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TNO-report V94.692

Acute (4-hour) inhalation toxicity study with CGA 329'351 in rats

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At the request of:
Ciba-Geigy Ltd., Basle
Switzerland

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943140

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354687

Study director:
5.1.2.e Woo

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20

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Summary

1. The acute inhalation toxicity of **CGA 329'351** was studied by nose-only exposure of one group of five male and five female rats to a test atmosphere containing 2.29 ± 0.03 g/m^3 **CGA 329'351** for a single 4-hour period. After exposure, the rats were kept for a 14-day observation period.

2. Slight shallow breathing was observed in all rats during the first three hours of exposure; clear restlessness was seen from the second hour of exposure and onwards. Two female rats additionally showed slight visually decreased breathing frequency during the last hour of exposure. Shortly after exposure, hunched appearance was seen in four female rats; slight visually decreased breathing frequency and incoordination were seen in one female. No abnormalities were seen during the 14-day observation period, except for a fatty and yellow discoloured fur and a small alopecic area in one female rat.

3. No abnormalities in body weight gain were observed.

4. At necropsy, no abnormalities were found.

5. From the results of the present study, it was concluded that the 4-hour LC50 value of **CGA 329'351** was higher than 2.29 g/m^3.

Contents

Statement of GLP compliance

We, the undersigned, hereby declare that this report constitutes a true and complete representation of the procedures followed and of the results obtained in this study by TNO Nutrition and Food Research Institute, and that the study was carried out under our supervision.
The study was carried out in accordance with the OECD Principles of Good Laboratory Practice.

5.1.2.e Woo

Ir 5.1.2.e Woo
(Study director) Date 3 January 1995

5.1.2.e Woo

Dr 5.1.2.e Woo
(Management) Date 3 January 1995

Quality Assurance Statement

On : Acute (4-hour) inhalation toxicity study with **CGA 329'351** in rats

Report Number : V94.692

Date : January, 1995

The experimental phase of this study was inspected by the Quality Assurance Unit of TNO Nutrition and Food Research Institute as follows:

Date of inspection:
1 December 1994

Date of report:
1 December 1994

This report was audited as follows:

Date of audit:
27 December 1994

Date of report:
27 December 1994

I, the undersigned, hereby declare that this report provides an accurate record of the procedures employed and the results obtained in this study; all inspections were reported to the study director and the management on the dates indicated.

5.1.2.e Woo

Ing. 5.1.2.e Woo
(Quality Assurance Officer)

Date: 3 January 1995

STAATSTOEZICHT OP DE VOLKSGEZONDHEID

VETERINAIRE HOOFDINSPECTIE

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE

Pursuant to the Netherlands GLP Compliance Monitoring Programme a general inspection for compliance with the OECD Principles of Good Laboratory Practice was carried out on February 2-12, 1993 at the

TNO Toxicology and Nutrition Institute
Department of Biological Toxicology
and
Department of Experimental Biology
(Clinical Chemistry and Biotechnique Sections)
Utrechtseweg 48
3704 HE ZEIST, The Netherlands.

As a result of this inspection it is herewith confirmed that the afore-mentioned departments are currently operating in compliance with the OECD Principles of Good Laboratory Practice.

Rijswijk, March 22, 1993

5.1.2.e Woo
[redacted]

Central Veterinary
Public Health Inspectorate

Testing facility

The toxicity study was conducted by:

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1 Introduction

At the request of Ciba-Geigy Ltd., Basle, Switzerland, an acute (4-hour) inhalation toxicity study with **CGA 329'351** was carried out in rats.

2 Experimental

The study was conducted according to a protocol, entitled: "Protocol for an acute (4-hour) inhalation toxicity study with **CGA 329'351** in rats", approved by the study director on 1 November 1994.

The protocol had been drafted in accordance with:

- EEC Directive 92/69/EEC, Annex, Part B: Methods for the determination of toxicity, B.2. Acute inhalation toxicity, dated 31 July 1992,
- OECD Guideline for Testing of Chemicals no. 403, Acute inhalation toxicity, adopted 12 May 1981 and,
- Test Guideline 81-3 of subdivision F of the EPA Pesticide Assessment Guidelines, which support the data requirements of 40 CFR (1989) part 798.1150, Acute inhalation toxicity.

2.1 Test material

The test material was supplied by the sponsor. One plastic bottle, containing ca. 1 kg of test material was received on 9 November 1994. The test material was stored at room temperature.

CGA 329'351 is a viscous, brown liquid and has the following characteristics (as given by the sponsor):

Batch no.	: OP.4
TNO internal reference number	: 94-1586
Purity	: 97.1%
Vapour pressure	: 2.9 x 10E-3 Pa (at 20°C)
	5.2 x 10E-3 Pa (at 25°C)
Density	: 1.125 kg/l at 20°C
Storage conditions	: room temperature

2.2 Test animals

The animals used were male and female SPF-reared, Wistar derived (Crl:WI(WU)BR) rats delivered by 5.1.2.e Woo, Wiga, Sulzfeld, Germany. The animals arrived on 8 November 1994, at an age of ca. 5-6 weeks. They were taken in their unopened shipping containers to animal room 15.05, were checked for overt signs of ill health and anomalies, and were kept in quarantine for 3 days. After approval of the lot (negative titers to microorganisms tested), they were transferred on 11 November 1994 to the inhalation facilities to animal room 06.15; 5-6 rats were randomly allocated to the cages, separated by sex and uniquely identified by ear tattoo. Total duration of the acclimatization period in the animal room was 20 days.
Just before the start of the exposure (1 December 1994), 5 male and 5 female rats were assigned to the study; mean body weights of the male and female rats were 297 g and 197 g, respectively. The group of animals was identified by a letter and a colour code. Within the group the individual animals were identified by earmark (tattoo).

2.3 Experimental conditions

2.3.1 Maintenance

During exposure the animals were deprived of food and water and housed individually in the holders.
Immediately after the exposure, the animals were returned to their living cages, 5 males or 5 females to a cage, and were held for an observation period of 14 days.

During the quarantine period animals were housed in room 15.05. During the remainder of the study, except during exposure, the animals were housed in animal room 06.15. They were housed under conventional conditions in suspended stainless steel cages fitted with wire-mesh floor and front. The number of air changes was about 10 per hour. The temperature was between 20.0 and 23.0 °C, relative humidity was between 38 and 68%. A 12-hour light and 12-hour dark cycle was maintained.

2.3.2 Diet and drinking water

All rats received the Institute's powdered, cereal-based rodent diet as well as tap water.
Each batch of this diet is analysed by the supplier (SDS Special Diets Services, Witham, England) for nutrients and contaminants. Results are made available to TNO Nutrition and Food Research Institute. Potable water for human consumption (quality guidelines according to Dutch legislation based on EEC Council Directive 80/778/EEC) was supplied by N.V. Waterleidingbedrijf Midden-Nederland (WMN). Results of the routine physical, chemical and microbiological examination of drinking water as conducted by the supplier are made available to TNO Nutrition and Food Research Institute. In addition, WMN periodically analyses water samples taken on the premises of TNO in Zeist for a limited number of variables.

2.4 Experimental procedures

The study was started on 1 December 1994 with one group consisting of 5 male and 5 female rats. This group was exposed by inhalation to a target concentration of at least 2 g/m^3. Since none of the rats died during exposure at this maximum attainable level or during the subsequent 14-day observation period, no more animals were exposed. The study was finished with the necropsy of the rats on 15 December 1994.

2.5 Exposure chamber

Animals were exposed to the test atmosphere in a nose-only inhalation chamber, a modification of the chamber manufactured by ADG Developments Ltd., Codicote, Hitchin, Herts. SG4 8UB, United Kingdom (see Figure 1). The inhalation chamber consisted of a cylindrical aluminium column, surrounded by a transparent cylinder. The column had a volume of ca. 50 l and consisted of a top assembly with two mixing chambers, underneath a rodent tube section and the exhaust section at the bottom. The rodent tube section had 20 ports for animal exposure. Two ports were used for test atmosphere sampling, particle size analysis, and/or measurement of oxygen concentration, temperature and relative humidity. The animals were secured in plastic animal holders (Battelle), positioned radially through the outer cylinder around the central column. The rats of each group were placed in an alternating order (male, female etc.). The remaining ports were

closed. Only the nose of the rats protruded into the interior of the column.
In our experience, the animal's body does not exactly fit in the animal holder which always results in some leak from high to low pressure side. By securing a positive pressure in the central column and a slightly negative pressure in the outer cylinder, which enclosed the entire animal holder, dilution of test atmosphere by air leaking from the animal's thorax to the nose was prevented.

The air flow was recorded eight times during exposure at regular intervals, by reading the pressure settings of the nebulizer (see section 2.4) and the rotameter (water mist in air for humidification). The settings of the nebulizer did not change during exposure (34 l/min), the mean dilution flow was 3.9 ± 0.1 l/min (total flow of 38 l/min).

Both temperature and relative humidity were monitored using a dry and wet bulb thermometer. They were recorded eight times at regular intervals (twice per hour) during the 4-hour exposure period. The mean temperature was 21.6±0.5 °C, the mean relative humidity was 65 ± 5 %. Oxygen concentration was checked twice during exposure and was 21.0 ± 0%.

2.6 Generation of the test atmosphere

The inhalation equipment was designed to expose rats to a continuous supply of fresh test atmosphere.
The test atmosphere was generated by nebulizing the test material into small droplets by using a compressed air driven nebulizer of the Institute's design. The nebulizer consisted of an atomizer and a glass jar, containing the test material. The atomizer coded DR 011 was purchased from Lechler (Germany).
To obtain a sufficiently large amount of respirable particles it proved to be necessary to reduce the viscosity by slightly heating the test material. In consultation with the sponsor, it was decided to use a temperature of ca. 50 °C. Therefore, the jar with the test material was placed in a water bath heated at 51 °C (recorded twice per hour).
The nebulizer was operated at a pressure of 3.0 bar. During operation, the test material was drawn through a suction tube into the atomizer. The spray thus generated was blown against a baffle which was fitted below the nozzle orifice in such a way that the larger droplets were removed by impaction. The impacted test material drained back into the test material supply at the bottom of

the jar. The resulting aerosol was passed to the inlet of the exposure unit where it was mixed with metered amounts of water mist in air which was recorded using a rotameter (once each 30 min). From there, the test atmosphere was directed downward through the mixing chambers towards the animals. At the bottom of the unit the test atmosphere was exhausted (see also Figure 1).

Before the start of the exposure, the rate of airflow through the nebulizer was established at the pressure used. The pressure settings were recorded at regular intervals (each 30 minutes), but they did not change (3.0 bar; see also section 2.5). In this way, the total exposure airflow was monitored indirectly through the aerosol generation system.

Animals were placed in the exposure unit 30 minutes after the start of exposure.

2.7 Analysis of the test atmosphere

2.7.1 Actual concentration

The actual concentration in the test atmosphere was determined four times (once each hour) by means of gravimetric analysis.
During preliminary experiments the amount of evaporation of the test material was checked as follows: A measured amount of test material was put on a glass fiber filter. Forced evaporation was obtained by passing dry pressurized air at ca. 5 l/min through the filter. At certain air volumes (25, 50, 75, 100 and 200 l) the weight of the filter was determined. No loss of weight was noticed, therefore, no evaporation had occurred.

During the exposure, representative samples were obtained by passing test atmosphere at ca. 5 l/min through fiber glass filters (Sartorius, diameter=45 mm). Before sampling the filters were weighed; immediately after sampling the filters were weighed again. The actual concentration was calculated by dividing the amount of test material present on each filter by the volume of the test atmosphere sample taken.

2.7.2 Nominal concentration

The nominal concentration was determined by dividing the total amount of test material used by the total volume of air passed through the inhalation chamber.

2.7.3 Particle size measurement

Particle size distribution measurement was carried out once during exposure using an 11-stage cascade impactor (Institute's design).

2.8 Observations and measurements

2.8.1 Clinical observations

The rats were visually inspected just before exposure, for reactions to treatment during the exposure, shortly after exposure, and at least once daily during the observation period.

2.8.2 Body weights

Body weights were recorded just prior to exposure (day 0), and on days 7 and 14.

2.8.3 Necropsy

At the end of the 14-day observation period, all rats were killed by exsanguination from the abdominal aorta under ether anaesthesia. All rats were necropsied and examined for gross pathological changes.

2.9 Retention of records

Raw data, the master copy of the final report and all other information relevant to the quality and integrity of the study will be retained in the archives of TNO Nutrition and Food Research Institute for a period of at least 15 years after submission of the final report.

2.10 Deviations from the protocol

- Test Guideline 81-1 of subdivision F of the EPA Pesticides Assessment Guidelines should read 81-3.
- The test material was not colourless but brown, which was confirmed by the sponsor.
- The relative humidity in the test atmosphere was 65 ± 5%, viz. slightly higher than the stipulated range of 40-60%.

These deviations were not considered to have influenced the outcome of the study.

3 Results

3.1 Analytical results

3.1.1 Actual concentration

The actual concentration of **CGA 329'351** during the exposure based on gravimetric analyses is given in Table 1. Mean concentration was 2.29 ± 0.03 g/m^3.

3.1.2 Nominal concentration

The nominal concentration was calculated to be 2.62 g/m^3 indicating a generation efficiency of ca. 88%.

3.1.3 Particle size measurement

The particle size distribution is given in Table 2. Particle size measurement during exposure to **CGA 329'351** showed that almost all particles in the animals' breathing zone were of the respirable size range. The Mass Median Aerodynamic Diameter (MMAD) was calculated to be 2.1 μm with a mean Geometric Standard Deviation (GSD) of 1.4 μm.

3.2 Behaviour, clinical signs and mortality

Observation of the rats was limited during exposure due to the stay in restraining tubes; however, slight shallow breathing was observed in all rats during the first three hours of exposure. Clear restlessness was observed in all rats from the second hour of exposure and onwards. Two female rats (nos. 85 and 91) additionally showed slight visually decreased breathing frequency during the last hour of exposure. Shortly after exposure, hunched appearance was seen in four female rats (nos. 81, 89, 91, 93); slight visually decreased breathing frequency and incoordination were seen in one female rat (no. 91).
No abnormalities were seen during the 14-day observation period, except for one female rat (no. 85) that showed a fatty and yellow discoloured fur during days 1-3 and a small alopecic area on the right front limb during days 4-12.

3.3 Body weights

No abnormalities in body weight gain were observed (Table 3).

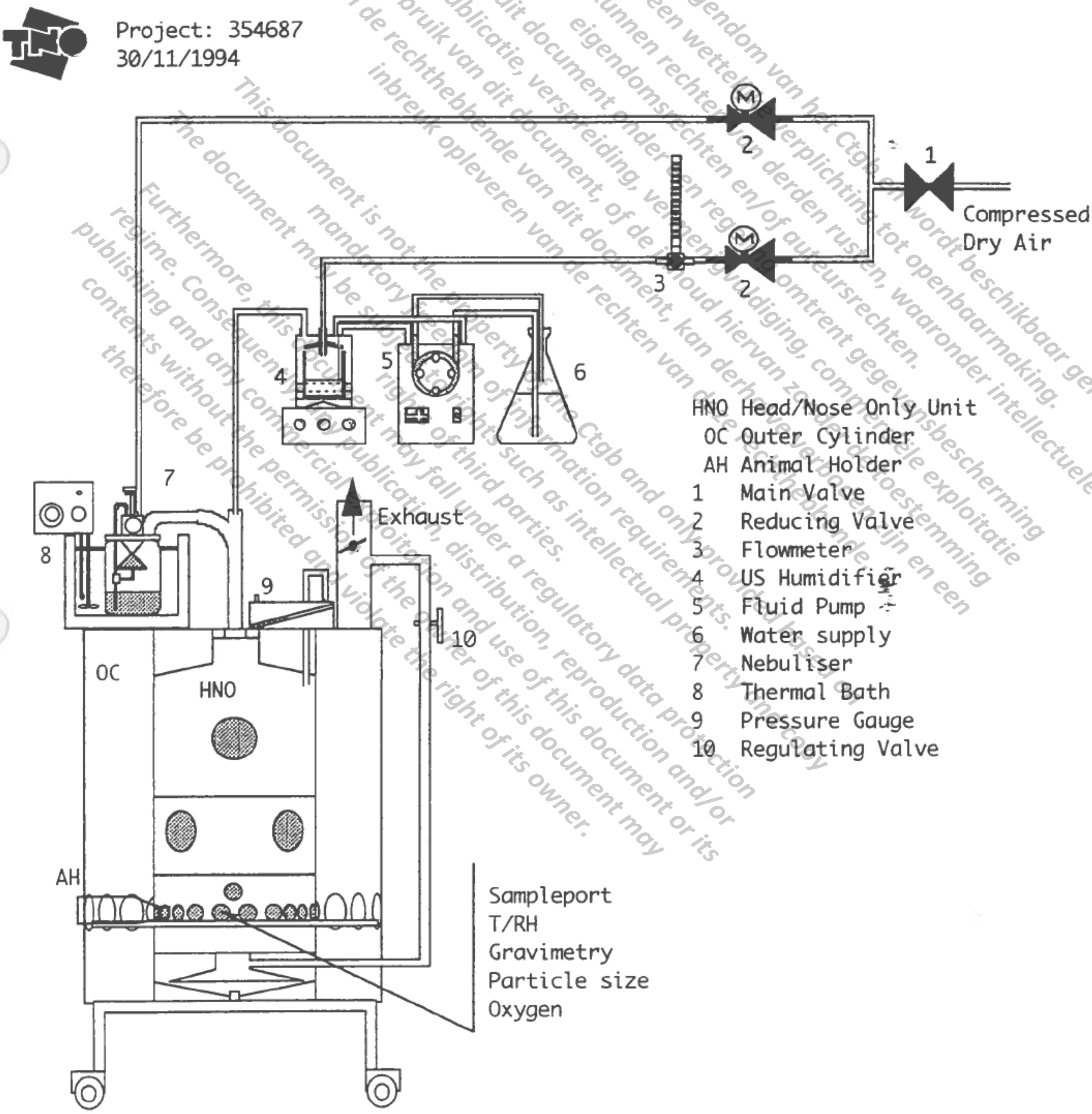
3.4 Necropsy

No abnormalities were seen at necropsy.

4 Discussion and conclusion

The aim of the present study was to determine the 4-hour LC50 value of **CGA 329'351**. Hereto one group of 5 male and 5 female rats was exposed to the highest attainable concentration, viz. 2.29 ± 0.03 g/m^3 **CGA 329'351** during a single period of four hours. Since none of the rats died at this concentration level, the 4-hour LC50 value was higher than 2.29 g/m^3.

Figure 1 - Schematic diagram of the generation and exposure system



CGA 329'351 ACUTE (4-HOUR) INHALATION RAT

TNO Nutrition and Food Research Institute

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Table 1 - Actual concentration measured by gravimetric analysis

Sample time (h)	Volume sampled (l)	Aerosol concentration (g/m^3)
10.30	10	2.31
11.30	10	2.32
12.30	10	2.26
13.30	10	2.28
mean±sd		2.29±0.03

CGA 329'351 ACUTE (4-HOUR) INHALATION RAT

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Table 2- Aerodynamic particle size distribution of CGA 329'351 in test atmosphere

Particle size (μm)	Weight (mg)	Distribution (%)
<1.0	0.08	0.6
1.0	0.39	2.8
1.4	1.39	9.9
1.8	3.34	23.8
2.4	3.94	28.0
2.8	2.22	15.8
3.1	1.22	8.7
3.4	0.98	7.0
3.8	0.39	2.8
4.2	0.08	0.6
>4.2	0.02	0.1
total	14.05	~100 %

Gasvolume used for cascade sample was 8 l; sample flow was 5.4 l/min.

Mass Median Aerodynamic Diameter (MMAD): 2.1 μm

mean Geometric Standard Deviation (GSD): 1.4 μm

CGA 329'351 ACUTE (4-HOUR) INHALATION RAT

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Study:354687

Table 3- Individual and mean body weights of male and female rats exposed to **CGA 329'351** test atmosphere during four hours

Animal No.	Day 0	Day 7	Day 14
MALES			
66	300.7	323.2	346.7
70	298.2	318.0	338.0
72	293.2	311.5	341.7
76	298.0	323.4	355.1
80	292.4	319.3	341.9
mean ± sd	296.5±3.6	319.1±4.9	344.7±6.6
FEMALES			
81	194.1	206.0	211.7
85	199.5	205.5	213.3
89	203.5	210.9	224.6
91	196.5	203.5	214.3
93	193.4	202.2	208.2
mean ± sd	197.4±4.2	205.6±3.3	214.4±6.1