

8ENQ-0695-13470



**SOLVAY
AMERICA**

June 26, 1995



INIT 86/27/95

RECEIVED
OPPT CBIC

95 JUN 27 AM 10: 53

United States Environmental
Protection Agency
Document Processing Center (TS-790)
Office of Toxic Substances
Room 105, East Tower
401 M Street, S.W.
Washington, D.C. 20460

By Federal Express

**ORIGINAL
Contains No CBI**

Attention: TSCA Section 8(e) Coordinator

Re: Solvay Performance Chemicals -- TSCA Section 8(e)
-- 3-chloro-1-2-propane diol ("Alpha-chlorohydrin")



88950000254

Dear Sir:

This letter and the enclosures are being submitted on behalf of Solvay Performance Chemicals (the "Company"), pursuant to Section 8(e) of the Toxic Substances Control Act, as amended. The Company recently received the enclosed unpublished studies from Solvay Duphar B.V., one of our European affiliates. A copy of each of the following studies is enclosed:

1. Waalkens-Berendsen, D.H.; Arts, J.H.E., (1992), *Final report for an acute 2-week inhalation toxicity and male fertility study with Alpha-chlorohydrin in rats*, TNO-Report No. V 91.298; and
2. Waalkens-Berendsen, D.H.; Arts, J.H.E., (1992), *Final report for an acute 6-hour inhalation toxicity and male fertility study with Alpha-chlorohydrin in rats*, TNO-Report No. V 90.014.

The Company wishes to bring the enclosed information to the attention of the Environmental Protection Agency (the "EPA") because it is not aware of any other similar study results involving the route of inhalation for Alpha-chlorohydrin, and the unpublished nature of the information makes it unlikely that the information has otherwise been brought to the EPA's attention.

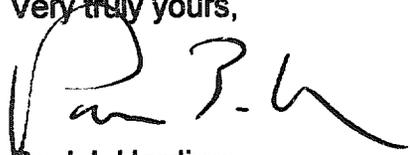
RECEIVED
OPPT-NCIC
95 JUN 29 AM 9: 11

U.S. Environmental Protection Agency
June 26, 1995
Page 2

Please acknowledge your receipt of the enclosures by signing, date stamping and returning the copy of this letter in the enclosed prepaid Federal Express envelope.

If you should have any questions concerning this submission, please do not hesitate to contact the undersigned at (713) 525-6026.

Very truly yours,

A handwritten signature in black ink, appearing to read "Paul J. Harding". The signature is fluid and cursive, with a large initial "P" and "H".

Paul J. Harding
Attorney

PJH/jlv

Enclosures

95 JUN 27 AM 10: 54

Report number V 90.014

Final report for an acute 6-hour inhalation toxicity and male fertility study with α -chlorohydrin in rats

Nothing from this issue may be reproduced and/or published by print, photoprint, microfilm or any other means without previous written consent from TNO. Submitting the report for inspection to parties directly interested is permitted.

In case this report was drafted under instruction, the rights and obligations of contracting parties are subject to either the 'Standard Conditions for Research Instructions given to TNO' or the relevant agreement concluded between the contracting parties on account of the research object involved.

© TNO

Authors:

Ir D.H. Waalkens-Berendsen
Ir J.H.E. Arts

At the request of:

Solvay & Cie

Project no.:

B89-8106

Study no.:

1234

Contains No CBI

Solvay Duphar Int. Doc. no.:

56645/62/91

Report no.:

S.9104

Date:

January, 1992

Number of pages:

63

Number of figures:

1

Number of tables:

14

Number of annexes:

5

Number of appendices:

8

SUMMARY

1. A single dose 6-hour inhalatory exposure study with α -chlorohydrin (0 and 16.7 ppm) was carried out in male and female rats, with special attention to male fertility. The male rats were mated with untreated females directly after exposure and after a period of 7 and 14 days. All exposed animals were killed after 14 days. The number of implantations in the unexposed mated females were counted on day 11 of pregnancy.
2. No clinical signs or mortalities were observed during exposure or during the recovery period.
3. No adverse effect of α -chlorohydrin on body weight was observed.
4. Mean absolute organ weights (lungs, kidneys, testes and epididymides) and organ weights relative to body weight were similar in both groups.
5. The number of matings immediately after exposure was decreased in both the control and the α -chlorohydrin group. This effect was most probably due to stress by the exposure and handling procedures. No effects on fertility or reproduction parameters were observed during the matings after a recovery period of 7 and 14 days.
6. On the basis of the results obtained in the present study it is concluded that:
 - no toxic effects can be shown after single exposure for 6 hours of male and female rats to 16.7 ppm α -chlorohydrin by inhalation
 - no adverse effects on male fertility can be shown after single exposure for 6 hours to 16.7 ppm α -chlorohydrin by inhalation.

	SUMMARY	2
	CONTENTS	3
	STATEMENT OF GLP COMPLIANCE	5
	STATEMENT OF THE TNO QUALITY ASSURANCE UNIT	6
	GOOD LABORATORY PRACTICE STATEMENT OF COMPLIANCE	7
	CONTRIBUTORS	9
1	INTRODUCTION	10
2	PROCEDURES	11
2.1	Test substance	11
2.2	Animals and animal identification	11
2.3	Group sizes, frequency and duration of administration of the test material	12
2.4	Route of administration of test material	13
2.5	Exposure chambers	13
2.6	Maintenance	13
2.7	Exposure level	14
2.8	Generation of test atmosphere	14
2.9	Test atmosphere control	14
2.10	Mating procedures	17
2.11	Observations, analyses and measurements	17
2.12	Statistics	20
2.13	Deviations of the protocol	20
3	RESULTS	21
3.1	Analytical results	21
3.2	Clinical signs and mortalities	22
3.3	Body weight	22
3.4	Mating and reproduction data	22
3.5	Organ weight	23
3.6	Pathology	23
4	CONCLUSIONS	24
5	RETENTION OF RECORDS, SAMPLES AND SPECIMEN	25
6	REFERENCES	25

TABLES

1 Individual and mean concentrations of α -chlorohydrin in the test atmosphere determined by gas chromatographic analysis; samples were taken at the inlet of the cylinder	26
2 Values obtained by total carbon analysis during exposure of rats to α -chlorohydrin. Monitoring was performed continuously halfway the cylinder, checks were made at the inlet and outlet of the cylinder	27
3 Mean parental body weights (males)	28
4 Mean parental body weights (females)	29
5 Mean parental body weight change (males)	30
6 Mean parental body weight change (females)	31
7 Summary of cohabitation, first mating	32
8 Summary of cohabitation, second mating	33
9 Summary of cohabitation, third mating	34
10 Summary of C-section data	35
11 Mean organ weights (males)	36
12 Mean organ weights (females)	37
13 Mean organ weights relative to terminal body weight (males)	38
14 Mean organ weights relative to terminal body weight (females)	39

FIGURE

1 Schematic diagram of the generation and exposure system	40
---	----

ANNEX

1 Percentage composition of cereal-based Civo-stock diet	41
2 Nutrient composition of cereal-based Civo-stock diet	42
3 Contaminants regularly determined in cereal-based Civo-stock diets and in Civo-tapwater	43
4 Levels of contaminants in cereal-based Civo-stock diets	44
5 Levels of contaminants in Civo-tapwater	45

APPENDICES

1 Individual parental body weights	46
2 Individual parental body weight change	51
3 Mating list	55
4 Individual female reproduction data and mean fetal weight data, first mating	56
5 Individual female reproduction data and mean fetal weight data, second mating	58
6 Individual female reproduction data and mean fetal weight data, third mating	60
7 Individual body and organ weights (males)	62
8 Individual body and organ weights (females)	63

STATEMENT OF GLP COMPLIANCE

We the undersigned hereby declare that the report following constitutes a true and faithful account of the procedures adopted and the results of this study. The study that was conducted by the Department of Biological Toxicology of the TNO-Toxicology and Nutrition Institute was performed in accordance with the current OECD Good Laboratory Practice Principles. Gas chromatographic analysis is excepted from this statement.

SUBMITTED BY:

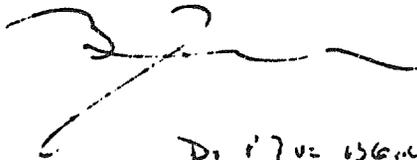

Ir D.H. Waalkens-Berendsen
Study Director/Reproduction Toxicologist

Date: *January 21, 1992*



Ir J.H.E. Arts
Inhalation Toxicologist

Date: *January 21, 1992*



8/6
Drs H.B.W.M. Koëter
For the management
Head of the Section of Reproduction and Genetic Toxicology
Dept. of Biological Toxicology

Date: *January 21, 1992*

TNO QUALITY ASSURANCE UNIT (TOXICOLOGY)
P.O. Box 360, 3700 AJ ZEIST, Netherlands

QUALITY ASSURANCE STATEMENT

On : An acute 6-hour inhalation toxicity and male fertility
study with α -chlorohydrin in rats

Report number: V 90.014

Date : January, 1992

Inspections were made of the various phases of this study and, in the case of repetitive operations, at pre-determined intervals. The dates on which the inspections were made and the dates on which the findings were reported to the Study Director and to Management are given below.

Date of inspection:

Date of reporting:

November 16, 1989

November 16, 1989

November 28, 1989

November 28, 1989

December 4, 1989

December 4, 1989

June 27-28, 1991

July 1, 1991

The final draft report has been audited according to the appropriate Standard Operating Procedure and is considered to be an accurate presentation of the methods and procedures employed and an accurate presentation of the findings.

Drs S. van Straten

QA Manager

Signature:

Date:



January 2, 1992



STAATSTOEZICHT OP DE VOLKSGEZONDHEID
VETERINAIRE HOOFDINSPECTIE

GOOD LABORATORY PRACTICE
STATEMENT OF COMPLIANCE

General inspections for compliance with the OECD Principles of Good Laboratory Practice were carried out at the

Department of Biological Toxicology
Institute CIVO-Toxicology and Nutrition TNO
Utrechtseweg 48
3704 HE Zeist
The Netherlands

June 15-26, 1987;
December 21-22, 1987;
September 12-19, 1988 and
February, 14-17, 1989

as part of the Netherlands GLP Compliance Monitoring Programme.

Based on the latter inspection it is confirmed that the aforementioned laboratory is currently operating in compliance with the Principles of Good Laboratory Practice.

Rijswijk, April 19, 1989



W. H. Koenigmann
Public Health Officer in General Service
(Section GLP)



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 March 4-12, and May 7, 1991 at the

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

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



Rijswijk, June 6, 1991

Drs Th. Heider

Central Veterinary
Public Health Inspectorate
(Section GLP)

CONTRIBUTORS

Major contributions to this study were made by:

Study director	: Ir D.H. Waalkens-Berendsen
Deputy study director	: Dr A.E. Smits-van Prooije
Assistant study director	: Mrs M.W. van Marwijk
Inhalation toxicologist	: Drs P.G.J. Reuzel
	: Ir J.H.E. Arts
Senior inhalation technician	: Mr R.A. van Bommel
Inhalation technician	: Mr F. Hendriksma
Research Assistants	: Mrs M. Balvers-van den Adel
	Mrs M.F. Hollanders-Krakowczyk
	Mr P.D. van den Heuvel
Biotechnicians	: Mrs A. Brand
Records keeping	: Mrs R. Dekker
Quality Assurance Manager	: Drs S. van Straten
Management	: Drs H.B.W.M. Koëter
Statistics	: Drs A.J.M. Hagenaars
Veterinary care	: Dr M.H.M. Kuypers

1

INTRODUCTION

At request of Solvay & Cie an acute 6-hour inhalation toxicity and male fertility study with α -chlorohydrin in rats was performed. The study was conducted according to the protocol dated October 31, 1989. The study was performed as far as applicable in accordance with the OECD Guidelines for testing of chemicals no.403, adopted 12 May, 1981.

The following time table was applied:

Arrival of animals	: 7 November, 1989
Start of the study	: 16 November, 1989
End of the in-live part	: 12 December, 1989
Interim results available	: 21 December, 1989
Interim report available	: February, 1990
QA-audited draft report	: July 3 1991
Second draft report	: October 21, 1991
Final report	: January, 1992

2 PROCEDURE

2.1 Test substance

Two brown-glass bottles each containing approximately 1000 ml of α -chlorohydrin have been received from Duphar B.V., Weesp, the Netherlands on 11 October, 1989.

Each of the bottles was coded with a number (TCE89J10A and TCE89J10B). The bottle coded TCE89J10A was used for the study.

Characteristics as provided by the sponsor:

Active ingredient	: α -chlorohydrin (3-chloro-1-2-propane diol)
Ratio of enantiomers	: R-enantiomer : S-enantiomer = 50 : 50 (+ 1)
CAS-number	: 96-24-2
General appearance	: colourless liquid
Molecular weight	: 110.54
Vapour pressure at 298 K	: 2.2 Pa
Saturated vapour level	
at 25 °C	: 22 ppm
Boiling point	: 486 K at 101.3 kPa
Melting point	: 233 K
Dynamic viscosity	: 215 mPa.s at 293 K
Log P o/w	: -0.25 (calculated)
Explosive limits	: 11 - 32 % (v/v)
Density of the liquid	: 1.32 at 293 K
Purity	: > 99.5%
Storage	: at 4-6 °C

2.2 Animals and animal identification

2.2.1 Animals

Fifteen males and 160 females SPF-reared, Wistar derived rats were obtained from a colony at Charles River Wiga GmbH, Sulzfeld, FRG. At arrival on November 7, 1989 the age of the males was about 17 weeks and the age of the

females was about 13 weeks. The animals were checked for overt signs of ill health and anomalies immediately upon arrival. There was an acclimatization period of 9 days.

The inhalation part of the study was carried out with ten young male rats of proven fertility and ten young adult virgin female rats. For mating of the exposed males another 30 females were used. These females were selected for pro-oestrus from 150 females.

2.2.2 Animal identification and allocation procedure

Identification of the exposed animals was as follows: Each group of animals was given a letter and colour code. Within each group the animals were individually identified by cage number and ear tattoo number. The females used for mating were identified with an ear tattoo number. Females used for selection for mating were identified by cage number and a ear tattoo number (odd numbers from 1-299).

The animals were allocated to the groups according to a randomization list based on body weight provided by a computersystem.

2.3 Group sizes, frequency and duration of administration of the test material

The study was identified as computer assay 1234.

The inhalation part of the study comprised two groups, one control group and one group exposed to α -chlorohydrin, both groups consisting of 5 males and 5 females. The rats were exposed to the test substance for one single period of six hours.

Dose group	Exposure level	No. of males (animal no.) ¹	No. of females (animal no.) ²	Colour code
A	control	5 (A 2-10)	5 (A 1-9)	white
B	high dose	5 (B12-20)	5 (B11-19)	blue

¹ males were identified by even numbers

² females were identified by odd numbers

In addition, 150 adult virgin females, which were not allocated to any of the groups and were not exposed to the test substance, were used for mating with treated males.

2.4 Route of administration of test material

The test material was administered to the animals by inhalation. This route of administration was chosen because possible human exposure to the test material at the workplace is by inhalation.

2.5 Exposure chambers

Animals were exposed total body in horizontally placed glass tube inhalation chambers of the Institute's design with a capacity of 15 L each. In each chamber 10 animals were exposed. The animals were housed individually. The position of the animals within the chambers was noted. The all glass construction of the chamber enables observation of the animals during exposure. Before the exposure was started the animals were allowed to acclimatize for at least half an hour.

The total air flow through the chambers was about 14 L/min.

The chambers were illuminated externally by normal laboratory lighting. Relative humidity and temperature in the exposure chambers were determined each hour by means of a dry and wet bulb thermometer. At the outlet of the cylinder the temperature was maintained between 24 and 25 °C and the relative humidity varied between 43-49 % and 49-63 % for the control and exposure cylinder, respectively.

2.6 Maintenance

Before and after exposure, the rats were housed under conventional conditions in an animal room in suspended stainless steel cages (45x32x18cm) fitted with wire-mesh floor and front. Immediately after exposure, the animals were returned to their living cages (males individually and females as five per cage) and were held for an observation period of 14 days. The temperature and relative humidity in the animal holding room were controlled at 22± 3°C and 30-70%, respectively. Lighting was artificial with a sequence

of 12 hours light (on 7.30 a.m.; off 19.30 p.m.) and 12 hours dark. The number of air changes was about 10 per hour. During the exposure the animals had no access to food or water. After exposure, the animals were provided ad libitum with the Institute's stock diet and with unfluoridated tap water from an automatic drinking water system. The percentage composition of the stock diet is given in Annex 1. The levels of nutrients in the stock diet are periodically determined and are given in Annex 2. The contaminants periodically determined in stock diet and in drinking water are tabulated, together with tentative maxima in Annex 3. Actual levels of contaminants in stock diet for rats and in drinking water are given in Annexes 4 and 5, respectively.

2.7 Exposure level

There was one exposure level. The concentration level of α -chlorohydrin should be as close as possible to the saturated level as provided by the sponsor (22 ppm). In order to prevent aerosol formation as a result of oversaturation the target concentration was slightly below this level.

2.8 Generation of the test atmosphere

A nearly saturated test atmosphere was generated by passing 14 L air/min through a bubbler filled with α -chlorohydrin (Figure 1). The test material in the bubbler was kept at a temperature of 35 °C. The test material laden air flow was passed through a condenser which was kept at a temperature of 23 °C to remove a possible excess of test material by condensation. Next, this flow was passed through a glass bulb, which was surrounded by heating lines in order to slightly heat the test atmosphere to vaporize possible aerosol particles. The glass bulb was placed just before the inlet of the cylinder.

2.9 Test atmosphere control

Infrared analysis

Based on information provided by the sponsor, it was concluded that infrared analysis was not appropriate to monitor the test atmosphere.

Total carbon analysis

Before the study was started, the response of the flame ionization detector (FID) of the total carbon analyser (Ratfisch) was calibrated. Calibration gases were prepared by injecting known volumes of α -chlorohydrin in Tedlar bags filled with 30 l of nitrogen. The bags were slightly heated overnight to ensure complete evaporation of the test material. However, due to the very low quantities of α -chlorohydrin used, it could not be seen whether all the test material in the bags had evaporated. In addition, it could not be ruled out that condensation had occurred during cooling down to ambient temperature. In this way it was not possible to calibrate the total carbon analyser to determine the concentration in the test atmosphere. On the other hand, gas chromatographic analysis could not easily be used as monitoring system due to the time needed for each analysis (ca. 25 min).

In co-operation with the sponsor, therefore, it was decided to use total carbon analysis for monitoring the steady state of the concentration only, and a gas chromatograph for establishing the actual concentration by analysing test atmosphere samples afterwards.

Continuous monitoring during exposure was performed at a sample point halfway the cylinder; sample checks were also taken twice at the inlet and outlet of the cylinder.

A Ratfisch RS 55 total carbon analyser with heatable sampling line was used. The settings of this analyser were as follows:

hydrogen	: 450 mbar
air	: 620 mbar
back pressure	: 200 mbar
oven temperature	: 180 °C
sampling line temperature:	160 °C

Gas chromatographic analysis

Gas chromatographic analysis was performed by the Analysis Department, TNO-Biotechnology and Chemistry Institute, Zeist, the Netherlands.

Samples were taken at the inlet of the cylinder.

Test material was collected by bubbling 33 - 60 l of the test atmosphere through one washing bottle filled with 40 ml of water. From a preliminary

experiment (without animals), it was concluded that a second impinger was not necessary, since barely any material was found in this impinger (below detection limit). Sampling rate turned out to be 1 per hour; 6 samples were obtained. After an overnight stay at c. 4°C, 1 µl aliquots of the washing bottles were injected into the gas chromatograph. All samples but one were analysed in duplo.

The gas chromatograph used was a Carlo Erba HRGC 5300 with FID (flame ionization detection).

Gas chromatographic conditions were:

- column : length 30 m, internal diameter 0.33 mm
film thickness 0.5 µm
- stationary phase : DBWax
- column temperature : initially 120 °C, raised with 10°C/min to 240 °C
- carrier : Helium
- pressure : 60 kPa
- split flow : 5 ml/min
- purge flow : 0.8 ml/min
- injector type : split
- injector temperature : 250 °C
- detector type : flame ionization
- detector temperature : 230 °C
- flow/pressure H₂ : 30 ml/min, 50 kPa
- flow/pressure air : 300 ml/min, 100 kPa
- electronic integrator: Perkin Elmer
- retention time : 8.7 min.

Calibration was performed by direct injection of a standard solution of 150 mg/l α-chlorohydrin in water onto the sampling column. This was repeated four times. The detector response (peak area) for each sample and standard solution was determined. The actual concentrations of the test atmosphere samples were related to the mean response of the standard solution.

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 cylinder.

2.10 Mating procedures (to determine male fertility)

2.10.1 Mating on day 1

At the end of the exposure period the exposed males were placed overnight with untreated young adult virgin pro-oestrus females. Matings were on a one-male to one-female basis. In the morning preceding the mating the oestrus cycle of all untreated females selected for mating purposes was determined. For this purpose vaginal smears were taken. The smears were fixed and stained according to the method of Papanicolaou (ref.1). All smears were microscopically examined for pro-oestrus. From the females which were in pro-oestrus 10 females were randomly selected and placed with the α -chlorohydrin treated or the control males. Vaginal smears were taken the next morning to determine whether mating had occurred. This day was considered to be day 0 of pregnancy. From that time on all mated females were housed individually in suspended stainless steel cages (37x21x15cm) provided with a wire-mesh floor and front. After mating, males were again housed individually.

2.10.2 Mating on day 7 and 14

On day 7 and 14 after exposure all treated and control males were allowed to mate again. The same mating procedure for selecting untreated pro-oestrus females and subsequent mating were followed as described in 2.10.1.

2.11 Observations, analyses and measurements

a. Clinical signs

The general health status of the rats was checked before the start of the exposure and during the exposure. During the 14-day observation period after exposure the rats were inspected at least once daily. All signs of ill health, reaction to treatment, and mortality were recorded.

b. Mortalities

A necropsy was to be performed on any animal dying during the study. The thoracic and abdominal cavities were to be opened by a ventral mid-line incision and the major organs were examined. Organs or tissues showing severe macroscopic abnormalities were to be removed and fixed in a buffered 4% formaldehyde solution. Microscopic examination was to be conducted if considered necessary.

c. Body weights

The body weights of all exposed animals were recorded just before the start of the exposure to the test substance or control atmosphere and weekly thereafter until scheduled kill.

d. Food and water consumption

Food and water consumption were not measured.

e. Autopsy of unexposed mated females

The unexposed mated females were killed by ether anaesthesia followed by decapitation on day 11 of pregnancy.

The following observations were made:

1. number of implantations
2. macroscopic abnormalities of major maternal organs of the abdomen and thoracic cavity

f. Autopsy of exposed males

On the day after the last mating all males were killed by exsanguination from the abdominal aorta under ether anaesthesia.

All animals were examined macroscopically for abnormalities.

The weights of the following organs were noted:

testes
epididymides
lungs
kidneys

The following organs were fixed in buffered 4% formalaldehyde solution for (possible) microscopical examination:

testes and epididymides (in Bouin's fixative)
kidneys
lungs
trachea
nose

Other tissues showing severe macroscopic abnormalities were removed and fixed in buffered 4% formaldehyde solution.

g. Autopsy of exposed females

Two weeks after the single exposure all exposed females were killed by exsanguination from the abdominal aorta under ether anaesthesia.

All animals were examined macroscopically for abnormalities.

The weights of the following organ were noted:

kidney
lungs

The following organs were fixed in buffered 4% formalin for (possible) microscopical examination:

kidneys
lungs
trachea
nose

Other tissues showing severe macroscopic abnormalities were removed and fixed in buffered 4% formaldehyde solution.

h. Histopathology of tissues of exposed animals

If fertility was affected the testes and epididymides, were to be microscopically examined. Histopathology of nasal tissue, lungs and trachea were to be performed if there was an indication of irritation during this study. Histopathology of lungs was to be performed if there was an effect on

lung weight. If there are no indications for nasal irritation during the present study, but there are during the subsequent 14-day study, the noses of the present study will also be examined microscopically.

2.12 STATISTICS

The results of this study were analysed using the following methods:

- Body weights and organ weights (absolute and relative) by the Student t-test (ref.2)
- Fertility index by the Fisher exact probability test (ref.3)
- Number of implantations by the Mann/Whitney U-test (ref.4)

As a level of significance was considered: $P < 0.05$

2.13 DEVIATIONS FROM THE PROTOCOL

Contributors

Drs P.G.J. Reuzel left the Institute in November 1990 and was replaced by Ir J.H.E. Arts as inhalation toxicologist.

Autopsy of unexposed mated females

Only the total number of implantations was counted instead of the number of live and dead implantations.

Microscopic examination of the kidneys of the exposed animals

Due to a mistake the kidneys were not microscopically examined. However, in consultation with the sponsor it was decided as no effects were observed in the 14-day study this mistake need not be rectified.

The above mentioned deviations are not considered to have influenced the integrity of the outcome of the study.

3 RESULTS

3.1 Analytical results

The individual concentration values determined by gas chromatographic analysis are given in Table 1.

The mean actual concentration measured at the inlet of the cylinder turned out to be 16.7 ± 2.7 ppm (mean \pm SD), which is about 24 % lower than the estimated saturated vapour concentration of 22 ppm. Beside the large variation in response of the standard solution injections (19%), there was also a large variation in concentration in all samples (2.7 ppm corresponds to 16 %). In addition, the difference between each duplicate measurement was generally large which resulted in a relatively low reliability of the analytical results.

Although it was not possible to measure the actual concentration using a total carbon analyser due to calibration problems, it was possible to monitor the concentration continuously using this device. Recorder outputs were obtained in scale units and are given in Table 2.

After the first hour of exposure, in which the recorder output was increasing rapidly, a more or less stable increase up to 31 scale units was obtained. The differences in output between the different cylinder locations did suggest a decrease in concentration from the inlet to the outlet. The difference between the inlet and halfway the cylinder was not large, and the decrease in values at the end of the cylinder was within the standard deviation.

The time-related increase of the concentration measured by total carbon analysis was not at all reflected in the actual concentration measured at the inlet by gas chromatographic analysis. In the latter case, a time-related increase in concentration was not observed.

Both the steadily increasing total carbon readings during the course of the experiment and the decrease in values from the inlet to the outlet measured by total carbon analysis might be explained by adsorption of test material onto the walls of the cylinder. The steadily increasing readings might also be due to adsorption and saturation onto the walls of the sample tube and the total carbon analyser.

The nominal concentration calculated by dividing the total amount of test material used by the total volume of air passed through the inhalation cylinder was 218 mg/m³ (= 47 ppm). The discrepancy between the actual and nominal concentration might be explained by adsorption of the test material onto tubing and cylinder walls.

3.2 Clinical signs and mortalities

No clinical signs or mortalities were observed during the exposure or the recovery period.

3.3 Body weight (Tables 3-6, Appendices 1 and 2)

One male of the α -chlorohydrin group (B18) lost weight during the acclimatization period and was therefore excluded from the study. Therefore, the results of the matings of this animal were also excluded.

No statistically significant difference was observed in the mean bodyweight and mean body weight changes of the exposed males and females when compared to the animals of the control group.

3.4 Mating and reproduction data (Tables 7-10, Appendices 3-6)

During the first mating immediately after 6-hour exposure only 2 of the 5 control males and 2 of the 4 α -chlorohydrin males mated with the unexposed females. One female of the control group and both females of the α -chlorohydrin group, appeared to be pregnant at autopsy on day 11 of pregnancy. No difference in the mean number of implantations was observed.

After a recovery period of 7 days all males of both the control and the α -chlorohydrin group mated overnight with the females. At autopsy on day 11 of pregnancy all females appeared to be pregnant. No statistically significant difference was observed in the mean number of implantations between both groups.

After a recovery period of 14 days all males of both the control and the α -chlorohydrin group mated overnight with the females. At autopsy on day 11 of pregnancy all females of the control group and 3 of the 4 females of the α -chlorohydrin group appeared to be pregnant. No statistically significant

difference was observed in the mean number of implantations of both the control and the α -chlorohydrin group.

3.5 Organ weight (Tables 11-14, Appendices 7-8)

The mean organ weights (lungs, kidney, testes and epididymides) absolute and relative to body weights of the exposed animals and the control animals were similar.

3.6 Pathology

Upon gross examination no abnormalities were observed.

4

CONCLUSIONS

No clinical signs or mortalities were observed during exposure or the recovery period.

No adverse effect of α -chlorohydrin on body weight was observed

Mean absolute and relative organ weights (lungs, kidneys, testes and epididymides) were similar in both groups.

The number of matings immediately after exposure was decreased in both the control and the α -chlorohydrin group. This effect was most probably due to stress by the exposure procedures and handling procedures. No effects on fertility or reproduction parameters were observed during the matings after a recovery period of 7 and 14 days, respectively.

On basis of the results obtained in the present study it is concluded that:

- no adverse effects can be shown after single exposure for 6 hours of male and female rats to approximately 16.7 ppm α -chlorohydrin by inhalation on general health
- no adverse effects on male fertility can be shown after single exposure for 6 hours to 16.7 ppm α -chlorohydrin by inhalation.

5 RETENTION OF RECORDS, SAMPLES AND SPECIMENS

All records containing raw data, the master copy of the final report and all other information relevant to the quality and integrity of the study, together with the wet specimens, paraffin blocks and microscopic slides will be retained in the archives of the Department of Biological Toxicology for a period of at least 5 years after termination of the study. At the end of the 5 year storage period, the Sponsor will be consulted whether the records and materials can be discarded or should be stored for an additional period or have to be transferred to the archives of the Sponsor.

6 REFERENCES

1. Papanicolaou, G.N.(1957) C.A. Bull.Cancer Prog., 7, 124-135.
2. Dixon, W.J.(1988) BMDP statistical soft-ware manual, University of Berkeley, 534-535.
3. Siegel, S.(1956) Non Parametric Statistics for the Behavioral Sciences, McGraw-Hill Kogakusha Ltd, 96-104.
4. Siegel, S.(1956) Non Parametric Statistics for the Behavioral Sciences, McGraw-Hill Kogakusha Ltd, 116-127.

TNO TOX. & NUTR. INST.

1992-01-21/iw