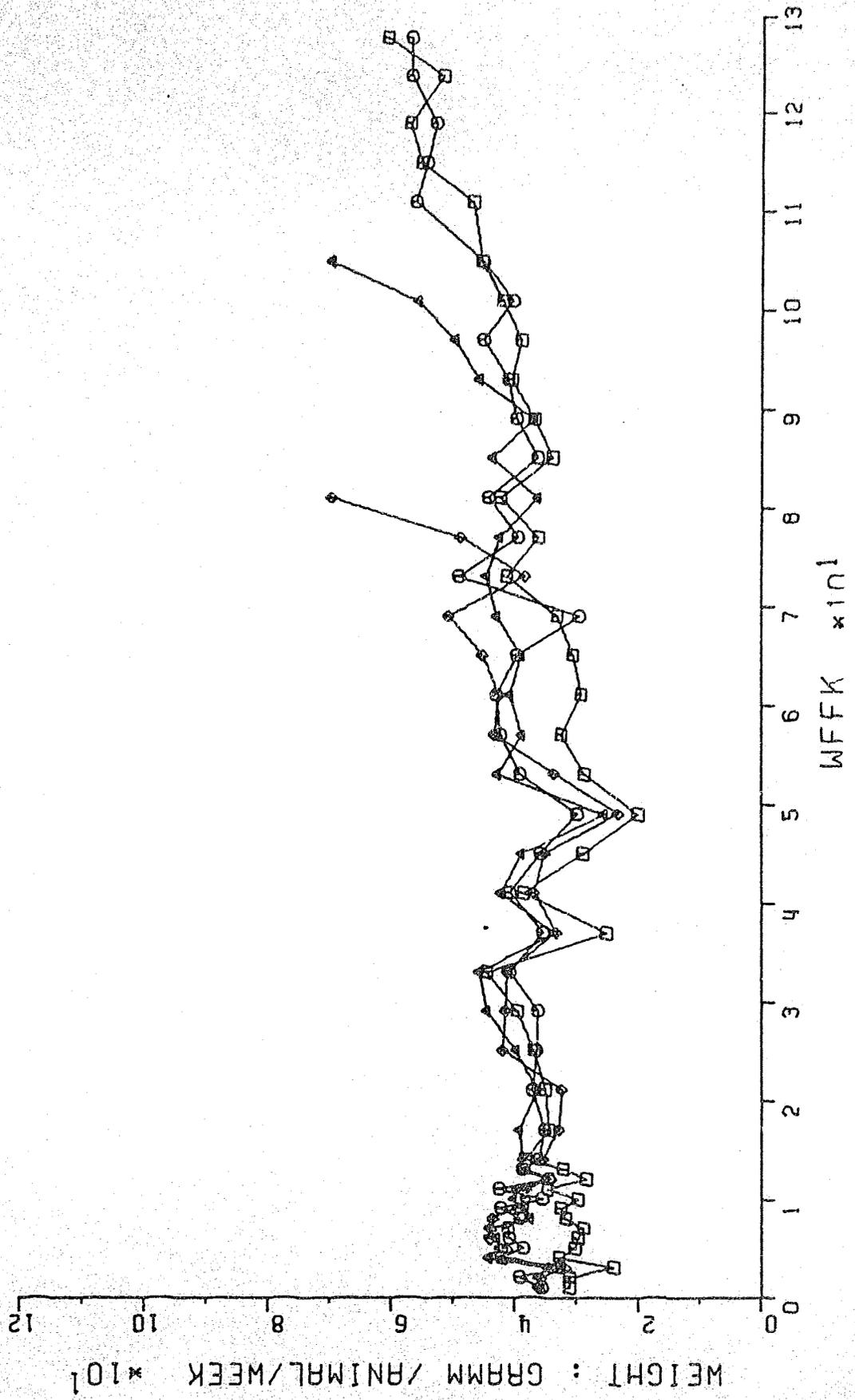


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M E A N F O O D C O N S U M P T I O N

=====  
SPEC.: MOUSE MAG 1 = EXP. : M04762/1  
SEX.: FEMALE  
COMP.: N-FORM.-4-CHL.-0-TOL

GROUP 1 = 0.00 PPM  
GROUP 2 = 20.00 PPM  
GROUP 3 = 100.00 PPM  
GROUP 4 = 500.00 PPM



Compound: N-formyl-4-chloro-o-toluidine

Hist.No.: EX 235

## PATHOLOGY

### Method

#### Macroscopical and Histological Examination:

Detailed autopsy, unless advanced autolysis and cannibalism prevented it, was carried out in all mice from the control and all treated groups which died or were sacrificed during the test period.

Small tissue portions of heart, aorta, trachea, lungs, spleen, lymph nodes, salivary glands, stomach, small and large intestine, liver, pancreas, adrenals, kidneys, urinary bladder, testes, epididymis, prostate, seminal vesicles, coagulating glands or ovaries and uterus, striated muscle, skin, bone with bone marrow, eye, Harderian gland, brain and any tissue with signs of possible tumour formation or other abnormalities were fixed in buffered 4 % neutral formalin.

The fixed tissue samples were embedded in paraffin wax and sectioned at 3 - 5  $\mu$ . The routine stain was haematoxylin and eosin. The paraffin sections from brain were stained with cresylviolet and those from the liver and spleen were additionally stained for iron by the Perl's method. Additional frozen sections from liver were specifically stained for fat with Sudan III.

### Results

#### Macroscopical Findings

All details regarding the gross anatomical changes are listed among the macroscopical findings in individual mice.

There were numerous gross anatomical lesions including nodules and tumorous masses seen in both treated and control mice, especially in those that survived longer than one year of age. Increased number of haemorrhagic masses was seen in the subcutaneous tissue, in the retroperitoneum and in some internal organs in mice from the 20 ppm, 100 ppm and 500 ppm groups. Other lesions occurred in comparable numbers among both controls and test animals.

Compound: N-formyl-4-chloro-o-toluidine

00107

### Microscopical Findings

All details regarding the histological changes are listed in microscopical findings in individual mice. The incidence of various tumours and the number of tumour bearing and tumour free mice in all the groups of this study are tabulated on pages 263 to 266.

In mice from 20 to 500 ppm concentration level groups an increased incidence of haemorrhagic malignant tumours of vascular origin in the subcutaneous tissue, in the retroperitoneum and in some organs was observed upon histological examination. These tumours, histologically classified as malignant haemangioendotheliomas, were found in 7 mice (6 males and 1 female) of the 20 ppm group, in 41 mice (17 males and 24 females) of the 100 ppm group and 98 mice (40 males and 38 females) of the 500 ppm group. In some animals the tumours were of multiple origin and occasionally metastases were found. This type of tumour is rarely encountered in aged mice from our breeding colony. In the present study it occurred only in one control animal (41 m).

There was not only a dose dependant increase in the incidence of malignant haemangioendotheliomas in the treated mice from the 20 to 500 ppm groups but these tumours occurred in animals from the highest concentration level group (500 ppm) markedly earlier than in mice from the intermediate (100 ppm) and low (20 ppm) concentration level groups. In the 500 ppm group the majority of mice bearing malignant haemangioma died earlier than after 450 days of the treatment (30/30 males and 26/38 females). In the 100 ppm group only 4 out of 41 (2/17 males and 1/24 females) mice with malignant haemangiomas died before the 500th day of the treatment. In the 20 ppm group all mice with this type of malignant vascular tumour were older than 2 years.

The benign variant of this tumour (benign haemangioma) was seen in 19 animals: 11 mice (3 males and 8 females) were from the control, 4 mice (1 male and 3 females) from the 20 ppm, 3 mice (2 males and 1 female) from the 100 ppm and 1 mouse (1 male) from the 500 ppm concentration group.

The vascular tumours (haemangiomas and haemangioendotheliomas) of the type that occurred in the mice of the present study appear to be peculiar to this rodent species. The haemangiomas classified as benign, although without the overt characteristics of malignancy, are nevertheless locally invasive. For the assessment of the total incidence of vascular tumours it may appear justified therefore to group both the benign and the malignant variety together.

Apart from significantly higher incidence of malignant vascular tumours in mice treated with 20, 100 and 500 ppm frequency and type of other neoplasms occurring in this study were not influenced by the treatment.

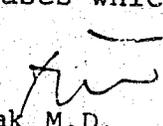
Compound: N-formyl-4-chloro-o-toluidine

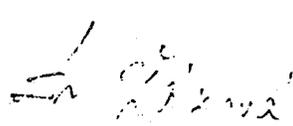
So called "Potter lesions" occurred in the lymph nodes of 18 mice. Eight animals (4 males and 4 females) were from the control, 6 (3 males and 3 females) from the 20 ppm, 1 animal (1 female) from the 100 ppm and 3 mice (1 male and 2 females) from the 500 ppm concentration group. The youngest animal showing Potter lesion in its lymph nodes was a male from the control group died at the 193rd experimental day. The incidence of Potter lesions was not influenced by the treatment.

In 1943 Potter and his coworkers described these lesions in lymph nodes as "histological changes preceding spontaneous lymphatic leukaemia in mice". The neoplastic nature of this affection has never been proved (Dunn, 1954), however. The peculiar histopathology, showing localised reticulum cell hyperplasia, is discussed in the comprehensive papers of Dunn (1954) and Robb-Smith (1938) along with reticulum cell neoplasms although it presents quite a distinct entity. In our experience, the Potter lesion as encountered in mice used in the present study is a benign affection pertained to particular lymph nodes and showing neither local progression nor generalisation.

These lesions do not increase in incidence with age and there is no relation to lymphatic leukaemia. In our opinion and that of others (Dunn, 1954 and Stewart, 1976) the nature of Potter lesion is hyperplastic rather than neoplastic. For reasons of histological consistency alone Potter lesion was included in the table of total tumour incidence.

A variety of microscopical lesions, found at a similar frequency among both control and test animals and described as degenerative or inflammatory in nature are attributed to naturally occurring diseases which are common in aged mice.

  
F. Zak M.D.  
Section Head Pathology

  
N. Zakova M.D.  
Pathologist

#### REFERENCES

- Dunn, T.B.: Normal and pathologic anatomy of the reticular tissue in laboratory mice, with a classification and discussion of neoplasms. J. Natl. Cac. Inst. 14, 181-1433, 1954
- Potter, J.H., J. Victor, E.N. War:  
Histological changes preceding spontaneous lymphatic leukaemia in mice.  
Am. J. Path. 19, 239-253, 1943.
- Robb-Smith, A.H.T.:  
Reticulosis and reticulosarcoma. A histological classification.  
J. Path. and Bact. 47, 457-480, 1938.
- Stewart, H.L. (1976): Personal communication.

Compound: N-formyl-4-chloro-o-toluidine

GENERAL OCCURRENCE OF TUMOURS AT AUTOPSIES DURING 24 MONTHS OF THE STUDY

	Group 1 0 ppm			Group 2 20 ppm			Group 3 100 ppm			Group 4 500 ppm			Total		
	m	f	m+f	m	f	m+f	m	f	m+f	m	f	m+f	m	f	m+f
Total number of animals in group	50	50	100	50	50	100	50	50	100	50	50	100	50	50	100
Died or sacrificed up to 2 years	29	30	59	30	36	66	43	49	92	50	50	100	50	50	100
Autopsy not performed	4	3	7	1	3	4	2	7	9	3	2	5	3	2	5
Animals with 1 tumour	9	8	17	6	8	14	16	21	37	30	38	68	38	68	106
Animals with 2 tumours	4	1	5	3	3	6	7	6	13	11	4	15	11	4	15
Animals with 3 tumours	1	-	1	-	1	1	1	2	3	3	-	3	3	-	3
Animals with 4 tumours	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-
Number of tumour bearing mice	14	9	23	9	12	21	25	29	54	44	42	86	44	42	86
Animals without tumours	11	18	29	20	21	41	16	13	29	3	6	9	3	6	9

00101

Compound: N-formyl-4-chloro-o-toluidine

TUMOUR INCIDENCE IN MICE DIED OR SACRIFICED DURING 24 MONTHS OF THE STUDY

	Group 1 0 ppm			Group 2 20 ppm			Group 3 100 ppm			Group 4 500 ppm			Total		
	m	f	m+f	m	f	m+f	m	f	m+f	m	f	m+f	m	f	m+f
	Haemangioendothelioma malignant	-	-	-	-	-	-	15	23	38	40	38	78	55	61
Haemangioendothelioma benign	2	3	5	-	-	-	1	1	2	1	-	1	4	4	8
Reticulumcellsarcoma generalised	2	1	3	-	1	1	-	1	1	1	1	2	3	4	7
Reticulumcellsarcoma localised	7	-	-	-	1	1	-	-	-	-	-	-	-	-	1
Lymphoreticular lymphoma, lymph nodes	5	-	5	2	1	3	1	2	3	2	2	4	10	5	15
Lymphatic leukaemia	1	1	2	-	1	1	1	2	3	1	1	2	3	5	8
Myeloid leukaemia	-	-	-	-	1	1	-	-	-	-	-	-	-	1	1
Anaplastic sarcoma	1	-	1	-	-	-	-	-	-	-	-	-	1	-	1
Fibrosarcoma	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
Fibroleiomyoma, uterus	-	-	-	-	1	1	-	-	-	-	-	1	1	-	1
Lymphangioma	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Potter's lesion*, Lymph nodes	1	2	3	3	3	6	-	1	1	1	2	3	5	8	13

\*) Lymphohistiocytic reticulosis

Compound: N-formyl-4-chloro-o-toluidine

	Group 1 0 ppm			Group 2 20 ppm			Group 3 100 ppm			Group 4 500 ppm			Total		
	m	f	m+f	m	f	m+f	m	f	m+f	m	f	m+f	m	f	m+f
Interstitial cell tumour, testicle	1	-	1	-	-	-	-	-	-	-	-	-	1	-	1
Granulosa cell tumour, ovary	-	1	1	-	-	-	-	1	1	-	-	-	-	2	2
Hepatocellular carcinoma	1	-	1	-	-	-	-	1	1	1	1	1	2	1	3
Hepatoma	3	-	3	1	2	3	7	4	11	9	20	9	6	26	
Adenoma, lungs	1	1	2	4	4	8	8	2	10	2	1	3	15	8	23
Adenoma, Harderian's gland	2	1	3	1	2	3	3	1	4	1	1	2	7	5	12
Adenoma, lacrimal gland	-	-	-	-	-	-	1	-	1	-	-	-	1	-	1
C-cell adenoma, thyroid	-	-	-	1	-	1	-	-	-	-	-	-	1	-	1
Total of all tumours	20	10	30	12	17	29	37	39	76	61	130	107	112	112	242

Compound: N-formyl-4-chloro-o-toluidine

Macroscopical Findings in Individual Mice  
(during 24 months of the study)

Group 1 - 0 ppm - Control

- 1 m Died on the 351th experimental day.  
Advanced autolysis, no autopsy performed.
- 3 m Sacrificed on the 703rd experimental day.  
Lymph nodes slightly enlarged.
- 6 m Died on the 657th experimental day.  
Liver with tumorous nodules. Abdominal lymph nodes enlarged, 1,5 x 1 x 1 cm (1 g).
- 10 m Died on the 646th experimental day.  
Lymph nodes markedly enlarged, especially submandibulary.  
Lungs with haemorrhages. Advanced autolysis. Partially eaten by the cage mates.
- 12 m Died on the 639th experimental day.  
Seminal vesicles enlarged.
- 13 m Died on the 235th experimental day.  
Advanced autolysis. No autopsy performed.
- 14 m Died on the 656th experimental day.  
In the liver: haemorrhagic nodules. In the abdominal cavity 5 ml of haemorrhagic fluid.
- 17 m Sacrificed on the 704th experimental day.  
No changes seen.
- 20 m Sacrificed on the 703rd experimental day.  
Spleen enlarged, 2 x 1 x 0,5 cm (0,7 g).
- 21 m Died on the 552nd experimental day.  
Spleen enlarged, 1,4 x 1 x 0,5 cm (0,7 g).

ADENDUM: GENERAL OCCURRENCE OF TUMOURS IN LIFESPAN STUDY WITH N-FORMYL-4-CHLORO-O-TOLUIDINE  
(after 24 months treatment period)

	Group 1 0 ppm		Group 2 20 ppm		Group 3 100 ppm		Group 4 500 ppm		Total		
	m	f	m	f	m	f	m	f	m	f	m+f
Total number of animals/group	50	50	50	50	50	50	50	50	200	200	400
Died or sacrificed up to 2 years	29	30	30	36	43	49	50	50	152	165	317
Died or sacrificed after 2 years	21	20	20	14	7	1	-	-	48	35	83
Autopsy not performed	4	1	1	-	2	-	-	-	7	1	8
Animals with 1 tumour	8	7	10	5	2	-	-	-	20	12	32
Animals with 2 tumours	5	6	6	7	3	1	-	-	14	14	28
Animals with 3 tumours	1	4	2	-	-	-	-	-	3	4	7
Number of tumour bearing mice	14	17	18	12	5	1	-	-	37	30	67
Animals without tumours	3	2	1	2	-	-	-	-	4	4	8

ADENDUM: TUMOUR INCIDENCE IN A LIFESPAN FEEDING STUDY WITH N-FORMYL-4-CHLORO-O-TOLUIDINE IN MICE

(after 24 months treatment period)

	Group 1 0 ppm		Group 2 20 ppm		Group 3 100 ppm		Group 4 500 ppm		Total		
	m	f	m	f	m	f	m	f	m	f	m+f
Haemangioendothelioma, malignant	1	-	6	1	2	1	-	-	9	2	11
Haemangioma, benign	1	5	1	3	1	-	-	-	3	8	11
Fibroma	1	-	-	-	-	-	-	-	1	-	1
Reticulum cell sarcoma	2	2	4	1	1	-	-	-	7	3	10
Lymph node: lymphoreticular lymphoma	1	2	-	3	-	-	-	-	1	5	6
Lymph node: Potter lesion	3	2	-	-	-	-	-	-	3	2	5
Fibrosarcoma	-	2	2	-	-	-	-	-	2	2	4
Ovary: benign tumours	-	3	-	1	-	-	-	-	-	4	4
Pituitary: adenoma	-	1	-	-	-	-	-	-	-	1	1
Harderian gland: adenoma	1	2	3	-	-	-	-	-	4	2	6
Harderian gland: carcinoma	-	-	-	1	-	-	-	-	-	1	1
Thyroid: follicular adenoma	-	-	1	-	-	-	-	-	1	-	1

ADENDUM: TUMOUR INCIDENCE IN A LIFESPAN FEEDING STUDY WITH N-FORMYL-4-CHLORO-O-TOLUIDINE IN MICE  
 (after 24 months treatment period)

	Group 1 0 ppm		Group 2 20 ppm		Group 3 100 ppm		Group 4 500 ppm		Total			
	m	f	m	f	m	f	m	f	m	f	m+f	
Thyroid: carcinoma	-	1	-	-	-	-	-	-	-	-	1	1
Lungs: adenoma	5	5	5	2	3	-	-	-	13	7	20	
Lungs: carcinoma	2	-	2	-	-	-	-	-	4	-	4	
Stomach: adenoma	-	-	-	1	-	-	-	-	-	-	1	1
Stomach: carcinoma	-	-	-	1	-	-	-	-	-	-	1	1
Liver: hepatoma	4	1	3	2	1	1	-	-	8	4	12	
Liver: carcinoma	-	-	1	-	-	-	-	-	1	-	1	1
Mammary gland: adenoma	-	-	-	2	-	-	-	-	-	-	2	2
Skin: basosquamous carcinoma	-	3	-	-	-	-	-	-	-	-	3	3
Skin: squamous cell carcinoma	-	-	-	1	-	-	-	-	-	-	1	1
Skin (adnexa): carcinoma	-	2	-	-	-	-	-	-	-	-	2	2
Total of all Tumours	21	31	28	19	8	2	-	-	57	52	109	

TOTAL GENERAL OCCURRENCE OF TUMOURS IN LIFESPAN STUDY WITH N-FORMYL-4-CHLORO-O-TOLUIDINE

	Group 1 0 ppm		Group 2 20 ppm		Group 3 100 ppm		Group 4 500 ppm		Total		
	m	f	m	f	m	f	m	f	m	f	m+f
Total number of animals/group	50	50	50	50	50	50	50	50	200	200	400
Died or sacrificed up to 2 years	29	30	30	36	43	49	50	50	152	165	317
Died or sacrificed after 2 years	21	20	20	14	7	1	-	-	48	35	83
Autopsy not performed	8	4	2	3	4	7	3	2	17	16	33
Animals without tumours	14	20	21	23	16	13	3	6	54	62	116
Animals with 1 tumour	17	15	16	13	18	21	30	38	81	87	168
Animals with 2 tumours	9	7	9	10	10	7	11	4	39	28	67
Animals with 3 tumours	2	4	2	1	1	2	3	-	8	7	15
Animals with 4 tumours	-	-	-	-	1	-	-	-	1	-	1
Number of tumour bearing mice	28	26	27	24	30	30	44	42	129	122	251

00263

TOTAL TUMOUR INCIDENCE IN A LIFESPAN FEEDING STUDY WITH N-FORMYL-4-CHLORO-O-TOLUIDINE IN MICE

	Group 1 0 ppm		Group 2 20 ppm		Group 3 100 ppm		Group 4 500 ppm		Total		
	m	f	m	f	m	f	m	f	m	f	m+f
Haemangi endothelioma, malignant	1	-	6	1	17	24	40	38	64	63	127
Haemangioma, benign	3	8	1	3	2	1	1	-	7	12	19
Lymphangioma	-	-	-	-	-	-	1	-	1	-	1
Fibroma	1	-	-	-	-	-	-	-	1	-	1
Fibroleiomyoma	-	-	-	1	-	-	-	-	-	-	1
Reticulum cell sarcoma	4	3	4	3	1	1	1	1	10	8	18
Lymph node: lymphoreticular lymphoma	6	2	2	4	1	2	2	2	11	10	21
Lymph node: Potter lesion	4	4	3	3	-	1	1	2	8	10	18
Lymphatic leukaemia	1	1	-	1	1	2	1	1	3	5	8
Myeloid leukaemia	-	-	-	1	-	-	-	-	-	1	1
Fibrosarcoma	-	2	2	-	-	-	1	-	3	2	5
Anaplastic sarcoma	1	-	-	-	-	-	-	-	1	-	1

00264

TOTAL TUMOUR INCIDENCE IN A LIFESPAN FEEDING STUDY WITH N-FORMYL-4-CHLORO-O-TOLUIDINE IN MICE

	Group 1 0 ppm		Group 2 20 ppm		Group 3 100 ppm		Group 4 500 ppm		Total		
	m	f	m	f	m	f	m	f	m	f	m+f
Testicle: interstitial cell tumour	1	-	-	-	-	-	-	-	1	-	1
Ovary: benign tumours	-	4	-	1	-	1	-	-	-	6	6
Pituitary: adenoma	-	1	-	-	-	-	-	-	-	1	1
Lacrimal gland: adenoma	-	-	-	-	1	-	-	-	1	-	1
Harderian gland: adenoma	3	3	4	2	3	1	1	1	11	7	18
Harderian gland: carcinoma	-	-	-	1	-	-	-	-	-	1	1
Thyroid: c-cell adenoma	-	-	1	-	-	-	-	-	1	-	1
Thyroid: follicular adenoma	-	-	1	-	-	-	-	-	1	-	1
Thyroid: carcinoma	-	1	-	-	-	-	-	-	-	1	1
Lungs: adenoma	6	6	9	6	11	2	2	1	28	15	43
Lungs: carcinoma	2	-	2	-	-	-	-	-	4	-	4

TOTAL TUMOUR INCIDENCE IN A LIFESPAN FEEDING STUDY WITH N-FORMYL-4-CHLORO-O-TOLUIDINE IN MICE

	Group 1 0 ppm		Group 2 20 ppm		Group 3 100 ppm		Group 4 500 ppm		Total		
	m	f	m	f	m	f	m	f	m	f	m+f
Stomach: adenoma	-	-	-	1	-	-	-	-	-	1	1
Stomach: carcinoma	-	-	-	1	-	-	-	-	-	1	1
Liver: hepatoma	7	1	4	4	8	5	9	-	28	10	38
Liver: carcinoma	1	-	1	-	-	1	1	-	3	1	4
Mammary gland: adenoma	-	-	-	2	-	-	-	-	-	2	2
Skin: basosquamous carcinoma	-	3	-	-	-	-	-	-	-	3	3
Skin: squamous cell carcinoma	-	-	-	1	-	-	-	-	-	1	1
Skin (adnexa): carcinoma	-	2	-	-	-	-	-	-	-	2	2
Total number of tumours	41	41	40	36	45	41	61	46	187	164	351

Compound: N-formyl-4-chloro-o-toluidine

Macroscopical Findings in Individual Mice  
(after 24 months)

Group 1 - 0 ppm - Control

- 2 m Died on the 783rd experimental day.  
Lungs mottled, spleen enlarged. On the right side of the neck skin swollen with small ulcer.
- 4 m Sacrificed on the 843rd experimental day.  
Liver mottled, with nodular structure. Seminal vesicles enlarged.
- 5 m Died on the 817th experimental day.  
On the left side of the neck small nodule in the subcutis with ulceration of the overlying skin.
- 7 m Died on the 835th experimental day.  
Liver and lungs mottled.
- 8 m Died on the 827th experimental day.  
The animal was partially eaten by the cage mates. Skin on the left side of the head thickened. In the liver nodules.
- 9 m Sacrificed on the 831st experimental day.  
Lungs with nodules. In the heart whitish firm nodules.
- 11 m Sacrificed on the 849th experimental day.  
No changes seen.
- 15 m Died on the 769th experimental day.  
Skin on the neck swollen, with focal alopecia. Cervical lymph nodules enlarged. Lungs with whitish nodules. Left testicle mottled.
- 16 m Sacrificed on the 849th experimental day.  
Renal pelvis of the left kidney dilated, seminal vesicle enlarged with stone.
- 18 m Died on the 806th experimental day.  
Liver mottled, skin on the back thickened with alopecia. Advanced autolysis.
- 19 m Died on the 743rd experimental day.  
Advanced autolysis. No autopsy performed.