

**8EHQ-0895-1177**

Certified Mail P 633 101 653  
Return Receipt Requested



August 3, 1995

RECEIVED  
OPPT NCIC  
95 AUG 10 PM 3:33

OPPT Document Processing Center (TS-790)  
Attn.: Section 8(e) Coordinator  
Office of Pollution Prevention and Toxics (OPPT)  
U. S. Environmental Protection Agency  
401 M Street, SW  
Washington, DC 20460

**ORIGINAL**

**Contains No CBI**

Ladies and Gentlemen:

Subject: Supplement to EPA Document Control Number 8EHQ-0291-1177,  
Iron Pentacarbonyl

Enclosed please find the final report for the 28-day inhalation study of iron pentacarbonyl which was the subject of the referenced submission.

The report is entitled "Study on the Inhalation Toxicity of Eisenpentacarbonyl as a Vapor in Rats - 28 Day Test."

If you have any questions, please call me at 313-246-6211.

Very truly yours,

BASF CORPORATION

Daniel C. Steinmetz  
Product Stewardship and Product Safety



mk  
Enc.

RECEIVED  
OPPT NCIC  
95 AUG 22 AM 8:33

D-W6700 Ludwigs-  
hafen, FRG

kli-db; 1885

JUL 24 1995

**REPORT**

Study on the inhalation toxicity  
of

**EISENPENTACARBONYL**

as a vapor in rats  
28-day test

Project No.: 46I0094/90021

Report

VOLUME I

**Contains No CBI**

This report consists of Volume I (Report), Volume II (Tables),  
Volume III (Pathology) and Volume IV (Supplement).

*not CBI.  
Per Submitter  
EAS*

Dieses Dokument enthält Betriebs- und Geschäftsgeheimnisse der  
BASF. Es ist Eigentum der BASF und darf nur zu dem von BASF  
vorgesehenen Zweck verwendet werden. Jede andere oder darüber  
hinausgehende Verwendung, Verwertung, Weitergabe, Vervielfäl-  
tigung oder Veröffentlichung bedarf der Einwilligung der BASF.

This document contains manufacturing and trade secrets of BASF.  
It is the property of BASF and may be used only for that pur-  
pose for which it was intended by BASF. Every other or addition-  
al use, exploitation, reproduction, publication or submission  
to other parties require the written permission of BASF.

Report; Project No.: 46I0094/90021

---

From the Department of Toxicology of  
BASF Aktiengesellschaft, D-W6700 Ludwigshafen, FRG  
Head: Prof. Dr.med. Dr.rer.nat. H.-P. Gelbke

Study Director  
(conduct of study, summary  
and editing):

*Klimisch, July 18, 1995*  
.....  
Dr.rer.nat. H.-J. Klimisch

Clinical chemistry and  
hematology:

*Deckardt, July 17, 1995*  
.....  
Dr.phil.nat. K. Deckardt

Pathology:

*Bahlemann, July 18, 1995*  
.....  
Dr.med.vet. R. Bahlemann

Head of Experimental  
Toxicology:

*Hildebrand, 18. VII. 95*  
.....  
Dr.med.vet. B. Hildebrand

Report; Project No.: 46I0094/90021

---

**GLP STATEMENT**

**Title of study:** Study on the inhalation toxicity of Eisenpenta-  
carbonyl as a vapor in rats, 28-day test

**Project No.:** 46I0094/90021

This study was conducted in accordance with the GLP-provisions of the "Chemikaliengesetz" (Chemicals Act; Bundesgesetzblatt 1990, Teil I, 22.03.90; FR Germany) and with the "OECD Principles of Good Laboratory Practice" (Paris, 1981).

*M. Meier* 18. IV. 95  
.....  
(Head of Experimental  
Toxicology)

*H. M. ...* July 18, 1995  
.....  
(Study Director)

Report; Project No.: 46I0094/90021

**STATEMENT  
 OF THE QUALITY ASSURANCE UNIT**

Number of test substance: 90/94

Name of test substance: Eisenpentacarbonyl

Title: Study on the inhalation toxicity of Eisenpentacarbonyl as a vapor in rats, 28-day test

The Quality Assurance Unit inspected the study, audited the final report, and reported findings to the Study Director and to Management.

Phase of study/inspection	Date of inspection	Report to Study Director and to Management
Protocol:	July 26, 1990	Jan. 4, 1991
Conduct of study:	Dec. 19, 1990	Jan. 4, 1991
	Dec. 20, 1990	Jan. 4, 1991
	Jan. 3, 1991	Jan. 4, 1991
Audit of the report:	May 5, 1993	May 5, 1993

Remarks: Parts of analytics were inspected independently by the Quality Assurance Unit of the analytical laboratory.

Ludwigshafen/Rhein.

*July 21, 1995* *U. Wandelt*

.....  
 U. Wandelt  
 (Quality Assurance Unit)

**CONTENTS**

	Page
- Volume I -	
TITLE PAGE	I
SIGNATURES	II
GLP STATEMENT	III
STATEMENT OF THE QUALITY ASSURANCE UNIT	IV
CONTENTS	V - XII
1. SUMMARY	1
- Scope of examinations	1
- Results	2
2. INTRODUCTION	3
- Aim of the study	3
- Selection of concentrations	3
- Guidelines; dates of the study	4
3. MATERIAL AND METHODS	5
3.1. Test substance	5
3.2. Test animals	6
3.3. Housing, diet and drinking water	7
3.4. Test groups, concentrations and time table	8
3.5. Experimental procedure	9
3.5.1. Generation of an inhalation atmosphere	9
3.5.2. Exposure system; exposure of the animals	10
3.5.3. Measurement of the operation conditions (air flows, relative humidity, temperature and pressure conditions) of the exposure system	11
3.6. Analytical investigations of inhalation chamber atmospheres	12
3.7. Clinical examinations	13
3.7.1. Body weight	13
3.7.2. Body weight change	13
3.7.3. Clinical signs and findings	13
3.7.4. Lethality	13

Report; Project No.: 46I0094/90021

	Page	
3.8.	Clinical chemistry, hematology and urinalyses	14
3.8.1.	Hematological examinations	15
3.8.2.	Carboxyhemoglobin	17
3.8.3.	Clotting analyses	17
3.8.4.	Clinicochemical examinations	17
3.8.5.	Urinalyses	20
3.9.	Pathology (see separate pathology report, Volume III)	22
3.10.	Statistical evaluation	22
3.10.1.	Clinical examinations	22
3.10.2.	Clinical chemistry and hematology	22
3.10.3.	Urinalyses	23
4.	<b>RESULTS AND ASSESSMENT</b>	24
4.1.	Clinical examinations	24
4.1.1.	Body weight	25
4.1.2.	Body weight change	25
4.1.3.	Lethality (exposure- and post-exposure observation period)	25
4.1.4.	Clinical signs and findings (preflow-, exposure- and post-exposure observation period)	26
4.1.5.	Assessment of clinical examinations	28
4.2.	Results: concentration measurements and measured values of the operation conditions of the exposure and generator systems	29
4.2.1.	Concentration monitoring with the IR-Spectrometer	29
4.2.2.	Operation conditions in the exposure systems	29
4.2.3.	Operation conditions in the generator system	30
4.3.	Clinical chemistry, hematology and urinalyses	31
4.3.1.	Hematological examinations	32
4.3.2.	Clotting analyses	32
4.3.3.	Enzymes	32
4.3.4.	Blood chemistry	32
4.3.5.	Urinalyses	33
4.3.6.	Discussion and conclusion	33
4.4.	Pathology (see separate pathology report, Vol. III)	33
5.	<b>RETENTION OF RECORDS</b>	34
6.	<b>LITERATURE</b>	35

Report; Project No.: 46I0094/90021

---

ANNEX: Summary Tables Table

**CLINICAL EXAMINATIONS**

Key to the abbreviations, page 37

**Body weights** 001 - 004  
- Test groups: 0, 1, 2, 4 and E

**Body weight change** 005 - 008  
- Test groups: 0, 1, 2, 4 and E

**CLINICAL CHEMISTRY, HEMATOLOGY AND URINALYSES**

Key to the abbreviations, page 39 - 40

**Hematological examinations** 009 - 010  
- males 011 - 012  
- females

**Differential blood count** 013 - 016  
- males 017 - 020  
- females

**Reticulocytes** 021 - 022  
- males 023 - 024  
- females

**Carboxyhemoglobin** 025  
- males 026  
- females

**Clotting analyses** 027 - 028  
- males 029 - 030  
- females

**Enzymes** 031 - 032  
- males 033 - 034  
- females

**Blood chemistry** 035 - 038  
- males 039 - 042  
- females

**Urinalyses** 043 - 044  
- males 045 - 046  
- females

**FIGURES**

generator system figure 1 and 2  
exposure system figure 3

**ANALYTICAL INVESTIGATIONS OF INHALATION CHAMBER  
ATMOSPHERES**

The individual values may be found in Volume II.

All data on PATHOLOGY may be found in the separate

Report; Project No.: 4610094/90021

---

**CONTENTS**

- Volume II -

Page

TITLE PAGE  
 CONTENTS

I  
 II - IV

**A CLINICAL EXAMINATIONS**

Table

Individual values

Key to the abbreviations, page A2

**Body weight**

- males

- females

A 001 - A 006  
 A 007 - A 012

**B CLINICAL CHEMISTRY, HEMATOLOGY AND URINALYSES**

Key to the abbreviations, pages B2 - B7

Tables

**Hematological examinations**

- individual values

- male animals

- female animals

B 001 - B 002  
 B 003 - B 004

**Differential blood count**

- individual values

- male animals

- female animals

B 005 - B 008  
 B 009 - B 012

**Reticulocytes**

- individual values

- male animals

- female animals

B 013 - B 014  
 B 015 - B 016

**Carboxyhemoglobin**

- individual values

- male animals

- female animals

B 017 - B 018  
 B 019 - B 020

Report; Project No.: 46I0094/90021

---

Table

**Clotting analyses**

- individual values
- male animals
- female animals

B 021 - B 022  
B 023 - B 024

**Enzymes**

- individual values
- male animals
- female animals

B 025 - B 026  
B 027 - B 028

**Blood chemistry**

- individual values
- male animals
- female animals

B 029 - B 032  
B 033 - B 036

**Urinalyses**

- individual values
- male animals
- female animals

B 037 - B 040  
B 041 - B 044

Report; Project No.: 46I0094/90021

---

**C ANALYTICAL DATA AND EXPOSURE PARAMETERS**

Key to the abbreviations, page C2

Daily means concentration (Fe(CO) <sub>5</sub> ) study means ± standard deviation	C 001
Daily means concentration (CO) study means ± standard deviation	C 002
Daily means chamber parameters, study means ± standard deviation	C 003 - C 008
Daily means generator parameters study means ± standard deviation	C 009

Report; Project No.: 46I0094/90021

---

**CONTENTS**

- Volume III -

	Page
TITLE PAGE	I
TABLE OF CONTENTS	1
MATERIAL AND METHODS	2 - 3
RESULTS	4 - 7
DISCUSSION	8
CONCLUSIONS	9
LITERATURE	10
LIST OF ABBREVIATIONS	11
ABSOLUTE WEIGHTS - MEAN VALUES (MALE)	12
ABSOLUTE WEIGHTS - MEAN VALUES (FEMALE)	13
RELATIVE WEIGHTS - MEAN VALUES (MALE)	14
RELATIVE WEIGHTS - MEAN VALUES (FEMALE)	15
INCIDENCE OF GROSS LESIONS	16 - 17
INCIDENCE OF MICROSCOPIC FINDINGS	18 - 19
ABSOLUTE WEIGHTS - INDIVIDUAL VALUES	20 - 29
RELATIVE WEIGHTS - INDIVIDUAL VALUES	30 - 39
SINGLE ANIMAL SHEET (GROSS LESIONS AND MICROSCOPIC FINDINGS)	40 - 99

**CONTENTS**

- Volume IV -

	Page
TITLE PAGE	I
CONTENTS	II

I      Analysis of the substance; report of Dec. 11, 1990  
 Reanalysis of the substance; report of Feb. 6, 1991

Report: Project No.: 4610094/90021.

1. **SUMMARY**

**Scope of examinations**

Eisenpentacarbonyl vapor (FEC; iron pentacarbonyl) was tested for its inhalation toxicity in Wistar rats. 5 female and 5 male rats per group were exposed to FEC-concentrations of 10 ppm, 3 ppm, 1 ppm, 0.3 ppm and 0.1 ppm 6 hours/day for a period of up to 28 days (20 exposures). Because of occurring lethality exposure was stopped after 1 or 2 treatments in test groups 10 and 3 ppm respectively. The surviving animals of these test groups were observed without further exposure. A control group of 5 female and 5 male rats inhaled clean air under similar exposure conditions. Target concentrations, number of animals, number of exposures and duration of post-exposure period are shown in the following table:

Test group (Target concentration ppm)	Number of animals		Number of exposures	Post-exposure period
	female	male		
0 (0)	5	5	20	-
4 (0.1)	5	5	20	-
E (0.3)	5	5	20	-
1 (1)	5	5	20	-
2 (3)	5	5	2	about 4 weeks
3 (10)	5	5	1	3 days*

\* all animals dead

FEC and carbonmonoxide (CO) concentrations in the inhalation chambers were analysed by spectrophotometry. Technical parameters of chamber atmospheres were recorded by an automated measuring system.

The body weights of the animals were determined weekly during the exposure- and post-exposure period. Clinical signs and findings were recorded in general before, during and after exposure on exposure days and once per working day in the preflow and post-exposure observation period. A check for dead animals was made daily.

Blood was taken from all animals at the end of the study. Numerous clinicochemical and hematological parameters and various enzyme activities were measured and a clotting time analysis was performed. During the exposure period blood was taken for COHB analysis. Urinalysis was performed. The animals were sacrificed; a complete necropsy including weighing of certain organs and a gross-pathologic evaluation was performed. Selected organs/tissues were examined histologically.

Report; Project No.: 4610894/90021

**Results**

Analytical concentration of FEC and CO in the atmospheres are given in the following table.

Test group	Target FEC concentration (ppm)	Measured FEC concentration $\pm$ SD (ppm)	Measured CO concentration $\pm$ SD (ppm)
0	-	-	-
4	0.1	0.10 $\pm$ 0.01	0.20 $\pm$ 0.30
E	0.3	0.30 $\pm$ 0.01	0.21 $\pm$ 0.31
1	1	1.00 $\pm$ 0.02	0.22 $\pm$ 0.29
2	3	2.91 $\pm$ 0.01	0.86 $\pm$ 0.07
3	10	9.85	4.87

Mean relative humidities in the atmospheres of test groups 0, 4, E and 1 ranged from 55 to 60%, mean chamber temperatures from 21.1 to 21.7°C.

One exposure to 10 ppm (test group 3) caused 100% lethality and 2 exposures to 3 ppm (test group 2) 50% lethality (3 male and 2 female animals) during a period of 4 days.

In test group 3 (10 ppm) the animals showed symptoms of respiratory distress and irritation as well as reduced general state. Pathological examinations revealed signs of irritation in the upper respiration tract and severe lung damage. In some animals lymphocyte depletion of the spleen occurred.

In test group 2 (3 ppm) the surviving animals showed accelerated respiration up to study day 9. No influence on body weight development was seen in the post-exposure observation period. No substance related changes in hematological or clinico-chemical parameters occurred. The same kind of lung damage as in test group 3 was found during pathological examination in the animals that died or were sacrificed prematurely. The surviving animals still showed increased absolute and relative lung weights and some histopathologic changes in the lungs at the end of the study period.

No substance related clinical signs and findings or influence on body weight development was seen in test groups 4, E and 1. No substance induced effects on hematological and clinicochemical parameters occurred besides a slight but mostly statistically significant elevation of CO-hemoglobin levels which is not considered as of toxicological relevance. Pathological evaluation also did not reveal any morphological alterations besides a slight but statistically significant increase in absolute and relative lung weights of the male animals of test group 1 ppm.

**Conclusions:**

Concentrations of 10 and 3 ppm of FEC vapours inhaled 6 hours per working day in this 28-day study caused considerable lethality probably by severe damage to the lungs already after 1 or 2 exposures. At 1 ppm the only effect that might possibly be related to the substance exposure was an increase in absolute and relative lung weights of male animals at the end of the study period. Concentrations of 0.3 ppm or below did not cause toxic effects.

The "No Observed Adverse Effect Concentration" (NOAEC) therefore is judged to be 0.3 ppm under the conditions of this test.

Report: Project No.: 46I0094/90021

## 2. INTRODUCTION

### Aim of the study

Only few information and toxicity data on iron penta-carbonyl (Eisenpentacarbonyl FEC) are published in the literature. (c.f. Selection of concentration). Dose-response-relationships and a no-observed-adverse effect-concentration were not determined. It is the aim of the study to characterize the toxic potential and toxic potency of Eisenpentacarbonyl vapor under the condition of a 28-day-inhalation study in rats.

### Selection of concentrations

Rats (Gage et al., 1970) died after up to 18 exposures over 5.5 hour exposures to 15 ppm FEC. Clinical examination revealed particularly lethargy and respiratory symptoms. The lung was found to be the target organ (lung edemas and congestions). At a concentration of 7 ppm and under the same exposure conditions no organ changes were described. Based on these findings, the following concentrations were selected:

- high concentration: 10 ppm
- intermediate concentration: 3 ppm
- low concentration: 1 ppm

A NOEL was expected with the low concentration, which is equivalent to 10 times the MAK value.

Because of severe toxic effects (lethality) at 10 ppm during the first and at 3 ppm during the second exposure, treatment was discontinued for these groups and the surviving animals were observed without further exposure. Two additional groups were exposed to lower concentrations. In summary the following groups belonged to the study:

Test group (Target concentration ppm)	Number of animals		Number of exposures	Post-exposure period
	males	females		
0 (0)	5	5	20	-
4 (0.1)	5	5	20	-
E (0.3)	5	5	20	-
1 (1)	5	5	20	-
2 (3)	5	5	2	about 4 weeks
3 (10)	5	5	1	3 days*

\* all animals dead

Report; Project No.: 4610094/90021.

---

**Guidelines; dates of the study**

The study was carried out based on the OECD guidelines for Testing of Chemicals, Section 4; Health Effects; Paris 1981, method 412 (OECD 1981).

The test was performed during the following time period:

Nov. 5, 1990            (arrival of the animals)

Dec. 19, 1990        (start of sacrifice)

Report; Project No.: 46I0094/90021

---

**3. MATERIAL AND METHODS**

**3.1. Test substance**

Name of test substance: Eisenpentacarbonyl

Chemical name: Eisenpentacarbonyl

CAS No.: 13463-40-6

Abbreviation: FEC

Test substance No.: 90/94

Batch No.: from continuous production

Date of manufacture: Oct. 20, 1990

Purity: Iron 28.3% (nearly theoretical Fe contents for  $(Fe(CO)_5)$ )

Physical state/  
appearance: liquid/orange-yellow

Storage conditions: exclusion from light and oxygen, in a refrigerator

Stability: Stability was ensured for the period of the study under the specified storage conditions (see report Feb. 6, 1991, Supplement Vol. IV)

Further details of the test substance may be found in the raw data.

Report; Project No.: 46I0094/90021

3.2. Test animals

Male and female SPF-Wistar rats/Chbb:THOM, supplied by Dr. K. Thomae GmbH, D-W7950 Biberach, FRG, were used at an age of about 7 weeks (on delivery).

The animals were delivered on Nov. 5, 1990. They were clinically free from any signs of disease. The animals of test groups 0, 1, 2 and 3 only were allocated to the groups at random (WTALOC randomization program supplied by Instem). The purpose of this was to obtain test groups of equal weight.

At randomization, the mean body weights of the animals of the test groups 0, 1, 2 and 3 were:

Test groups	Body weight $\pm$ se (g)	
	Male	Female
0	248 $\pm$ 2.8	176 $\pm$ 2.3
1	248 $\pm$ 3.5	176 $\pm$ 2.6
2	246 $\pm$ 3.1	175 $\pm$ 2.5
3	248 $\pm$ 2.7	176 $\pm$ 2.3

se = standard error of the mean

Due to high lethality, exposure of test groups 2 and 3 was discontinued and two new test groups, 4 and E, were set up. These groups consisted of animals from the replacement group, which were not randomized at allocation to the test groups.

Test groups	Body weight $\pm$ se (g)	
	Male	Female
4	246 $\pm$ 3.1	176 $\pm$ 2.4
E	243 $\pm$ 1.1	171 $\pm$ 1.4

se = standard error of the mean

Report; Project No.: 46I0094/90021

---

3.3. Housing, diet and drinking water

During the period when the rats were not exposed they were housed singly in wire cages (type DK III of Becker, Castrop-Rauxel, FRG).

The animals were kept in fully air-conditioned rooms in which a temperature in the range of 20 - 24°C and relative humidity in the range of 30 - 70% were ensured by means of a central air-conditioning system.

A light/dark rhythm of 12 hours was maintained:

- 06.00 - 18.00 hours light
- 18.00 - 06.00 hours dark

Deviations from these specifications that might have had any adverse effect on the results of the study did not occur.

The animals were offered KLIBA rat/mouse laboratory diet 24-343-4 10 mm pellets, Klingentalmühle AG, CH-4303 Kaiseraugst, Switzerland, and tap water ad libitum. During exposure food and water were withdrawn.

Details of the feed batch used may be found in the raw data.

**Feed analysis**

The feed used in the study was assayed for chemical and microbiological contaminants.

In view of the aim and duration of the study the contaminants occurring in commercial feed might not have influenced the results.

**Drinking water analysis**

The drinking water is regularly assayed for chemical contaminants by the municipal authorities of Frankenthal and the Technical Services of BASF Aktiengesellschaft as well as for the presence of germs by a contract laboratory.

In view of the aim and duration of the study there are no special requirements exceeding the specification of drinking water.

Report; Project No.: 4610094/90021

3.4. Test groups, concentrations and time table  
 Time table

Year	Test group					
	0	1	2	3	4	E
1990						
Start of preflow	Nov. 13	Nov. 13	Nov. 13	Nov. 13	Nov. 13	Nov. 13
End of preflow	Nov. 19	Nov. 19	Nov. 19	Nov. 19	Nov. 19	Nov. 19
Start of exposure	Nov. 20	Nov. 20	Nov. 20	Nov. 20	Nov. 25	Nov. 25
End of exposure	Dec. 18	Dec. 18	Nov. 22	Nov. 20	Dec. 20	Dec. 20
Start of post-exposure observation	-	-	Nov. 23	Nov. 21	-	-
End of post-exposure observation	-	-	Dec. 18	Nov. 23	-	-
Sacrifice	Dec. 19	Dec. 19	Dec. 19	*	Dec. 21	Dec. 21
Number of exposures	20	20	2	1	20	20

\* all animals dead

Test groups and concentrations may be seen from the following tables.

The animals were identified by ear tattoo.

Males

Test group	Target concentration ppm	Number of animals	Animal No.
0	Air control	5	1 - 5
4	0.1	5	71 - 75
E	0.3	5	76 - 80
1	1	5	6 - 10
2	3	5	11 - 15
3	10	5	16 - 20

Females

Test group	Target concentration ppm	Number of animals	Animal No.
0	Air control	5	21 - 25
4	0.1	5	81 - 85
E	0.3	5	86 - 90
1	1	5	26 - 30
2	3	5	31 - 35
3	10	5	36 - 40

In the pathology the test group numbers would be indicated by the index letter E.

Report: Project No.: 46I0094/90021

---

3.5. Experimental procedure .

3.5.1. Generation of an inhalation atmosphere  
(see Annex, schematic figures 1 and 2)

Special safety instructions had to be respected because of the explosivity of the material in presence of oxygen.

Inhalation atmospheres were generated by passing nitrogen through a thermostated steel cylinder filled with liquid FEC. The nitrogen iron pentacarbonyl vapors were then diluted with compressed air, mixed by passing through a glass mixing stage, and distributed after further dilution with blast air to the inhalation chambers at the appropriate concentration ratios (Annex, schematic figure No. 1 and 2).

The following parameters were adjusted:

temperature of the thermostate (steel cylinder with FEC):	20 ± 2°C
temperature of the thermostate (glass mixing stage):	20 ± 2°C
stream of compressed air in glass mixing stage:	2.0 - 3.0 m <sup>3</sup> /h
nitrogen flow through steel cylinder with FEC:	max. 0.2 m <sup>3</sup> /h

Report: Project No.: 4610094/90021

3.5.2. Exposure system; exposure of the animals

**Whole-body exposure system**  
(see Annex, schematic figure No. 3)

The animals were kept singly in wire cages that were located in a glass-steel inhalation chamber, volume  $V \approx 1,400$  l (manufacturer: BASF Aktiengesellschaft).

In order to accustom the animals to the exposure conditions they were exposed to supply air in whole-body exposure systems on 5 days before the exposure period (preflow period). Then all test groups were exposed for 6 hours on workdays and Nov. 25, 1990 (sunday).

The following table shows the exposure time period and the number of exposures for all test groups:

Test group	Time period	number of exposure
0	Nov. 20 - Dec. 18, 1990	20
1	Nov. 20 - Dec. 18, 1990	20
2	Nov. 20 - Nov. 22, 1990	2
3	Nov. 20, 1990	1
E	Nov. 25 - Dec. 20, 1990	20
4	Nov. 25 - Dec. 20, 1990	20

The animals did not have access to water or feed during the exposure.

**Air conditions**

**Test group 0**

A slight positive pressure was maintained by adjusting the air flow of the exhaust air system. This ensured that no laboratory air reached the control animals.

**Test groups 1; 2; 3; E and 4**

A slight negative pressure was maintained by adjusting the airflow of the exhaust air system. This ensured that the laboratory was not contaminated as the result of any leakages from the inhalation chambers.

Report; Project No.: 4610094/90021

The following air flows were set:

Test group	Supply air (Blast air) [m <sup>3</sup> /h]	Exhaust air [m <sup>3</sup> /h]
0	28.0 ± 2	27.5 ± 2
1	27.8 ± 2 26.5 ± 2*	28.5 ± 2
2	27.5 ± 2	28.5 ± 2
3	26.5 ± 2	28.5 ± 2
E	27.5 ± 2	28.5 ± 2
4	27.8 ± 2	28.5 ± 2

\* from Nov. 25, 1990 onwards

**3.5.3. Measurement of the operation conditions (air flows, relative humidity, temperature and pressure conditions) of the exposure system**

The air flow rates, pressure conditions within the chambers, as well as relative humidities and temperatures in the inhalation system were measured continuously by an automated measuring system, were monitored against preset limits and partially regulated. The generator parameters temperature and compressed air were also recorded by means of this system.

Principles of recording with the automated measuring system (Details may be found in the operating instructions and SOP: LDV 600-618, 690, 691).

The measured values of the various parameters were recorded at the appropriate measuring points with suitable measuring equipment (sensors, orifice plates etc.), standardized (0-20 or 4-20mA) and transferred to the instrumentation consoles.

There the measured parameters were indicated in an analog way (where this is provided for) and some were used as actual value for regulating the specific parameter.

Report: Project No.: 4610094/90021

---

In addition the measured values were scanned about every 12 seconds, converted from analog to digital, transferred to a personal computer and displayed on the screen. The computer checked the arriving values against the preset threshold values, calculated daily means and standard deviations for each parameter and generated daily protocols. Furthermore the start and the end of exposure is provided in the daily protocol. Malfunctions that led to any exceeding of threshold values caused reports on the daily protocol that indicated the type of disturbance, the affected measuring point as well as the duration and extreme value reached.

The daily records are considered raw data. If values above or below the preset limits occurred for longer than ten minutes, 5-minute values were printed (see: SOP LDV 603) and are considered as raw data.

3.6. Analytical investigations of inhalation chamber atmospheres

for details see separate report Dr. Böck, ZSZ, BASF Aktiengesellschaft, in the "Analytical investigations at inhalation chamber atmosphere" Annex.

Report; Project No.: 4610094/90021

---

3.7. Clinical examinations .

3.7.1. Body weight

The body weight of the animals of the test groups 0, 1, 2 and 3 was checked at the beginning of the preflow period, one day before beginning of the exposure period (day -1), at the beginning of the exposure period (day 0) and then, as a rule, once a week. The body weight of the animals of the test groups 4 and E was checked at the beginning of the exposure period (day 0) and then, as a rule, once a week.

The body weight data from study days -7 and -1 are not given in the tables (No. 001 - 008) due to technical reasons.

3.7.2. Body weight change

The difference between the body weight on the day of weighing and the body weight at the beginning of exposure period was calculated as a group mean. This value was defined as body weight change.

3.7.3. Clinical signs and findings

Behavior and state of health of the test animals were checked, as a rule, 3 times on exposure days during exposure period, once on exposure days during preflow period and once on working days during post-exposure observation period.

3.7.4. Lethality

A check for dead animals was made daily.

Report: Project No.: 4610094/90021

3.8. Clinical chemistry, hematology and urinalysis

Blood was taken from the retroorbital venous plexus in the morning from non-fasted, not anesthetized animals. The blood samplings and the subsequent analyses of the blood and serum samples were carried out in a randomized sequence (with the exception of the blood sampling for carboxyhemoglobin examination). The list of randomization instructions was compiled with a computer using a random number generator.

Time schedule of blood and urine collection

Male	Female	Examination	Nominal days
Dec. 7, 1990 (groups 0, 1, 4 and E for carboxyhemoglobin only)	Dec. 7, 1990 (groups 0, 1, 4 and E for carboxyhemoglobin only)	Blood sampling	17 days**
Dec. 19, 1990 (only groups 0, 1 and 2)	Dec. 19, 1990 (only groups 0, 1 and 2)	Blood sampling	29 days
Dec. 21, 1990 (only groups 4 and E)	Dec. 21, 1990 (only groups 4 and E)	Blood sampling	29 days**
Dec. 16, 1990 (only groups 0, 4, 1 and E)	Dec. 16, 1990 (only groups 0, 4, 1 and E)	Urine collection	18/13 days

\*\* Due to technical reasons (Test group 4 and E were started additionally at a later time) the data of groups 4 and E must be listed and evaluated under nominal day 17 instead of 12 or under nominal day 29 instead of 26.

The assays of blood and serum parameters were performed under internal laboratory quality control conditions with commercial reference controls to assure reliable test results.

The results of the clinicochemical and hematological examinations are expressed in units of the International System (SI).

The following examinations were carried out in 5 animals per sex in groups 0, 1, 4 and E and in all surviving animals of test group 2.

Report: Project No.: 610094/90021

---

3.8.1. Hematological examinations

The following parameters were determined in blood with EDTA-K<sub>2</sub> as anticoagulant using a particle counter (S Plus model, by Coulter, Krefeld, FRG):

- leukocytes
- erythrocytes
- hemoglobin
- hematocrit
- mean corpuscular volume
- mean corpuscular hemoglobin
- mean corpuscular hemoglobin concentration
- platelets

The data obtained were transferred to a computer (VAX 11/780; supplied by DEC, Munich, FRG).

The differential blood count and the reticulocytes were evaluated visually. The data were transferred to the computer.

The methods used can be seen from the following table:

Report: Project No.: 4610094/90021

HEMATOLOGICAL EXAMINATIONS

Parameter	Unit	Method	References
Leukocyte count (WBC)	giga/l	measurement according to Coulter principle	Coulter Counter working instructions; laboratory modification
Erythrocyte count (RBC)	tera/l	measurement according to Coulter principle	
Hemoglobin (HGB)	mmol/l	hemoglobin cyanide; photometric measurement; 525 nm	
Hematocrit (HCT)	l/l	calculation: MCV-erythrocytes	
Mean corpuscular volume (MCV)	fl	measurement according to Coulter principle	
Mean corpuscular hemoglobin (MCH)	fmol	calculation: <u>hemoglobin</u> erythrocytes	
Mean corpuscular hemoglobin concentration (MCHC)	mmol/l	calculation: <u>hemoglobin</u> hematocrit	
Platelet count (PLT)	giga/l	measurement according to Coulter principle	
Differential blood count	% and giga/l	stained according to Wright; microscopic evaluation	Evaluation according to: Schermer, S., "The Blood Morphology of Laboratory Animals", F.A. Davis Co. Philadelphia (1967)
Reticulocytes (RETI)	% (10 <sup>-3</sup> erythrocytes)	vital staining with methylene blue; microscopic evaluation	Zeile G., Baake, M. and Henrici, G., "Kompendium der praktischen Hämatologie" [Compendium of Practical Hematology], GIT Verlag Ernst Giebler, Darmstadt, FRG, page 196 (1980); laboratory modification

Report: Project No.: 4610094/90021

**3.8.2. Carboxyhemoglobin**

Carboxyhemoglobin was determined by absorbance measurement using a hemoximeter ("OSM 3" model, by Radiometer, Copenhagen). The data obtained were transferred to a computer (VAX 11/780; supplied by DEC, Munich, FRG).

**3.8.3. Clotting analyses**

The clotting analyses were carried out using a ball coagulometer (KC 10 model, by Amelung, Lemgo, FRG) and the results transferred off-line to the computer.

The following parameter was determined:

- thromboplastin time (Hepato Quick's test)

The following table lists the method used:

Parameter	Unit	Method	References
Thromboplastin time (Hepato Quick's test) (HQT)	seconds	citrated blood with calcium thromboplastin	Fischer, M. and Falkensammer, Ch., Klin. Wschr. 86, 577-583 (1974)

**3.8.4. Clinicochemical examinations**

An automatic analyzer (Hitachi 737; by Boehringer, Mannheim, FRG) was used to examine the clinicochemical parameters.

The values obtained were transferred to a computer (VAX 11/780; by DEC, Munich, FRG).

The following parameters were determined:

1. Enzymes
  - alanine aminotransferase
  - aspartate aminotransferase
  - alkaline phosphatase
  - serum- $\gamma$ -glutamyltransferase

Report; Project No.: 4610094/90021

## 2. Blood chemistry

- sodium
- potassium
- chloride
- inorganic phosphate
- calcium
- urea
- creatinine
- glucose
- total bilirubin
- total protein
- albumin
- globulins
- triglycerides
- cholesterol
- magnesium

The methods used can be seen from the following table:

## 1. ENZYMES

Enzyme (systematic name and system number)	Unit	Method, wavelength and measuring temperature	References
Alanine aminotransferase (ALT) (L-alanine: 2-oxoglutarate aminotransferase; EC 2.6.1.2.)	µkat/l	kinetic UV test, 340 nm, 37°C	Recommendations of the German Society for Clinical Chemistry: "Standardization of methods for determining enzyme activities in biological liquids".
Aspartate aminotransferase (AST) (L-aspartate: 2-oxoglutarate aminotransferase; EC 2.6.1.1.)	µkat/l	kinetic UV test 340 nm, 37°C	J. Clin. Chem. Clin. Biochem. 8, 650-660 (1970);
Alkaline phosphatase (ALP) (orthophosphoric acid monoester phosphohydrolyase; EC 3.1.3.1.)	µkat/l	kinetic color test, 415 nm, 37°C	J. Clin. Chem. Clin. Biochem. 9, 464-465 (1971); J. Clin. Chem. Clin. Biochem. 10, 182-192 (1972) BM working instructions
γ-Glutamyltransferase (GGT) (γ-glutamyl)peptide: aminoacid-γ-glutamyltransferase; EC 2.3.2.2.)	nkat/l	kinetic color test 415 nm, 37°C	Szasz. G. et al. J. Clin. Chem. Clin. Biochem. 12, 228 (1974) BM working instructions

BM = Boehringer, Mannheim, FRG

Report; Project No.: 4610094/90021

## 2. BLOOD CHEMISTRY

Parameter	Unit	Method	References
Sodium (NA)	mmol/l	ion selective electrodes (ISE)	Hitachi 737 - working instructions
Potassium (K)	mmol/l		
Chloride (CL)	mmol/l	mercury(II)-thiocyanate method	Analogous to Küffer, H., Richterich, R., Kraft, R., Peheim, E. & Colombo, J.P., J. Clin. Chem. Clin. Biochem. 13, 203 - 211 (1975)
Inorganic phosphate (INP)	mmol/l	molybdate reaction	Henry, R.J. in: "Clinical Chemistry", Harper and Row Publishers, New York (1974); BM working instructions
Calcium (CA)	mmol/l	o-cresolphthalein complex without deproteinization	Ray Sarkar, B.C. and Chauhan, U.P.S., Anal. Biochem. 20, 155 (1967); BM working instructions
Urea (UREA)	mmol/l	enzymatic determination with the urease/glutamate dehydrogenase method	Neumann, U. and Ziegenhörn, J.: XVI, Nordiska kongressen för klinisk kemi och klinisk fysiologi 1977, Oulu, Finland; BM working instructions
Creatinine (CREA)	µmol/l	kinetic Jaffé method without deproteinization	Bartels, H. et al., Clin. Chim. Acta 31, 193 (1972); BM working instructions
Glucose (GLUC)	mmol/l	hexokinase/glucose-6-phosphate dehydrogenase method	Schmidt, F.H., Klin. Wschr. 39, 1244-1247 (1961); BM working instructions
Total bilirubin (TBIL)	µmol/l	DPD method	Wahlefeld, A.W. et al., Scand. J. Clin. Lab. Invest. 29, Suppl. 126 (1972) Abstract 11.12; BM working instructions
Total protein (TPROT)	g/l	biuret method	Weichselbaum, T.E., Amer. J. Clin. Path. 10, 40 (1966); BM working instructions
Albumin (ALB)	g/l	bromocresol green method	Doumas et al., Clin. Chim. Acta 31, 87 (1971); BM working instructions
Globulins (GLOB)	g/l	difference between total protein and albumin	
Triglycerides (TRIG)	mmol/l	enzymatic color test with lipase esterase/glycerokinase/glycerol-3-phosphate oxidase/4-aminophenazone	mod. method by Wahlefeld, A.W., in "Methoden der enzymatischen Analyse" [Methods of enzymatic analysis] (Bergmeyer, H.U., ed.) Vol. II, 3rd ed., Verlag Chemie Weinheim, FRG, pp. 1878-1882 (1974); BM working instructions
Cholesterol (CHOL)	mmol/l	enzymatic determination with cholesterol esterase/cholesterol oxidase/4-aminophenazone (CHOD-PAP method)	Siedel, J. et al., J. Clin. Chem. Clin. Biochem. 19, 838 (1981); BM working instructions
Magnesium (MG)	mmol/l	xylidylblue method	Mann, C.K. and Yoe, J.H., Anal. Chem. 28, 202 - 205 (1956) Bohnon, C., Clin. Chim. Acta 1, 811 - 817 (1962)

BM = Boehringer, Mannheim, FRG

Report: Project No.: 46I0094/90021

---

**3.8.5. Urinalyses**

With the exception of the Volume, appearance, sediment examination and the specific gravity, all the urine constituents were determined semiquantitatively using test strips (Combur-9-test RL, by Boehringer, Mannheim, FRG) and a reflection photometer (Urotron RL9 model by Boehringer, Mannheim, FRG).

The specific gravity was determined using a urine refractometer.

The sediment was evaluated microscopically.

The following examinations were carried out:

- volume
- appearance
- nitrite
- pH
- protein
- glucose
- ketones
- urobilinogen
- bilirubin
- blood
- specific gravity
- sediment

Details of the methods can be seen from the following table:

Report: Project No.: 46I0094/790024

## URINALYSIS

Parameter	Method	References
Nitrite	p-arsanilic acid diazonium salt; tetrahydroxybenzo(h)-quinolin-3-ol	Test strip book by Boehringer, Mannheim, FRG (1977)
pH	methyl red and bromothymol blue	
Protein	tetrabromophenol-phthaleinethylester (TBPE)	
Glucose	GOD-POD reaction	
Ketones	sodium nitroprusside	
Urobilinogen	p-methoxyaniline-diazonium-salt	
Bilirubin	2,5-dichloroaniline diazonium salt	
Blood	2,5-dimethylhexane-2,5-dihydroperoxide, tetramethylbenzidine	
Specific gravity	refractometer	Hamilton or Atago operating instructions
Sediment	microscopy	Hallmann, L., "[Clinical Chemistry and Microscopy] 10, ed., 233-246, Georg Thieme, Stuttgart, FRG (1966)
Appearance	by visual assessment	
Volume	graduated tubes	

Report; Project No.: 4610094/90021

---

3.9. Pathology (see separate pathology report, Vol. III)

3.10. Statistical evaluation

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

3.10.1 Clinical examinations

Groups 0, 1, 4, E

For body weight data a non-parametric one-way analysis was performed using the Kruskal-Wallis test [Siegel 1956]. If the resulting p-value was equal or less than 0.05, a pairwise comparison of each dose group with the control group was carried out. This comparison was performed via the Mann-Whitney U-test [Siegel 1956] for the hypothesis of equal medians. If the results of this test were significant, labels (\* for  $p \leq 0.05$ , \*\* for  $p \leq 0.02$ , \*\*\* for  $p \leq 0.002$ ) were printed together with the group means in the tables.

Both tests were performed two sided.

Groups 0, 2

For body weight data a comparison of the dose group with the control group was carried out using the Mann-Whitney U-Test [2] for the hypothesis of equal medians. If the results of this test were significant, labels (\* for  $p \leq 0.05$ , \*\* for  $p \leq 0.02$ , \*\*\* for  $p \leq 0.002$ ) were printed together with the group means in the tables. The test was performed two-sided.

3.10.2. Clinical chemistry and hematology

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

Groups 0, 1, 4, E:

For all parameters excepting the differential blood count, a non-parametric one-way analysis was performed using the KRUSKAL-WALLIS test [Siegel 1956]. If the resulting p-value was equal or less than 0.05 a pairwise comparison of each dose group with the control group was carried out. This comparison performed via the MANN-WHITNEY-U-test [Siegel 1956] for the hypothesis of equal medians. If the results of this test were significant, labels (\* for  $p \leq 0.05$ , \*\* for  $p \leq 0.02$ , \*\*\* for  $p \leq 0.002$ ) were printed together with the group means in the tables.

Both tests were performed two-sided.

Report: Project No.: 4610094/90021

---

Groups 0, 2:

For all parameters, excepting the differential blood count, a comparison of the dose group with the control group was carried out using the Mann-Whitney U-test [2] for the hypothesis of equal medians. If the results of this test were significant, labels (\* for  $p \leq 0.05$ , \*\* for  $p \leq 0.02$ , \*\*\* for  $p \leq 0.002$ ) were printed together with the group means in the tables.

The test was performed two-sided.

3.10.3. Urinalyses

With the exception of the volume and appearance, the scale for these parameters is divided into 4 sections (0, 1, 2, 3).

For the parameter "Nitrite" only a division in two sections (0, 1) is made.

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

A pairwise comparison of each dose group with the control was carried out using Fisher's exact test [2] for the hypothesis of equal proportions. If the results of this test are significant, labels (\* for  $p \leq 0.05$ , \*\* for  $p \leq 0.01$ ) were printed in the tables.

Report; Project No.: 4610094/99021

---

#### 4. RESULTS AND ASSESSMENT

##### 4.1. Clinical examinations

The results of the study may be found in the following Summary Tables (test group means) in the "CLINICAL EXAMINATIONS" Annex.

Table

Key to the abbreviations, page 37

Body weight

- males

001 - 002

- females

003 - 004

Body weight change

- males

005 - 006

- females

007 - 008

The pertinent tables of the individual body weights may be found in Part A of Volume II of this report.

Report: Project No.: 4618094/90024

---

#### 4.1.1. Body weight

The body weight of the male and female animals of test groups E, 1 and 2 and of the male animals of test group 4, compared to the control group 0, were not statistically significantly different. The body weight of the female animals of the test group 4 (0.1 ppm) was statistically significantly higher ( $p \leq 0.02$ ) on day 7 when compared to control group 0.

Test group 3 (10 ppm) could not be assessed due to high lethality.

#### 4.1.2. Body weight change

The body weight change of the male and female animals of test groups 4, E, 1 and 2 were, compared to the control group 0, statistically not significantly different.

Test group 3 (10 ppm) could not be assessed due to high lethality.

#### 4.1.3. Lethality (exposure- and post-exposure observation period)

No deaths were recorded in test groups 0, 4, E and 1 at any time of the study.

The following animals of test group 2 (3 ppm) died or were sacrificed in a moribund state:

study day 2: animal No. 34 died before second exposure  
study day 2: animal No. 13 died during second exposure  
study day 3: animal No. 32 died during post-exposure  
observation period  
study day 3: animal No. 11 sacrificed in a moribund state  
study day 3: animal No. 14 sacrificed in a moribund state

The exposure of test group 2 was discontinued after the second exposure and the surviving animals were further observed until the end of the study.

The following animals of test group 3 (10 ppm) died or were sacrificed in a moribund state after one exposure (The exposure of test group 3 was discontinued after the first exposure on study day 0):

study day 1: animal Nos. 16 - 20 died  
study day 1: animal Nos. 36, 37 died  
study day 2: animal No. 38 died  
study day 3: animal No. 39 died  
study day 3: animal No. 40 sacrificed in a moribund state

Report: Project No.: 4610094/90021

---

**6.1.4. Clinical signs and findings (preflow; exposure- and post-exposure observation period)**

**Preflow period**

The animals showed no clinical signs and findings different from normal

**Exposure period**

Test groups 0; 4 (0.1 ppm); E (0.3 ppm); 1 (1 ppm)

The animals showed no clinical signs and findings different from normal

**Test group 2 (3 ppm):**

**- Study day 0 (first exposure)**

Before, during and after exposure:  
The animals showed no clinical signs and finding different from normal

**- Study day 1 (no exposure (holiday))**

**- Study day 2 (second exposure)**

Before, during and after exposure:  
The animals showed piloerection and accelerated respiration

**Test group 3 (10 ppm):**

**- Study day 0 (first exposure)**

Before the first exposure:  
The animals showed no clinical signs and findings different from normal.

During the first exposure:  
The animals showed piloerection

After the first exposure:  
The animals showed slight apathy and piloerection

**- Study day 1 (no exposure (holiday))**

Report: Project No.: 4610094/90021

**Post-exposure observation period****Test group 2 (3 ppm):**

- Study day 3  
All surviving animals showed piloerection and accelerated respiration.  
  
Two animals showed additionally squatting posture.
- Study days 4 to 5  
The surviving animals showed piloerection and accelerated respiration
- Study days 6 to 9  
The surviving animals showed accelerated respiration
- Study days 10 up to the end of the study  
The surviving animals showed no clinical signs and findings different from normal

**Test group 3 (10 ppm)**

- Study day 2  
the surviving animals showed abdominal respiration, piloerection, reddish crusts on the edges of the noses (blood test positive), deteriorated general state and squatting posture
- Study day 3  
The surviving animals showed abdominal respiration, piloerection, deteriorated general state and squatting posture
- Study days 4 onwards  
All animals of test group 3 were dead

Report; Project No.: 4610004/90021

---

4.1.5.

**Assessment of clinical examinations****Lethality**

No lethality was seen at concentrations 0.1 - 1 ppm. 50% of the animals exposed to 3 ppm died or were killed in a moribund state after up to two exposures and 2 days without exposure. From the animals exposed to 10 ppm 100% died or were killed in a moribund state after one exposure and up to 3 days post-exposure observation period.

**- Body weight, body weight change**

In test group 0.1 ppm - 3 ppm no treatment-related effects on body weight development were observed.

**- Clinical signs and findings**

No treatment-related effects different from normal were observed in the test groups 0.1 - 1 ppm. At 3 ppm and 10 ppm dose-related effects on general-state (3 and 10 ppm), changes in respiration behaviour (10 ppm) and irritative effects on the noses (10 ppm) were observed. During the post-exposure period animals of test group 10 ppm did not recover until their death, whereas surviving animals of test group 3 ppm showed no clinical signs different from normal from day 10 onwards.

Report: Project No.: 4610094/90021

4.2. Results: concentration measurements and measured values of the operation conditions of the exposure and generator systems

The results of the measurements may be found in Volume II, Part C, C 001 - C 009.

4.2.1. Concentration monitoring with the IR-Spectrometer

Study means with standard deviations were calculated from the daily means of the individual concentrations of all test groups. The following study means were obtained with the IR-Spectrometer:

Test group	Target concentration Fe(CO) <sub>5</sub> [ppm]	Analytical concentration ± standard deviation	
		Fe(CO) <sub>5</sub> [ppm]	CO [ppm]
4	0.1	0.10 ± 0.01	0.20 ± 0.30
E	0.3	0.30 ± 0.01	0.21 ± 0.31
1	1	1.00 ± 0.02	0.22 ± 0.29
2	3	2.91 ± 0.01	0.86 ± 0.07
3	10	9.85	4.87

4.2.2. Operation conditions in the exposure systems

An evaluation of all measured values (temperature, relative humidity and chamber pressure in the inhalation systems) may be seen from the following table.

Report: Project No.: 4610094/90021

Test group	t ± s	§ ± s	p ± s
0	21.7 ± 0.2	58.8 ± 2.8	10.6 ± 0
4	21.7 ± 0.2	58.2 ± 1.5	- 9.9 ± 0.1
E	21.1 ± 0.2	60.2 ± 1.2	- 9.8 ± 0.1
1	21.1 ± 0.3	55.1 ± 2.7	-13.4 ± 1.2
2	21.7 ± 0.4	61.0 ± 2.8	- 8.6 ± 1.9
3	21.2	57.0	-12.6

t = chamber temperature [°C]  
 § = rel. humidity in the chamber [%]  
 p = chamber pressure [Pa]  
 s = standard deviation

Individual values may be found in the record sheets and are kept with the raw data.

4.2.3. Operation conditions in the generator system

Thermostate-temperature  
 (steel cylinder with FEC) 20.0 ± 0.3°C  
 Thermostate-temperature  
 (glass mixing stage) 20.3 ± 0.1°C  
 flow of compressed air glass mixing stage 2.1 ± 0 m³/h  
 flow of nitrogen through steel cylinder 20.4 ± 0.6 l/h  
 rsp. 1.5 ± 0.1 l/h\*

\* from Nov. 25, 1990 onward

Individual values may be found in the record sheets and are kept with the raw data.

Report; Project No.: 4610094/90021

## 4.3. Clinical chemistry, hematology and urinalyses

The results of the study are shown, together with a key to the abbreviations, in the following Summary Tables in the Annex:

	Table
Key to the abbreviations, page 39 to 40	
<b>Hematological examinations</b>	
- male animals	009 - 010
- female animals	011 - 012
<b>Differential blood count</b>	
- male animals	013 - 016
- female animals	017 - 020
<b>Reticulocytes</b>	
- male animals	021 - 022
- female animals	023 - 024
<b>Carboxyhemoglobin</b>	
- male animals	025
- female animals	026
<b>Clotting analyses</b>	
- male animals	027 - 028
- female animals	029 - 030
<b>Enzymes</b>	
- male animals	031 - 032
- female animals	033 - 034
<b>Blood chemistry</b>	
- male animals	035 - 038
- female animals	039 - 042
<b>Urinalyses</b>	
- male animals	043 - 044
- female animals	045 - 046

The relevant tables on the individual values can be found in Part B of the tables (Volume II).

Report; Project No.: 4610094/90021

---

#### 4.3.1. Hematological examinations

A statistically significant increase in carboxyhemoglobin was found in the peripheral blood of the male animals of the test groups 4, E and 1 (0.1, 0.3 and 1 ppm) and in the blood of the female animals of test groups E and 1 (0.3 and 1 ppm) [Tabs. 025 and 026]. Although these findings were definitely caused by the test substance inhaled, the increase in carboxyhemoglobin is considered to be without any biological relevance, because the changes were marginal and a transient increase by up to 2% is generally considered to be of no toxicological significance.

In the other hematological examinations no substance-induced changes were detected.

#### 4.3.2. Clotting analyses

No substance-induced changes were observed.

#### 4.3.3. Enzymes

In the serum of the female animals of test group E (0.3 ppm) [Tab. 033] statistically significantly increased  $\gamma$ -glutamyltransferase activities were noted. Since the increase in the enzyme activity is marginal and is lacking dosage-relationship, this finding is assessed as being incidental.

No substance-induced changes were seen in the other enzymes examined.

#### 4.3.4. Blood chemistry

In the blood chemistry parameters no substance-induced changes were found. Only the serum of the female animals of test group 2 (3 ppm) [Tab. 041] showed a statistically significant decrease in creatinine concentration. This finding is assessed as being incidental, because at the beginning of the study the surviving animals of test group 2 were exposed to the test substance twice only and subsequently they were observed without exposure until the end of the study. Hence, it is improbable that the decreased creatinine level is due to the test substance inhaled.

Report; Project No.: 4610094/90021

---

#### 4.3.5. Urinalyses

No substance-induced changes were detected at urinalyses. The statistically significant increase in specific gravity observed in the urines of the male animals of test group E (0.3 ppm) [Tab. 043] is inconsistent and lacking dosage-relationship. Accordingly, this finding has no toxicological significance.

#### 4.3.6. Discussion and conclusion

No toxicologically relevant changes were found in the hematological and clinicochemical examinations.

#### 4.4. Pathology

(see separate pathology report, Volume III)

Report; Project No.: 4610094/90021

---

5. **RETENTION OF RECORDS**

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

Report; Project No.: 4610094/90021

---

6. **LITERATURE**

Gage et al.: Brit. J. Industr. Med. 1970, 27. 1 - 18

OECD Guideline for Testing of Chemicals No. 412

Section 4: Health Effects  
(1981) Paris, France ISBN 92-64-12221-4

SIEGEL, S. (1956): Non-parametric Statistics for the  
behavioral sciences, McGraw-Hill  
New York,