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Diacetyl FCC / Publication in The Wall Street Journal / Your E-Mail of 12.10.01

Dear Dr. Api:

Enclosed please find the toxicological report „Study on the acute inhalation toxicity LC_{50} of Diacetyl FCC as a vapor in rats 4-hour exposure" for your information. Please handle this report strictly confidential.

Sincerely,

BASF Aktiengesellschaft
Fine Chemicals Division
Regulatory Affairs/Product Stewardship

Frosch

Enclosure

38269

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Abteilung Toxikologie
Department of Toxicology
D-W6700 Ludwigshafen, FRG

ga-bh; 2315

JUN 0 8 1993

REPORT

Study on the acute inhalation toxicity LC_{50}
of

Diacetyl FCC

as a vapor
in rats
4-hour exposure

Project No. 13I0247/927010

Testing facility: BASF Aktiengesellschaft,
Department of Toxicology,
D-W6700 Ludwigshafen, FRG

This report consists of pages I - VI, 1 - 20.

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Department of Toxicology

LC50; Project No.: 13I0247/927010

ORGANIZATION

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

Sponsor: BASF Aktiengesellschaft, Ludwigshafen

Study Director: A.O. Gamer, May 24, 1993
Dr.med.vet. A.O. Gamer

Head of Section: Hirsch June 2, 1993
Dr.med.vet. P. Hirsch

Pathologist: June 1, 1993
Dr.med.vet. K.-O. Freisberg

III

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LC50; Project No.: 13I0247/927010

STATEMENT
OF THE QUALITY ASSURANCE UNIT

Number of test substance: 92/247

Name of test substance: Diacetyl FCC

Title of study: Study on the acute inhalation toxicity LC_{50} of Diacetyl FCC as a vapor in rats, 4-hour exposure.

The Quality Assurance Unit inspected the study, 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:	Jan. 20, 1993	Jan. 25, 1993
Conduct of study:	Jan. 25, 1993	Jan. 25, 1993

Ludwigshafen/Rhein, June 7, 1993

Dr.rer.nat. H. Fleig
(Head of Quality Assurance Unit)

IV

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LC_{50}; Project No.: 13I0247/927010

GLP STATEMENT

Title of study: Study on the acute inhalation toxicity LC_{50} of Diacetyl FCC as a vapor in rats, 4-hour exposure.

Project No.: 13I0247/927010

This study was conducted in accordance with the GLP provisions of the Chemikaliengesetz" ("Chemicals Act"; Bundesgesetzblatt, Teil I, 22.03.90) and with the "OECD Principles of Good Laboratory Practice" (Paris, 1981). However, there were was the following deviation from the requirements of the above mentioned principles:

- The report was not audited by QAU.

The validity of the data and of the overall results of the study is not considered to be adversely affected by the above mentioned deviations. If necessary the audit of the report on the basis of the archived raw data can be performed at any time later.

Kingsly, June 2, 1993
Head of section

A.O. Jones, May 24th, 1993
Study director

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CONTENTS

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1. SUMMARY

For determination of the acute inhalation toxicity (single 4-hour exposure) of Diacetyl FCC as a vapor, a study in male and female Wistar rats was performed according OECD Guideline, as well as EEC and EPA guidelines. The following concentrations were tested: 2.25; 5.2 and 23.9 mg/l. No mortality occurred at 2.25 mg/l. All animals died at 5.2 and 23.9 mg/l. The LC_{50} for male and female animals was estimated to be $2.25 < LC_{50} < 5.2$ mg/l.

In the low concentration clinical examination revealed eyelid closure, restlessness, later apathy, squatting posture and ruffled fur in all animals. Abdominal and dragging respiration as well as respiratory sounds and reduced general state was seen in single animals. No clinical signs could be detected from day 6 onward.

The mid as well as the high concentration resulted in an abundance of symptoms indicative for respiratory tract injury (see 5.2.1.). In the mid concentration group these symptoms developed mainly from day 1 onward in the surviving animals.

Body weight development of the low concentration males was depressed in the first half of the post-exposure period but at the end of the study the body weight had recovered. There was no effect on body weight development of the females. The body weight development of mid and high concentration groups could not be evaluated because of the poor survival.

During necropsy all animals that died showed general congestion. Focal hyperemia of the lungs and empty gastrointestinal tract were seen additionally in animals of the mid concentration group. Exposure to the high concentration led to atelectasis and bloody edema of the lungs, bronchial edema and intensified hydrothorax. No macroscopic pathologic findings were noted in the animals of the low concentration killed at the end of the study.

Histopathology in selected organs of single animals from the mid and high concentration groups revealed extensive hyperemia of the lung, necrosis in the proximal tubules of the kidney and centrilobular swelling of hepatocytes in the high concentration as well as moderate empyhsema and focal hyperemia of the lungs and peripheral swelling of hepatocytes in the mid concentration.

2. INTRODUCTION

Aim of the study:

To obtain quantitative animal data in order to assess a possible hazard by inhalation of the product.

Dates of the study:

The study was carried out from January 25, 1993 to February 16, 1993.

Selection of concentrations:

The selection of the concentration of test group 3 was based on available information about the toxicity of the substance; the other concentrations were selected in order to achieve an estimate of the LC_{50}.

3. MATERIAL

Name of test substance: Diacetyl FCC

Batch No.:	D1/9/92
Purity:	99.1%
Date of manufacture/ filling:	September 18, 1992
Physical state/ appearance:	liquid / yellow to green-yellow
Storage conditions:	was stored in a refrigerator
Stability:	On completion of all tests the stability of the substance will be verified by reanalysis. The result can be obtained from the sponsor (BASF AG).

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4. METHODS

The test method is based on the OECD Guidelines (1), method 403 Commission Directive 84/449 EEC (2) and EPA guidelines (3).

4.1. Test animals

Male and female Wistar rats (strain: SPF Wistar/Chbb: THOM; breeding facility: Dr. K. Thomae GmbH, D-W7950 Biberach, FRG) were used for the investigations.

Age at the beginning of the study: approx. 8 - 9 weeks

The mean body weight of the animals on day of exposure is given in chapter 5.2.2.

4.2. Identification

The animals were identified by color marking on the tail.

4.3. Housing and diet

The animals were offered KLIBA rat/mouse/hamster laboratory diet 24-343-4 10 mm pellets, Klingentalmühle AG, CH-4303 Kaiseraugst, Switzerland, and drinking water ad libitum during the post-exposure observation period.

The feed used in the study was assayed for chemical as well as for microbiological contaminants. In view of the aim and duration of the study the contaminants occurring in commercial feed might not influence the results.

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.

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

They were housed singly in cages type DK III of Becker,

Deviations from these specifications that might have had any adverse effect on the results would be reported in the results section.

4.4. Test groups

5 male and 5 female rats were used for each test group.

4.5. Exposure system

Whole-body inhalation system: IKA 02 (glass-steel construction), BASF Aktiengesellschaft, Volume V = 200 l: the animals were kept singly in compartmentalized wire cages, and were exposed in the chamber.

4.6. Generation of the inhalation atmosphere

4.6.1. Substance preparation

The test substance was dosed unchanged.

4.6.2. Technical equipment

Continuous infusion pump INFU 362 (INDIGEL/Switzerland), glass vaporizer with thermostat (BASF) and a glass mixing vessel (BASF).

4.6.3. Procedure

By means of the continuous infusion pump amounts of the test substance per test group were supplied to the heated vaporizer. The vapors that developed were mixed with supply air and passed into the inhalation system.

4.7. Exposure

The following air flows (supply air) were set:

test group	blast air [l/h]
1	3,000
2	3,000
3	3,000

The supply air was conditioned via a central air-conditioning system. The exposure system was placed in an air-conditioned laboratory.

By means of an exhaust air system the pressure ratios in the inhalation system were adjusted in such a way that the amount of exhaust air was higher (pressure below atmospheric). This ensured that no contamination of the laboratory occurred as result of leakages from the inhalation chamber.

The inhalation atmosphere was offered to the animals for inhalation for 4 hours.

4.8. Analytical investigations

4.8.1. Nominal concentration

The nominal concentration was calculated from the amount of substance consumed and the air flow.

4.8.2. Sampling

Apparatus: 2 absorption vessels and a fritted glass flask, connected in series filled with sorption solvent

BASF sampling station (with vacuum pump gas meter, impulse counter and automatic pump switch)

- Sorption solvent: Dimethylformamide (Merck) [DMF]
- Sampling velocity: 1.25 m/s
- Sampling volumes: 5 l - 7 l

These volumes were adjusted to achieve amounts of the test substance in the samples of the different test groups, which refer to the one-point calibration (c.f. 4.8.3.).

- Sampling position: immediately adjacent to the animals' noses
- Sampling probe diameter: 4 mm
- Sampling frequency: 1 sample per concentration group about hourly

4.8.3. Analytical determination method

For the quantitative determination of the vapor concentration a gas chromatographical method was used.

Equipment: GC HP 5840 A (Hewlett Packard)

The following gaschromatographical conditions were used:

column:	glass
length:	2 m
int. diam..:	2 mm
separation phase:	10% Ucon LB 550X
support material:	Chromosorb WAW/HP
size:	100/120 mesh
carrier gas:	helium
carrier gas flow rate:	26.9 ml/min
hydrogen:	30 ml/min
air:	242 ml/min
temperature program:	20°C/min after 6 min
furnace temp. start:	60°C
furnace temp.: end:	160°C
detector temp. (FID):	200°C
injector temp.:	200°C
internal standard (i.st.):	C_8KW
calibr. retention time i.st.:	4.6 min
calibr. retention time sample:	2.7 min
injection volume:	1 µl

Calibration

- Method set up

  During the set up of the analytical procedure a calibration curve was prepared in the solvent with the test substance to be investigated to show linearity in the suitable concentration range of the samples.

- Routine analysis during test period

  For routine analysis a one-point calibration of the analytical procedure was prepared daily.

Sample preparation

The obtained samples were taken up in approx. 40 ml DMF in a 50-ml calibrated flask. An internal standard was added using a pipette and the flask was filled up to the calibration mark.

The samples 1 - 4 of test group 3 were diluted with DMF sorption solvent at a ratio of 15 + 35.

Calculation of the concentration:

From the analytically determined mass values and the sample volumes of the inhalation atmosphere the concentrations were calculated in mg/l.

4.8.4. Temperature and humidity

The humidity in the inhalation systems was not measured due to technical reasons. It is assumed that deviations of humidity values from the guideline requirements (especially low humidity in dust aerosol) did not influence the test results, because of the relative short exposure time.

The temperatures in the inhalation systems were measured continuosly and recorded once. Only deviations from the temperature range (22 ± 2°C) of the OECD Guideline (1) will be reported in the result section.

4.9. Observation period

After the exposure period the surviving animals were observed for 14 days.

4.10. Clinical examinations

The body weight of the animals was checked before the beginning of the test, after 7 days and at the end of the observation period. Clinical findings were recorded for each animal separately several times during exposure and at least once on each workday in the observation period. A check for dead animals was made daily.

4.11. Pathology

At the end of the 14-day observation period the surviving animals were killed with CO_2 and were subjected to gross-pathological examination like all other animals which had

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4.12. Statistical evaluation

The statistical evaluation of the dose-response relationship was carried out using FORTRAN program AKPROZ: Depending on the data of the dose-response relationship obtained by way of experiment, this program is used to estimate the LC_{50} or to perform a Probit analysis (see (4)). Estimation of the LC_{50} will produce types LC_{50} greater, LC_{50} about, or LC_{50} smaller. If the results are Type LC_{50} greater or LC_{50} smaller, an additional binominal test is carried out (see (5)), in order to verify these statements statistically, if necessary.

4.13. Retention of records

The raw data and protocol, as well as the original of this report, are retained at BASF Aktiengesellschaft at least for the period of time specified in the GLP regulations. The specimens are handled according to the pathology SOP "procedure for acute tests".

5. RESULTS

5.1. Concentration and lethality

The means of the concentrations in the test groups and the lethality rates (number of animals that died / number of animals exposed) are presented in the following table.

Cumulated lethality on day	Test group (concentration)					
	1 (2.25 mg/l)		2 (5.2 mg/l)		3 (23.9 mg/l)	
	m	f	m	f	m	f
0	0/5	0/5	0/5	0/5	4/5	2/5
1	-	-	4/5	2/5	5/5	4/5
2	-	-	-	5/5	-	-
7	-	-	5/5	-	-	5/5
14	-	-	-	-	-	-
Total at end of the study	0/10		10/10		10/10	

m = male, f = female

- = lethality unchanged

0 = day of exposure

5.2. Clinical examinations

5.2.1. Clinical signs and findings

During exposure and in the post-exposure observation period:

Clinical signs and findings which were observed during (up to 4 hours) and after exposure (> 4 hours) are presented in the following tables.

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5.2.1.1. Maximum incidence of clinical findings depending on concentration

Table:

Male

Concentration (mg/l)	2.25	5.2	23.9
total number of animals	5	5	5
lethality (number of animals)	n.d.	5	5
respiration, abdominal	1		
respiration, dragging	1	5	5
gasping		5	5
respiratory sounds	1	5	2
eyelid, closure	5	5	5
nose, crust formation, bloody		1	
restlessness	5	5	
apathy	5	5	5
abdominal position		1	5
snout wiping			5
squatting posture	5	5	
piloerection	5		
reduced general state	1	5	

n.d. = not detected

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Table:

Female

Concentration (mg/l)	2.25	5.2	23.9
total number of animals	5	5	5
lethality (number of animals)	n.d.	5	5
respiration, abdominal	1		
respiration, dragging	1	5	5
gasping		5	5
respiratory sounds	1	5	3
eyelid, closure	5	5	5
restlessness	5	5	
apathy	5	5	5
abdominal position			5
snout wiping			5
squatting posture	5	5	1
piloerection	5		1
reduced general state	1	5	

n.d. = not detected

5.2.1.2. Time intervall of duration of the specific symptoms depending on concentration

Table:

Male

Concentration (mg/l)	2.25	5.2	23.9
total number of animals	5	5	5
animals without findings	d0; d6-d14	d0	n.d.
lethality (study days)	n.d.	d1; d3	d0; d1
respiration, abdominal	d2-d3		
respiration, dragging	d1	2 1/2 h - 4 h	1/2 h
gasping		d1 - d2	1 h - d0
respiratory sounds	d1	d1 - d2	d0
eyelid, closure	1/4 h - 4 h	1/4 h - 4 h	< 1/4 h - 4 h
nose, crust formation, bloody		d2	
restlessness	< 1/4 h	< 1/4 h	
apathy	1/4 h - 4h; d1 - d2	1/4 h - 4 h	< 1/4 h - 4h; d0
abdominal position		d2	1 h - 4 h; d0
snout wiping			< 1/4 h
squatting posture	d1	d1	
piloerection	d1 - d3		
reduced general state	d1	d1 - d2	

d0 = post-exposure on exposure day
h = hour
d = day
n.d. = not detected

No clinical observations were performed on public holidays or weekend by test groups 1 and 3.

Table:

Female

Concentration (mg/l)	2.25	5.2	23.9
total number of animals	5	5	5
animals without findings	d0; d6-d14	d0	n.d.
lethality (study days)	n.d.	d1 - d2	d0; d1; d3
respiration, abdominal	d2-d3		
respiration, dragging	d1	2 1/2 h - 4 h	1/2 h
gasping		d1	1 h - d2
respiratory sounds	d1	d1	d0 - d2
eyelid, closure	1/4 h - 4 h	1/4 h - 4 h	1/4 h - 4 h
restlessness	< 1/4 h	< 1/4 h	
apathy	1/4 h - 4h; d1 - d2	1/4 h - 4 h	< 1/4 h - 4 h; d0
abdominal position	-		1 h - 4 h; d0
snout wiping			< 1/4 h
squatting posture	d1	d1	d1 - d2
piloerection	d1 - d3		d1 - d2
reduced general state	d1	d1	

d0 = post-exposure on exposure day
h = hour
d = day
n.d. = not detected

No clinical observations were performed on public holidays or weekend by test groups 1 and 3.

5.2.2. Body weight

Mean body weight for all animals of all groups on the day of exposure: male animals ($\bar{x}$ ± sd) 293 ± 10.1 g, female animals ($\bar{x}$ ± sd) 204 ± 12.7 g. The exact body weight can be seen from the raw data.

Mean body weight	Male			Female		
	Day of exposure	After 7 days	After 14 days	Day of exposure	After 7 days	After 14 days
Test group 1 Weight in g Number of animals	283 5	281 5	328 5	191 5	201 5	210 5
Test group 2 Weight in g Number of animals	295 5	+	+	207 5	+	+
Test group 3 Weight in g Number of animals	301 5	+	+	215 5	+	+
Historical (air) control Weight in g	248	286	318	177	196	211

+ = all animals dead

5.3. Results of analytical measurements

5.3.1. Concentration measurements

The calibration curve of the substance in the solvent used had a linearity range from 14.5 to 33.9 mg/50 ml.

Each individual sample was analyzed. The following individual values for the concentration of the test groups were obtained: using the routinely prepared on-point-calibration.

Test group 1

Sample No.	Analyt. concentration mg/l
11	2.20
12	2.31
13	2.26
14	2.23
Mean	2.25
Fritted glass flask 15	0.0
Mean + fritted glass flask	2.25
Mean (rounded)	2.25
Standard deviation of the mean	± 0.047
Nominal concentration	2.61

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Test group 2

	Sample No.	Analyt. concentration mg/l
	6	4.70
	7	5.06
	8	4.90
	9	6.08
Mean		5.19
Fritted glass flask 10		0.01
Mean + fritted glass flask		5.20
Mean (rounded)		5.2
Standard deviation of the mean		± 0.61
Nominal concentration		5.9

Test group 3

	Sample No.	Analyt. concentration mg/l
	1	30.74
	2	20.68
	3	20.72
	4	23.26
Mean		23.85
Fritted glass flask 5		0.020
Mean + fritted glass flask		23.87
Mean (rounded)		23.9
Standard deviation of the mean		± 4.75
Nominal concentration		25.2

5.4. Statistical evaluation

LC_{50} values, 4-hour exposure

LC_{50} (male and female rats): 2.25 < LC_{50} < 5.2 mg/l

The statistical reliability is 99%.

5.5. Pathology

Necropsy findings:

Animals that died spontaneously (♂ + ♀):

Test groups 2 and 3 (5 ♂ and 5 ♀):
General congestion

Test group 2 (5 ♂ and 5 ♀):
Lungs: focal hyperemia

Test group 2 (2 ♂ and 5 ♀):
Gastrointestinal tract: empty

Test group 3 (5 ♂ and 4 ♀):
Lungs: focal atelectasis in all lobes;
bloody edema (1 ♀), bronchi: edema (5 ♂ and 4 ♀),
intensified hydrothorax

Microscopic findings:

Test group 3 (1 ♀):
Lung: extensive hyperemia
Kidneys: marked necrosis in the proximal part of the tubulus
Liver: centrolobular swelling of hepatocytes

Test group 2 (1 ♂):
Liver: peripheral swelling of hepatocytes
Lungs: moderate emphysema, focal hyperemia

Sacrificed animals:

Test group 1 (5 ♂ and 5 ♀):
No pathologic findings.

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6. LITERATURE

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