Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

Chemical name: 1,1-dichloroethylene; vinylidene chloride

EC Number: 200-864-0

CAS Number: 75-35-4

Index Number: 602-025-00-8

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Version number: V2 **Date: February 2022**

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1 HEALTH HAZARDS

Acute toxicity

1.1 Acute toxicity - oral route

1.1.1 Animal data

Species	Method	Results	Référence
Mice, male and female, (B6C3F1)	gavage	LD50 estimated between 50 and 500 mg/kg bw	NTP, 1982
Rat, male and female, (Fischer 344)	gavage	DL50 estimated >1000 mg/kg bw (male/female)	NTP, 1982
Mice, male, (Swiss OF1)	gavage	LD50 > 200 mg/kg bw	Ban M. *et al.*, 1995
Rat, male, (Holtzman)	gavage	LD50 = 1510 mg/kg bw (male)	Jenkins L.J. *et al.*, 1972
Rat, male and female, (Fischer 344)	gavage	LD50 = 1500 mg/kg bw (female) LD50 = 1800 mg/kg bw (male)	Ponomarkov V *et al.*, 1980
Mice, male and female, (Alderley Park)	gavage	LD50 (male) = 217 mg/kg bw LD50 (female) = 194 mg/kg bw	Jones B.K. *et al.*, 1978
Rat, strain and sex not specified	oral route	LD50 = 2500 mg/kg bw	Kennedy G.L. *et al.*, 1991

1.1.1.1 [NTP, 1982]

Study reference:

National Toxicology Program 1982: Technical report No. 228, Carcinogenesis bioassay of vinylidene chloride (CAS NO. 75-35-4) in F344 rats and B6C3F1 mice (gavage study) (publication), U.S. Department of health and human services. Public Health Service. National Institute of Health.

Detailed study summary and results:

Test type

At the time of this study period (1977), no guideline was available either for the test method used or for the GLP. Groups of mice were given a single oral dose of the test substance by gavage in corn oil at 5 dose levels.

Test substance

The test substance, as cited in the report "vinylidene chloride", was supplied by Dow Chemical Company, batch V83848, with an analytical purity of 99%.
The test substance was diluted in corn oil and administered by gavage to groups of 5 males and 5 females mice at six dose levels including a negative control group (0, 10, 50, 100, 500 and 1000 mg/kg).

Test animals

The study was conducted on mice (B6C3F1). Mice were 4 weeks-old at receipt, and were dosed after an acclimatation period of 5 weeks.

Administration/exposure

The test substance was administered by gavage. The animals were observed during 14 days following administration.

Results

No mortality was observed at 10 and 100 mg/kg, 1/5 female died at 50 mg/kg, 5/5 males and 3/5 females died at 500 mg/kg. No survival either male or female were observed at 1000 mg/kg. The LD50 is considered between 50 mg/kg (males: no lethality, females: 20 % lethality) and 500 mg/kg (males: 100% lethality, females: 60% lethality).

Table 1: summary of mortality data in mice (NTP, 1982)

Doses (mg/kg)	Mice	
	Male	Female
0	0/5	0/5
10	0/5	0/5
50	0/5	1/5
100	0/5	0/5
500	5/5	3/5
1000	5/5	5/5

1.1.1.2 [NTP, 1982]

Study reference:

National Toxicology Program 1982: Technical report No. 228, Carcinogenesis bioassay of vinylidene chloride (CAS NO. 75-35-4) in F344 rats and B6C3F1 mice (gavage study) (publication), U.S. Department of health and human services. Public Health Service. National Institute of Health.

Detailed study summary and results:

Test type

Groups of rats were given a single oral dose of the test substance by gavage in corn oil at 5 dose levels.

Test substance

The test substance, as cited in the report "vinylidene chloride", was supplied by Dow Chemical Company, lot No. UTLX83844.

Test animals

The study was conducted on F344/N rats (from the NCI Frederick Cancer Research Center), housed 5 per cage. Diets of Wayne Lab Blox and tap water via an Edstrom automatic watering system were provided ad-libitum. After an acclimatation period of 5 weeks, animals were assigned to groups according to a table of random numbers.

Administration/exposure

The test substance was diluted in corn oil and administered by gavage to groups of 5 male and 5 female rats at each of six dose levels including a negative control group (0, 10, 50, 100, 500 and 1000 mg/kg). The animals were observed during 14 days following administration.

Results

No mortality was observed at 50 and 100 mg/kg. One female rat receiving 500 mg/kg bw died. One male rat receiving 10 mg/kg bw died after 7 days, and two male rats receiving 1000 mg/kg bw died within 48 hours. The LD50 was considered above 1000 mg/kg bw for both male and female rats.

Table 2: summary of mortality data in rats (NTP, 1982)

Doses (mg/kg)	Rats	
	Male	Female
0	0/5	0/5
10	1/5	0/5
50	0/5	0/5
100	0/5	0/5
500	0/5	1/5
1000	2/5	0/5

1.1.1.3 [Jenkins, 1972]

Study reference:

Jenkins L.J., Trabulus Jr. M.J. and Murphy S.D. 1972: Biochemical effects of 1,1-Dichloroethylene in Rats: Comparison with Carbon Tetrachloride and 1,2-Dichloroethylene (publication), Toxicology and applied pharmacology, 23:501-510.

Detailed study summary and results:

Test type

At the time of this study period (1972), no guideline was available either for the method used or for the GLP. Groups of rats were given by gavage a single oral dose of the test substance in corn oil. The LD50 value was calculated by the method of Litchfield and Wilcoxon (1949).

Test substance

The test substance, as cited in the report "1,1-dichloroethylene", was supplied by K & K Laboratories, Inc., Plainview, New York. The batch or the analytical purity are not available.

Test animals

The study was conducted on adult male rats (Holtzman rats). They were housed 4-6 in a cage, in air-conditioned room and were provided with commercial rat diet (Purina Rat Chow, Ralstn, Purina Company, St Louis, Missouri) and tap water at libitum. Animals were transferred to individual cages for observation following administration of the test substance. No information on acclimation period duration was specified in the publication.

Administration/exposure

The test substance was administered by gavage as preparation in corn oil in a total volume of 2 mL/Kg. The tested dose levels were not specified in the publication. The LD50 values were calculated at 24 and at 96h after administration.

Results

No data on mortality was specified in the publication. The LD50 values in male rats calculated by the authors were 1550 and 1510 mg/kg bw, 24 and 96 hours after the administration, respectively.

1.1.1.4 [Ponomarkov, 1980]

Study reference:

Ponomarkov V., Tomatis L. 1980: Long-Term Testing of Vinylidene Chloride and Chloroprene for Carcinogenicity in Rats (publication), Oncology, 1980, 37, 136-141.

Detailed study summary and results:

Test type

No guideline was followed and the GLP compliance is not specified in this publication. Single doses of VDC were administered to groups of 4 rats. The LD50 was calculated according to the method described by Weil for male and female rats separately.

Test substance

The test substance, as cited in the report "vinylidene chloride" or VDC, was supplied by Merck-Schuchardt, Dramstadt, FRG, with a purity of 99%, containing 0.03% 4-methoxyphenol. The substance was dissolved in olive oil.

Test animals

The study was conducted on inbred BDIV rats (provided by Prof. H Druckey, Freiburg, FRG). The animals were maintained on Charles River pellets or Aliment Extralabo Biscuits (Pietrement), and water was available at libitum. No information on acclimation period duration was specified in the publication.

Administration/exposure

A single oral dose was administered in olive oil by gavage (using stomac tube) to a group of 4 rats. The tested dose levels were not specified.

Results

No data on mortality was given in the publication. The LD50 value, calculated following the method described by Weil, was 1 800 mg/kg bw for males and 1 500 mg/kg bw for females.

1.1.1.5 [Jones, 1978]

Study reference:

Jones B.K., Hathway D.E. 1978: Differences in metabolism of Vinylidene Chloride between mice and rats (publication), Br. J. Cancer, 37:411-417.

Detailed study summary and results:

Test type

At the time of this study, no guideline was available either for the method used or for the GLP. Groups of mice were given a single oral dose of the test substance by gavage in corn oil at 5 dose levels. LD50 values were calculated using Thompson's method (1947) of moving averages and interpolation.

Test substance

The test substance, as cited in the report "vinylidene chloride", was supplied by Imperial Chemical Industries Limited, Mond Division, Runcorn, Cheshire). No information on degree of purity or impurities or batch are available.

Test animals

The study was conducted on adult mice (Alderley Park strain, specific pathogen-free) about 5 weeks old, with a body weight of 25g, and kept on standard pellet diet. Five groups of 6 mice of each sex received 5 different test item concentrations prepared in corn oil.

Administration/exposure

The test substance was administered by gavage. No information on the control group, on the tested dose levels or on the post exposure observation period are available.

Results

The LD50 values obtained were 217 mg/kg bw for male and 194 mg/kg bw for females.

1.1.1.6 [Kennedy, 1991]

Study reference:

Kennedy G.L., Graepel Jr.G. and Graepel G.Jay 1991: Acute toxicity in the rat following either oral or inhalation exposure (publication), Toxicology Letters, 56:317-326.

Detailed study summary and results:

Test type

This study was conducted on 108 chemicals to determine the relationship between acute oral and acute inhalation data in the rat.

Test substance

The test substance was cited in the publication as "vinylidene chloride, CAS no. 75-35-4". No more information on the test substance is available.

Test animals

The study was conducted in rats. No more information on animals is available.

Administration/exposure

The substance was administered orally as a single dose. No more information is available.

Results

The LD50 value reported by the authors was 2500 mg/kg bw.

1.2 Acute toxicity - inhalation route

Species	Method	Results	Référence
Sprague-Dawley rat	Whole body vapour	LC_{50} for male and female: **28.35 and 40.78 mg/l** respectively.	Anonymous, 1979a
Sprague-Dawley rat, fasted	Whole body vapour	LC_{50} for fasted male and female: **1.63 and 26 mg/l** respectively.	Anonymous, 1979b
NMRI mouse, fasted	Whole body vapour	LC50 for fasted male and female**: 0.2 and 0.5 mg/l** respectively.	Anonymous, 1979c
Chinese hamster, fasted	Whole body vapour	LC_{50} for fasted male and female: **0.6 and 1.8 mg/l** respectively.	Anonymous, 1979e
Chinese hamster	Whole body vapour	LC_{50} for male and female: **6.59 and 11.69 mg/l** respectively.	Anonymous, 1979f
Male Sprague-Dawley rat	Whole body vapour	LC_{50} = **25.4 mg/l.**	Siegel, 1971
Male Holtzman rats, fasted or fed	Whole body vapour	Estimated LC50: Fed rats = **60 mg/l** Fasted rats = **2.4 ml/l**	Jaeger, 1974
CD rat	Whole body vapour	no LD_{50}, no mortality	Short, 1977a
CD-1 mouse	Whole body vapour	LC_{50} (male, 22-23 h) = **0.39 mg/l** LC_{50} (female, 22-23 h) = **0.42 mg/l**	Short, 1977a
NMRI mouse	Whole body vapour	LC50 for male and female**: 0.46 and 0.82 mg/l** respectively.	Anonymous, 1979d

1.2.1 Animal data

1.2.1.1 [Anonymous, 1979a]

Study reference:

See confidential Annex

Detailed study summary and results:

Test type

Groups of 10 rats/sex were exposed to VDC at 4 dose levels (7.94, 19.84, 35.71, 59.52 mg/l) by inhalation for 4h.

Test substance

Name of test material (as cited in study report): Vinylidene chloride and 1,1-dichloroethene. Analytical purity: 99.7%.

Test animals

The study was conducted in male and female Sprague-Dawley rat.

Administration/exposure

The substance was administered by whole body inhalation exposure (vapour) for 4h with a duration of observation period following administration of 14 days.

Results

The LC50 of VDC for male and female rats is respectively 28.35 and 40.78 mg/L air.

1.2.1.2 [Anonymous, 1979b]

Study reference:

See confidential Annex

Detailed study summary and results:

Test type

Groups of 10 rats/sex were exposed to VDC at 9 dose levels (0.4, 1, 2, 4, 6, 8, 20, 40, 48 mg/l) by inhalation for 4h.

Test substance

Name of test material (as cited in study report): Vinylidene chloride and 1,1-dichloroethene. Analytical purity: 99.7%.

Test animals

The study was conducted in male and female Sprague-Dawley rat.

Administration/exposure

The substance was administered by whole body inhalation exposure (vapour) for 4h with a duration of observation period following administration of 14 days.

Results

The LC50 of VDC for fasted male and female rats was 1.63 and 26 mg/l respectively.

1.2.1.3 [Anonymous, 1979c]

Study reference:

See confidential Annex.

Detailed study summary and results:

Test type

Groups of 10 mice/sex were exposed to VDC at 8 dose levels (0.04, 0.08, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mg/l) by inhalation for 4h.

Test substance

Name of test material (as cited in study report): Vinylidene chloride and 1,1-dichloroethene. Analytical purity: 99.7%.

Test animals

The study was conducted in male and female NMRI mice.

Administration/exposure

The substance was administered by whole body inhalation exposure (vapour) for 4h with a duration of observation period following administration of 14 days.

Results

The LC50 of VDC for fasted male and female mice was 0.2 and 0.5 mg/l respectively.

1.2.1.4 [Anonymous, 1979e]

Study reference:

See confidential Annex.

Detailed study summary and results:

Test type

Groups of 10-20 hamsters/sex were exposed to VDC at 8 dose levels (0.5, 0.8, 1, 2, 3, 4, 6, 8 mg/L) by inhalation for 4 hours.

Test substance

Name of test material (as cited in study report): Vinylidene chloride and 1,1-dichloroethene. Analytical purity: 99.7%

Test animals

The study was conducted in male and female Chinese hamsters.

Administration/exposure

The substance was administered by whole body inhalation exposure (vapour) for 4h with a duration of observation period following administration of 14 days.

Results

The LC50 of VDC for fasted male and female Chinese hamsters was 0.6 and 1.8 mg/l respectively.

1.2.1.5 [Anonymous, 1979f]

Study reference:

See confidential Annex.

Detailed study summary and results:

Test type

Groups of 10 hamsters/sex were exposed to VDC at 9 dose levels (0, 1, 2, 3, 4, 6, 8, 12, 16, 20 mg/L) by inhalation for 4 hours.

Test substance

Name of test material (as cited in study report): Vinylidene chloride and 1,1-dichloroethene. Analytical purity: 99.7%.

Test animals

The study was conducted in male and female Chinese hamsters.

Administration/exposure

The substance was administered by whole body inhalation exposure (vapour) for 4h with a duration of observation period following administration of 14 days.

Results

The LC50 of VDC for male and female Chinese hamsters was 6.59 and 11.69 mg/l respectively.

1.2.1.6 [Siegel, 1971]

Study reference:

Siegel, J., Jones, R.A., Coon, R.A., & Lyon, J.P. 1971: Effects on experimental animals of acute, repeated and continuous inhalation exposures to dichloroacetylene mixtures. Toxicol. appl. Pharmacol., 18: 168-174.

Detailed study summary and results:

Test type

Groups of 16 male rats were exposed for 4 hours to 1,1-dichloroethene to 4900 ppm and 6150 ppm (19.6 and 24.6 mg/L). After exposure, they were returned to their individual cages for a 2-week observation period. Mortality was recorded.

Test substance

Reference substance name: vinylidene chloride, obtained commercially. No more information on the test substance is available.

Test animals

The study was conducted in male Sprague-Dawley rats. No more information on animals is available.

Administration/exposure

The substance was administered by whole body inhalation exposure (aerosol) in air for 4h.

Results

The LC50 of VDC in male NMRI :O(SD) Sprague-Dawley rats is 25.4 mg/l.

1.2.1.7 [Jaeger, 1974]

Study reference:

Jaeger R.J., Conolly R.B., and Murphy S.D. 1974: Effect of 18-hr fast and glutathione depletion on 1,1-dichloroethylene-induced hepatotoxicity and lethality in rats. Exp. mol. Pathol., 20: 187-198.

Detailed study summary and results:

Test type

Five to 10 animals were exposed to concentrations of VDC in the range of 0.2 to 80 mg/L. One group was fasted for 18 hours (4 PM-10 AM) prior to exposure and the other group was supplied with food ad libitum. No more information is available.

Test substance

The test substance was cited in the publication as "1,1-dichloroethylene (1,1-DCE), also referred to as vinylidene chloride ". No more information on the test substance is available.

Test animals

Male Holtzman rats weighing between 250 and 400 g were used.

Administration/exposure

The substance was administered by whole body inhalation exposure (vapour) for 4h.

Results

The estimated 24 hr LC50 for fed rats was 60 mg/L and rats was 2.4 ml/L.

1.2.1.8 [Short, 1977a]

Study reference:

Short R.D., Winston J.M., Minor J.L., Hong C.B., Seifter J., & Lee, C.C. 1977a: Toxicity of vinylidene chloride in mice and rats and its alteration by various treatments. J. Toxicol. environ. Health, 3: 913-921.

Detailed study summary and results:

Test type

The toxicity of VDC was studied in 10 mice and rats per sex per dose exposed to 0.060, 0.12 and 0.24 mg/L of the vapors for 23 h/day.

Test substance

Name of test material (as cited in study report): Vinylidene chloride (VDC). Analytical purity: 99 %.

Test animals

The study was conducted in male and female CD rats. No more information on animals is available.

Administration/exposure

Animals were exposed by whole body inhalation to VDC for 22-23 hr/day for 7 days.

Results

no LD50 can be derived, as no mortality occurred in rats.

1.2.1.9 [Short, 1977a]

Study reference:

Short R.D., Winston J.M., Minor J.L., Hong C.B., Seifter J., & Lee, C.C. 1977a: Toxicity of vinylidene chloride in mice and rats and its alteration by various treatments. J. Toxicol. environ. Health, 3: 913-921.

Detailed study summary and results:

Test type

The toxicity of VDC was studied in 10 mice and rats per sex per dose exposed to 0.060, 0.12 and 0.24 mg/L of the vapors for 23 h/day.

Test substance

Name of test material (as cited in study report): Vinylidene chloride (VDC). Analytical purity: 99 %.

Test animals

The study was conducted in male and female CD1 mouse. No more information on animals is available.

Administration/exposure

Animals were exposed by whole body inhalation to VDC for 22-23 hr/day for 7 days.

Results

One-day LC50 values obtained were 390 mg/m and 420 mg/m3 in males and females, respectively.

1.2.1.10 [Anonymous, 1979d]

Study reference:

See confidential Annex.

Detailed study summary and results:

Test type

Groups of mice were exposed to VDC at several dose levels by inhalation for 4 hours.

Test substance

Vinylidene chloride. No information on purity and no more information on the test substance available.

Test animals

The study was conducted in NMRI mouse. No more information on animals is available.

Administration/exposure

The substance was administered by whole body inhalation exposure (vapour) for 4h with a duration of observation period following administration of 14 days.

Results

The LC50 for male and female are 0.46 and 0.82 mg/L respectively.

1.3 Serious eye damage/eye irritation

1.3.1 Animal data

1.3.1.1 [Anonymous 1979g]

Study reference:

See confidential Annex.

Detailed study summary and results:

Test type

One of eye of 2 rabbits was treated with 50 µl of undiluted VDC solution and not washed.

At the time of this study period (1978), no guideline was available either for the method used or for the GLP.

Test substance

The substance cited in the report was “vinylidene chloride” or “1,1-dichloroethylene”, with the formula CH2=CCl2, and a purity of 99.7% was specified.

Test animals

The study was conducted on 2 Vienna white rabbits, supplied by Firma GAUKLER, 6050 Offenbach. The weight at the beginning of the study was about 3.3 kg. The diet and water was available ad libitum. Animals did not have access to food or water during the treatment.

Administration/exposure

The test item was tested undiluted. One eye of each rabbits is treated with 50 µL of undiluted VDC solution and not washed. The same dose of physiological solution is applied in the non treated eye as control. As

there was no washing, the duration of the treatment lasted all the observation period. The post exposure observation periods was up to 8 days, with time points at 1h, 24h and 8 days after treatment.
The effects observed and the grading scale was defined in the report as follows: R= redness / Ö= edema / N= necrosis / + = sligh / + + = strong

Results and discussion

Table 3: Results of eye irritation of VDC from in vivo test on rabbits

Treatment applied	Effects observed		
	1h after application	**24h after application**	**8 days after application**
VDC (50 µL)	R +; Ö +	R +	No significant effect Slight irritation of the lining
Control (NaCl physiological solution)	No effect	No effect	No effect

One hour after treatment, slight redness and slight edema were observed. Only slight redness was still found 24h after application. After one week, no significant effect was noted except a slight irritation of the lining of the eye.

1.3.1.2 [Anonymous, 2010]

Study reference:

See confidential Annex.

Detailed study summary and results:

Test type

The irritation potential of the substance was assessed in an in vitro test on fresh bovine cornea.
The study complies to the current GLP and OECD guideline 437 (Bovine Corneal Opacity and Permeability test method or BCOP assay) requirements.

Test substance

The study was conducted on "1,1 dichlorethylene", CAS 75-35-4, batch OB 135, with a purity > 99%.

Test animals

The study was conducted on fresh isolated bovine cornea, supplied by Odenwaldschlachthof Brensbach, 64395 Brensbach, Germany. The Supplier collected fresh bovine cornea from the abattoir. Three corneae were used in each group (test item, negative control, positive control).

Administration/exposure

The test item was tested undiluted. The corneae opacity was measured using the OP-KiT opacitometer (Electro Design, 63- Riom France). In the second step of the assay, permeability of the cornea possibly caused by the test item, was measured at 490 nm (OD490) with a spectrophotometer.

Results and discussion

With the negative control (0.9% NaCl solution) neither an increase of opacity nor permeability of the corneae could be observed. The positive control (2-Ethoxyethanol) showed clear opacity and distinctive permeability of the corneae and therefore considered as severe eye irritant. The test item 1,1-dichloroethylene caused only a slight increase of opacity values but a distinct increase of the permeability values of the corneae compared with the results of the negative control. The calculated mean in vitro score was 43.90.

Table 4: Results of VDC tested in BCOP assay after 10 minutes incubation time

Test group	Opacity value *		Permeability OD_{490}**		In vitro score	Mean in vitro score	In vitro irritation scale
Negative control	-1	Mean 0.00	0.052	Mean 0.055	-0.22	0.82	Non eye irritant
	0		0.051		0.77		
	1		0.061		1.92		
Positive control	66.00		0.640		75.61	79.20	Severe eye irritant
	68.00		0.874		81.12		
	69.00		0.791		80.87		
VDC	10.00		2.312		44.69	43.90	Moderate eye irritant
	9.00		2.306		43.60		
	8.00		2.360		43.41		

*: Difference (t130-t0) of opacity / **: Optical density at 490 nm

On the basis of these results, the test item was not corrosive and was considered as moderate eye irritant.

1.4 Germ cell mutagenicity

1.4.1 In vitro data

Type of test	Description	Results	Reference
Gene mutation in bacterial cells	Ames test / No measures to prevent volatilization Strain TA100 without metabolic activation	Not mutagenic	Laumbach A.D. *et al.*, 1977
	Ames test Strain TA100 with and without metabolic activation	Mutagenic with metabolic activation	Simmon V.F. *et al.*, 1977
	Ames test / similar to OECD Guideline	Mutagenic with and without metabolic	Waskell L., 1978

	471 / in a closed system Strains TA98 and TA100 with and without metabolic activation	activation	
	Ames test / No measures to prevent volatilization Strains TA98, TA100, TA1535 and TA1537 with and without metabolic activation	Not mutagenic	Mortelmans K. *et al.*, 1986
	Ames test Strain E. coli K-12 with and without metabolic activation	Mutagenic with metabolic activation	Greim H. *et al.*, 1975
	Ames test / in a dessicator Strains TA100 and TA1530 with metabolic activation	Mutagenic with metabolic activation	Bartsch H. *et al.*, 1975
	Ara mutagenicity assay S. typhimurium strains: BA13 and BAL13 with and without metabolic activation	Mutagenic with metabolic activation	Roldan-Arjona T. *et al.*, 1991
	Ames Test S. typhimurium, TA1535 and TA100 With metabolic activation	Mutagenic	Baden et al., 1978
	Ames Test S. typhimurium, TA1535 and TA100 With metabolic activation	Mutagenic	Jones & Hathway (1978c)
	Reverse mutation and gene conversion S. cerevisae strain D7 With and without metabolic activation	Mutagenic with metabolic activation	Bronzetti et al. (1981)
	Reverse mutation and gene conversion, host-mediated assay S. cerevisae strain D7	Point mutations and mitotic gene conversion seen in the yeast recovered from kidney and liver, but not lung	
	Ames Test (VDC used as positive control) S. typhimurium, TA1535 and TA100 With metabolic activation	Mutagenic	Baden et al., 1982
	Ames Test S. typhimurium strains TA1535, TA1537, TA98, TA100 and TA92 E. coli WP2 With and without metabolic activation	Mutagenic with metabolic activation	Oesch et al., 1983
Gene mutation in mammalian cells	MLA V79 cells with and without metabolic activation	Not mutagenic	Drevon C. and Kuroki T. 1979
	MLA L5178Y cells with and without metabolic activation	Positive with metabolic activation Ambigous without metabolic activation	Mc Gregor D. *et al.*, 1991
DNA damage and repair	UDS Freshly isolated rat hepatocytes with metabolic activation	Positive	Costa A.K. *et al.*, 1984:
Chromosome aberrations	Similar to OECD guideline 473 CHL (Chinese hamster cell) with and without metabolic activation	Positive in presence of metabolic activation	Sawada M. *et al.*, 1987
	Sister Chromatid Exchange Assay CHL (Chinese hamster cell) with and without metabolic activation	Positive in presence of metabolic activation	Sawada M. *et al.*, 1987
Mitotic recombination and aneuploidy	Saccharomyces cerevisae, mitotic recombination assay Strains D7 and D61.M with and without metabolic activation	Strain D7 : Positive (+ S9 mix) Strain D61.M : Positive (+/- S9 mix)	Koch R. *et al.*, 1988
Other tests	Detection of one single-strand break in the double-stranded circular phage PM2 DNA molecule	Positive	Waskell L., 1978
	Mitotic chromosome malsegregation in Aspergillus nidulans	Positive	Crebelli R. *et al.*, 1992

1.4.1.1 [Laumbach (1977)]

Study reference:

Laumbach A.D., Lee S., Wong J. and Streips U.N. 1977: Studies on the mutagenicity of vinyl chloride metabolites and related chemicals (publication), Prev Detect Cancer Proc Int Symp 3RD 1976, 1:155-170.

Detailed study summary and results:

Test type

The genotoxicity of VDC was determined in a reverse mutation assay conducted without metabolic activation in Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100. The study was conducted according to the Ames method and was based on the OECD guideline 471.

Test substance

In the publication, the substance is described as being "vinylidene chloride". No information about the purity, batch number and supplier are available. VDC was prepared in DMSO as vehicle.

Administration/exposure

The Salmonella typhimurium strain TA100 was obtained from B. N. Ames (no information for the other strains). The bacteria cultures were grown in Nutrient Broth plus 0.5 mM NaCl overnight in a rotary incubator shaker at 37°C . A mixture of the test chemical (0.1 ml) in dimethyl sulfoxide (DMSO) and 2 ml of soft agar was added to 0.1 ml of the bacterial culture. The solutions were mixed thoroughly and overlaid onto minimal plates (Vogel-Bonner E medium). Control samples were prepared by omitting the test chemicals. For the positive control, 4-NQO (4-nitroquinoline-N-oxide) was added to the mixtures in place of the test chemicals. All plates were incubated for 48 hr at 37°C prior to the enumeration or revertant colonies. The study was conducted only in absence of metabolic activation. No information about test concentrations, negative controls, number of replicates are available.

Results and discussion

No individual results are available. No information about the presence of precipitate or toxicity is available.

According to the authors, vinylidene chloride did not induce any significant increase in the number of revertants in the strain TA100 in the absence of metabolic activation. No information is provided for the other tested strains.

1.4.1.2 [Simmon (1977)]

Study reference:

Simmon V.F. and Tardiff R.G. 1977: The mutagenic activity of halogenated compounds found in chlorinated drinking water (publication), Water chlorination, environmental impact and health effects, 2:416-431.

Detailed study summary and results:

Test type

The mutagenic activity of 128 chemicals identified in drinking water in the United States was tested on Salmonella typhimurium strain TA100 with and without metabolic activation and according to the Ames test protocol.

The study conditions were poorly described. VDC was tested at a concentration of 5% in desiccator as the positive substance. Test conditions (cytotoxicity, number of replicates, precipitation, negative controls …) and results were not detailed.

Test substance

In the publication, the substance is described as being "1,1-dichloroethylene". No information about the purity, batch number, supplier or vehicle are available.

Administration/exposure

The Salmonella typhimurium strain TA100 was tested with and without metabolic activation. No more details are available.

Results and discussion

The authors concluded that VDC was mutagenic on Salmonella typhimurium in the presence of metabolic activation, with a dose-dependent response.

1.4.1.3 [Waskell (1978)]

Study reference:

Waskell Lucy 1978: A study of the mutagenicity of anesthetics and their metabolites (publication), Mutation Research, 57:141-153.

Detailed study summary and results:

Test type

The mutagenicity of commonly used inhalational anesthesics and their metabolites was screened for mutagenicity in Salmonella typhimurium assay as developed by B. N. Ames. Vinylidene chloride was used as positive control. The study seems to have been performed according to standardised protocol, but test conditions (cytotoxicity, precipitation, negative controls …), results and purity of the test substance were not detailed. In addition, only 2 strains and one concentration were tested.

Test substance

The substance as described in the publication was vinylidene chloride or 1,1-dichloroethylene from Dow

Chemicals. No more information on the test item is available.

Administration/exposure

The test was conducted in duplicate in strains TA98 and TA100, with and without metabolic activation. The metabolic activation system was prepared from liver homogenate of rats induced with phenobarbital and Aroclor 1254, as described by Ames. As vinylidene chloride is volatile, it was tested in closed system. The mixture was put on petri dishes, and five holes were punched in the top of each petri dish to allow the test vapour access to the bacteria and microsomes. The plates were placed in the dessicator. Concentration in the dessicator was fixed at 5 %. The concentration of the substance in the sealed vessel was increased until killing of the bacteria was noted, confirming that the anesthetic was indeed contacting the bacteria on the petri dish.

Results and discussion

Only the results of TA100 are presented in the publication. The results for exposure periods of 10h, 24h and 48h are not reported. The results of negative controls and information regarding precipitation and cytotoxicity are not available. According to the authors, vinylidene chloride was more mutagenic with TA100.

Table 5: Results of VDC tested in the TA100 S. typhymurium strain

Substance	Tested concentrations	Number of revertants above control			
		exposure to the bacteria: 6h		exposure to the bacteria: 4h	
		Liver homogenate		Liver homogenate	
		+	-	+	-
Vinylidene chloride	**5% in air**	400	150	183	56

The authors concluded that VDC was mutagenic in the strain TA100 in the presence and absence of metabolic activation.

1.4.1.4 [Mortelmans (1986)]

Study reference:

Mortelmans K., Haworth S., Lawlor T., Speck W., Tainer B. and Zeiger E. 1986: Salmonella mutagenicity Tests: II. Results from the testing of 270 chemicals (publication), Environmental Mutagenesis,8(sup.7):1-119.

Detailed study summary and results:

Test type

A list of 270 chemicals were tested in Ames test. Four Salmonella typhimurium strains obtained from Bruce Ames were used (TA1535, TA1537, TA98 and TA100). The study was conducted in presence and in absence of metabolic activation, by the pre-incubation method.
The test was conducted similarly to the guideline OECD 471. However, only 4 strains were tested instead of 5 requested in the guideline. In addition, VDC is volatile and the test was conducted without desiccator and without any measures to prevent volatilisation.

Test substance

The tested substance as identified in the publication was "vinylidene Chloride", with the CAS number 75-35-4, from Matheson, Coleman and Bell, with a purity of 98%. The treatment formulations were prepared in DMSO as vehicle.

Administration/exposure

The main study was conducted on four Salmonella typhimurium strains obtained from Bruce Ames: TA1535, TA1537, TA98 and TA100. Based on results from a preliminary experiment conducted only on the strain TA100, five dose levels were tested: 0, 33.3, 100, 333.3, 1000 and 3333.3 µg/plate, with and without metabolic activation, in triplicate. Concurrent negative and positive controls were added with and without metabolic activation. The test was conducted by pre-incubation method: each test tube containing S-9 mix (metabolic activation) or buffer (in absence of metabolic activation), bacterial overnight culture, solvent (negative control) or positive controls or VDC, were mixed and incubated for 20 min at 37°C without shaking. Then top agar was added, and the tube was poured on petri dishes containing Vogel-bonner medium. The plates were incubated for 48h at 37°C. The S-9 mix fraction was prepared from male Sprague-Dawley rats and male Syrian hamsters induced by Aroclor 1254 (500 mg/kg body weight). The S-9 fraction, obtained by centrifugation of the liver homogenate, was prepared immediately prior each test according to Ames protocol.
The criteria used for data evaluation were the same as those described in Haworth et al., 19831, and are summarized as follows:
1/Mutagenic response: a dose-related, reproducible increase in the number of revertants over background, even if the increase was less than two-fold
2/Non-mutagenic response: when no increase in the number of revertants was elicited by the chemical
3/Questionable response: when there was an absence of clear-cut dose-related increase in revertants; when the dose-related increases in the number of revertants were not reproducible; or when the response was of insufficient magnitude to support a determination of mutagenicity.

Results and discussion

Table 6: Results of Ames test conducted on VDC

	TA100						TA1535						TA1537						TA98					
DOSE	NA		10% HLI		10% RLI		NA		10% HLI		10% RLI		NA		10% HLI		10% RLI		NA		10% HLI		10% RLI	
µg/plate	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM	MEAN	SEM
0	91	3.2	93	6	88	7	8	0.6	12	3.1	5	2	6	0.3	5	0.7	6	0.7	14	2.5	22	2.2	22	3.2
33.3	95	7.3	97	7.4	97	6.7	10	1.9	8	0.3	6	0.3	7	0.9	8	1.3	12	2	8	2.3	20	2.3	28	5.1
100	91	5.5	98	6.6	97	6.3	7	0.6	7	1.7	8	1.2	5	0.9	9	1.5	5	1.9	15	3.8	20	1.9	31	6.1
333.3	97	7.4	104	6.9	104	3.5	6	1	7	0.7	10	3	4	0.9	5	0.6	6	1.2	13	0.3	20	2.4	25	3.8
1000	106	2.2	103	9.5	120	8.9	8	2.5	6	1.7	9	2	4	0.9	7	3.5	6	1.5	17	2.1	22	2	24	3.1
3333.3	82	4	51	11.5	110	4	9	0	4	1.7	5	0.9	5	1.7	4	0.3	5	0.6	16	5.8	10	5	19	1.3
POS	500	10.5	290	21.3	1066	12.2	337	22.5	186	12.9	387	26.3	258	17.3	362	20.4	461	16.3	693	39	199	7.8	787	53.4

HLI: S9 fraction from hamsters / RLI: S9 fraction from rats

No increase in the number of revertants was observed in the four S. typhymurium strains (TA 1535, TA 1537, TA 98 and TA 100) when tested with VDC up to 3333.3 µg/plate, either in the presence or in the absence of metabolic activation**.**

1.4.1.5 [Greim (1975)]

Study reference:

Greim H., Bonse G., Radwan Z., Reichert D., Henschler D. 1975: Mutagenicity in vitro and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation (publication), Biochemical Pharmacology, 24:2013-2017.

Detailed study summary and results:

Test type

In this publication, the mutagenicity activity of the whole series of chlorinated ethylenes was tested in the strain E. coli K-12 with a metabolic activation system.

This study is closed to OECD 471, and restrictions were noted. Test conditions (purity, replicates, control) and results are not sufficiently detailed. In addition, only one strain was tested instead of 5 required in the guideline, and the strain used (E. coli K-12) is not included in the list of the recommended strains. Only one concentration was tested.

Test substance

The test item described in the publication is “1,1-dichloroethylene” obtained from Merck & Co., Darmstadt.

No information on the purity, impurities and batch number are available.

Administration/exposure

The test was conducted only on the strain E. coli K-12. In this strain, mutations can induce reversions from different auxotrophies (nad-, arg-, gal-) and in the MTR system leading to resistance against 5-methytryptophan. It is assumed that many types of DNA alteration, including deletions and changes involving gross DNA regions, will lead to viable detectable mutants.

An overnight culture was suspended on 1.5 mL incubate containing a metabolic activation system (liver

microsomes isolated from male mice pre-treated for 10 days with 0.1% phenobarbital in the drinking water) and 2.5 mM of test item. This concentration was selected from preliminary experiment so that they did not reduce cell survival by more than 20%, and was determined by GC analysis. After 2 hours of incubation in a shaking bath at 37°C, the reaction was terminated on ice. The incubate was diluted in saline and plated on appropriate selective media. Survival of the strain was determined by plating on the complete medium.
Survival was expressed in % from colony forming units (CFU) counted on the complete medium. Mutagenicity was expressed as % of spontaneous mutation rate in different operons from colony forming units (CFU) counted in the appropriate selective media

Results and discussion

To obtain 80-100% survival, the concentration of 2.5 mM was tested (survival obtained was 74 ± 7%). The authors indicated that no mutagenic activity was observed in the absence of metabolic activation. However, the corresponding data are not available. In presence of metabolic activation, mutations were observed. The highest mutations rate was detected in the Arginine gene (with a mutation rate of 229%).

Table 7: Mutagenicity of VDC in the strain E. coli K12 after incubation with metabolic activation system

Substance	Concentration in the medium at 37°C [a]	Survival of bacteria (%)	Mutation rate in different operons (%)			
			Gal +	Arg +	MTR	Nad +
1,1-Dichloroethylene	2.5 mM	74 ± 7	120 ± 14	229 ± 26	100	100

[a] concentrations determined by GC analyses

In conclusion, VDC induced mutations in this bacterial gene mutation assay in the presence of an external metabolic activation system and in the conditions of the test.

1.4.1.6 [Bartsch (1975)]

Study reference:

Bartsch H., Malaveille C., Montesano R., Tomatis L. 1975: Tissue-mediated mutagenicity of vinylidene chloride and 2-chlorobutadiene in Salmonella typhimurium (publication), Nature, 255(5510):641-643.

Bartsch H., Malaveille C., Camus A.M., Martel-Planche G., Brun G., Hautefeuille A., Sabadie N., Barbin A., Kuroki T., Drevon C., Piccoli C. and Montesano R. 1980: Validation and comparative studies on 180 chemicals with S. thyphimurium strains and V79 chinese hamster cells in the presence of various metabolizing systems (publication), Mutation Research, 76:1-50.

Detailed study summary and results:

Test type

In this study, the mutagenicity activity of the VDC was tested in Salmonella typhymurium strains TA1530 and TA100, by gaseous exposure. This study was conducted according to the procedure defined by Ames et al. and modified by Bartsch et al.. Bacteria strains were exposed to different concentrations of VDC in air (v/v) in presence of different tissues supernatant (liver, kidney and lung) from untreated and phenobarbitone-pretreated mice or rats.

The study is closed to OECD guideline 471. Test conditions (purity of test item, controls) and results are not sufficiently detailed. In addition, only two strains and three concentrations were tested.

Test substance

In the publication, the test substance was defined as vinylidene chloride (VDC), from Merck-Schuchardt, Darmstadt, Germany, containing 0.3 % of 4-methoxyphenol as antioxidant. Purity, impurities and batch number are not available.

Administration/exposure

The strains TA100 and TA1530 were exposed to 0, 0.2%, 2% and 20% of VDC in air (v/v:) for 4 hours. Petri dishes containing 9 000 g of liver supernatant, the NADPH-generating system and the bacteria in a soft agar layer were exposed to VDC in air in a dessicator at 37°C in the dark. The plates were tested in triplicate. Then VDC was removed under vacuum and replaced by air, and, after further incubation for up to 48h at 37°C, the revertant were counted. Controls assay were carried out by omitting the cofactors (NADP+, glucose-6-phosphate).

The metabolic activation system was prepared from different tissues (9000 g supernatant) of phenobarbitone-pretreated male OF-1 mice or from female BDVI rat with cofactors (NADP+ at 2.0 µmol per plate) and glucose-6-phosphate (at 2.5 µmol per plate).

The concentration of VDC dissolved in the aqueous phase was determined by gas-liquid chromatography. Tthe concentration of VDC in the aqueous phase after 2h of exposure to 0.2, 2 or 20% VDC in air (v/v) were 3.3×10^{-4}M, 3.3×10^{-3}M and 3.3×10^{-2}M, respectively.

Results and discussion

When the cofactors (NADP+, glucose-6-phosphate) were omitted, no mutagenic effect was observed.

The mutagenic response, which was greater in the TA100 strain, increased in both strains after exposure to up to 2% VDC in air.. Since exposure of TA100 to 2% VDC in the presence of hepatic microsomal fraction from untreated and phenobarbitone-pretreated mice caused a mutagenic response up to 4h, exposure to 2 or 20% VDC in air for 4h was used to also assay the ability of rat and mouse kidney and lung fractions to

convert VDC in mutagenic metabolites.

Phenobarbitone pre-treatment of the mice produced an increase in the mutagenic response with all the three organs, but it was more active with liver S9 fraction than S9 fraction from kidney or lung. The authors explained that the lower response observed at 20% VDC in air may result from inhibitory action of VDC and/or its metabolites on the microsomal enzymes. A much lower response was observed in rats than in mice with liver fraction, and there was only minimal activity with kidney or lung fraction.

The effect of 4-methoxyphenol, which used as stabiliser in VDC, was tested. When applied to 500 µg/plate, it is not mutagenic for either strain, in absence or in presence of metabolic activation system.

Table 8: Mouse and rat tissue mediated mutagenicity of VDC in S. typhymurium TA100

Experiment No.	Species	Phenobarbitone pre-treatment	Tissue (9000g supernatant) [a]	Co-factors [b]	2% VDC in air		20% VDC in air	
					Revertants per plate [c]	Relative activity [d]	Revertants per plate [c]	Relative activity [d]
1	OF-1 mouse male	Yes	Liver	+	500 ± 23	150	330 ± 29	75
2				-	23 ± 10	5	7 ± 5	2
3		No		+	330 ± 49	100	435 ± 46	100
4				-	16 ± 4	5	1 ± 3	0
5		Yes	Kidney	+	147 ± 15	45	173 ± 5	40
6				-	31 ± 7	9	17 ± 2	4
7		No		+	67 ± 2	20	125 ± 5	29
8				-	20 ± 3	6	16 ± 1	4
9		Yes	Lung	+	34 ± 4	10	48 ± 5	11
10				-	5 ± 4	1	10 ± 1	2
11		No		+	21 ± 5	6	37 ± 3	8
12				-	6 ± 9	2	14 ± 8	3
13	BDVI rat female	No	Liver	+	95 ± 7	30	77 ± 5	18
14				-	0	0	2 ± 2	0
15		No	Kidney	+	18 ± 4	5	16 ± 2	4
16				-	18 ± 2	5	21 ± 4	5
17		No	Lung	+	9 ± 2	3	11 ± 2	3
18				-	9 ± 7	3	12 ± 6	3

[a] Equivalent to 38 mg wet tissue per plate / [b] NADP+ (2.0 µmol per plate) and glucose-6-phosphate (2.5 µmol per plate)

[c] Mean values of 4 experiments, each using pooled tissues from 4 mice or 3 rats. The number of spontaneous mutations per plate (49 ± 2) has been subtracted from each value.

[d] Relative mutagenic activity was expressed by taking the value obtained in experiment 3 as 100.

In conclusion, VDC induced a mutagenic response in presence of metabolic activation under the test conditions. These results demonstrate that VDC can be converted in vitro into mutagenic metabolites by mouse liver, kidney and lung fractions.

1.4.1.7 [Roldan-Arjona (1991)]

Study reference:

Roldan-Arjona T., Garcia-Pedrajas M.D., Luque-Romero F.L., Hera C. and Pueyo C. 1991: An association between mutagenicity of the Ara test of Salmonella typhimurium and carcinogenicity in rodents for 16 halogenated aliphatic hydrocarbons (publication), Mutagenesis, 6(3):199-205.

Detailed study summary and results:

Test type

Sixteen halogenated aliphatic hydrocarbons compounds, including vinylidene chloride, were assayed for genotoxicity using the L-arabinose resistance test or "Ara mutagenicity assay" with Salmonella typhimurium, both in absence and in presence of metabolic activation. The Ara forward mutation test was performed as previously described by Hera and Pueyo et al., 1986 and Roldan-Arjona et al., 1989. This bacterial assay is based on the changes from L-arabinose sensitivity (Ara^s) to L-Arabinose resistance (Ara^r) of Salmonella typhimurium. The mutants and survivors following the treatment of a mixed population of strains BA13 (mutation indicator) and BAL13 (survival indicator) are selected on a medium containing L-arabinose and a carbon source.

Test substance

In the publication, the tested substance is identified as "vinylidene Chloride (VDC)", with the CAS number 75-35-4, from Merck, with a purity of 99.5%. The treatment formulations were prepared in DMSO as vehicle.

Administration/exposure

Two S. typhimurium strains were used: BA13 (Ruiz-Rubk et al, 1985) and BAL13 (RoMan-Arjona et al, 1989). The strain BA13 was used as the 'mutation indicator' strain. The only colonies growing in mutant plates are L-arabinose resistant mutants (Ara^r) derived from BA13. The strain BAL13 was used as "survival indicator" and only grows in survival plates. The S9 fraction was prepared according to Ames protocol from liver of rats induced with Aroclor 1254. VDC was tested using the pre-incubation method. The mixed bacteria suspension (containing strains BA13 and BAKL13) with VDC solution and S9 mix (in case of metabolic activation) or buffer (in absence of metabolic activation) was incubated for 20 minutes at 37°C. Thereafter, top agar was added for overlaying onto the mutant or survivor plates. The test was performed in duplicate plates, and every assay was repeated on at least two separate occasions.

A test was considered positive if the number of Ara^r mutant colonies was at least twice the value of the corresponding solvent control, over at least three dose levels, and a reproducible dose-response curve could be demonstrated. The mutagenic potency was expressed as the number of Ara^r induced mutants per micromole of compound and minute of exposure.

Results and discussion

No increase in the mutants was observed in absence of metabolic activation. An increase of 2.08 over the solvent control was noted in presence of metabolic activation.

Table 9: Mutagenicity of VDC in the Ara assay

		Number of mutants over the solvent control	
		In absence of metabolic activation	In presence of metabolic activation

Substance	Method	Result	Number of independent experiments	Result	Number of independent experiments
VDC	Pre-incubation	Negative	2	2.08 ± 0.86 [a]	2

[a] Average ± SD of Ara^r mutants/µmol x min.

These results showed that VDC induced mutagenic effects in presence of metabolic activation under the conditions of the test.

1.4.1.8 [Baden (1978)]

Study reference:

Baden J. M. , Kelley M., Simmon V. F., Rice S. A. and Mazze R. I. 1978: Fluroxene Mutagenicity Mutation Research, 58 183-191

Detailed study summary and results:

Test type

Fluroxene (2,2,2-trifluoroethyl vinyl ether) which contains the stabilizer N-phenyl-l-napthylamine, was tested for mutagenicity using four strains of S. typhimurium, TA1535, TA1537, TA98 and TA100, and one strain of E. coli, WP2.

Test substance

Vinylidene chloride (3%) was used as positive control, and obtained from Dupont.

Administration/exposure

50 µl of overnight culture of Salmonella (~109 organism/ml), 2 ml of molten top agar containing 0.1 µM histidine, and either 0.5 ml of metabolic system or control mixture were mixed and plated on glucose minimal medium. For the E. coli strain, WP2, no histidine was added to the top agar and the mixture was plated on 0.125% oxoid medium. Triplicate plates were prepared at fluroxene and trifluoroethanol vapor concentrations ranging from 0.1 to 30% and at N-phenyl-l-naphthylamine concentrations ranging from 0.01-1000 µg plate; each experiment was performed at least twice.

Vinylidene chloride (3%) was used as positive control. In an initial series of desiccator and liquid incubation experiments, vapor concentrations of fluroxene were verified by gas chromatography and were found not to vary from predicted values by more than 10% nor to vary significantly with time.

(1) Desiccator incubation experiments. Plates were incubated for 8 h at 37°C in desiccators containing the above vapor concentrations of commercial fluroxene. Plates were then removed from the desiccators and incubated for an additional 40 h. In other dessicator experiments, bacterial stratus TA1535, TA100 or WP2, were exposed to fluroxene vapor concentrations of 10 or 30% for 2, 8, 12, 24 and 48 h.

(2) Liquid suspension experiments. Bacteria with either metabolic system or buffer were exposed to either purified fluroxene, commercial fluroxene, Nphenyl-l-naphthylamine or trifluoroethanol in sealed tubes for 2 h at 37°C. Molten top agar was then added to each tube and the contents were plated. In several experiments, survival of the bacteria was determined by diluting an aliquot of the suspension mixture 10^6 fold before plating. Plates were incubated at 37°C for 48 h.

(3) Direct plate assay. Bacteria, N-phenyl-l-naphthylamine, top agar, and metabolic system or buffer, were mixed, plated immediately and incubated for 2 days at 37°C.

Results and discussion

Table 10: Fluroxene Mutagenicity, S-9 Prepared From Livers Of Different Species

Agent	Concentration	Rat				Mouse			
		TA1535		TA100		TA1535		TA100	
		U	I	U	I	U	I	U	I
Fluroxene	1%	0	5	2	-5	-25	-23	9	3
	5%	10	35*	1	26*	5	-	21	63*
	10%	-3	93*	10	152*	7	207*	93	131*
	30%	13	75*	-9	148*	1	105*	16	38*
Vinylidene chloride	3%	-5	88*	16	126*	-	-	-	269*

U, Livers prepared from uninduced animals

I, Livers prepared from animals treated 5 days prior to sacrifice within 500 mg/kg Aroclor 1254

*Greater than room air control p < 0 01

1.4.1.9 [Jones & Hathway (1978b)]

Study reference:

Jones B. K. And Hathway D. E. (1978): Tissue-Mediated Mutagenicity Of Vinylidene Chloride In Salmonella Typhimurium TA1535. Cancer Letters, 5. 1-6

Detailed study summary and results:

Test type

Test system of Ames modified to assess the mutagenicity of gases and vapours

Test substance

VDC was supplied by Imperial Chemical Industries Limited, Mond Division, Runcorn, Cheshire. VDC was used in a stabilized form, i.e., in the presence of 100 ppm of p-methoxyphenol, in order to minimize the risk of peroxide formation and spontaneous polymerization.

Administration/exposure

Disposable plastic Petri dishes containing Vogel Bonner basal medium (Difco) were overlain with 2.0 ml 'top agar,' containing 0.1 ml overnight culture of the bacterial tester strain and mammalian tissue post-mitochondria supernatant (S-9, diluted 1-3 with cofactor) prepared either from Aroclor 1254 induced rats (Sprague-Dawley male albino) and mice (Alderley Park Swiss-derived male albino) or from uninduced rats and mice, marmosets (Alderley Park colony, 12-month-old males) and humans (suitable autopsy samples). The seeded dishes were exposed to atmospheres of VDC and air inside gas-tight culture vessels during incubation at 37°C for 72 h. VDC was introduced, as a liquid, through gas-tight valves.

VDC was screened at a concentration of 5% in air since preliminary tests showed that such a concentration had no adverse effect upon the survival of the bacterial strain and this was the maximum acceptable level below the explosive concentration of VDC in air.

Results and discussion

Table 11: Mutagenicity of VDC in the tissue-mediated Ames test

		Revertants/plate expressed as an average value for 5 separate plates											
Mammal	S-9 fraction	5% VDC			5% VCM[b]			50% VCM			Control[c]		
		Mean	± SD[a]	(RMA)	Mean	± SD	(RMA)	Mean	± SD	(RMA)	Mean	± SD	(RMA)
Rat	Induced liver	23.6	4.7	4.7	48.4	3	9.7	80.4	4.8	16.1	5	2.4	1
	Induced kidney	4.4	2.1	2	29.2	6.1	13.5	47.4	10.4	21.5	2.2	1.3	1
	Uninduced liver	12.4	4.3	1.2	87.4	6.5	8.2	129	16.4	12.2	10.6	2.3	1
	Uninduced kidney	14.4	1.8	1	94	8.3	6.8	154.4	21.2	11.2	13.8	2.6	1
Mouse	Induced liver	58	12	18.1	70.2	9.2	21.9	114.2	16.2	35.7	3.2	1.9	1
	Induced kidney	31.6	5.8	22.6	41.4	6.5	29.6	70.6	11.4	50.4	1.4	1.3	1
	Uninduced liver	22.6	4.3	1.6	87.4	11	6.2	141.4	11.7	10.1	14	3.8	1
	Uninduced kidney	30.8	8.7	2.3	116.8	10.5	8.6	189.2	10	13.9	13.6	4.7	1
Marmoset	Uninduced liver	22	4.5	1	83.6	4.9	4	193.2	12.6	9.2	21	1.6	1
Human	Uninduced liver	27.2	5.4	3	96.6	6.2	10.5	170	20	18.5	9.2	4.2	1
	Uninduced kidney	162	5.1	1	99.4	10.5	6.1	192	22	11.9	16.2	4.4	1

[a]Where RMA refers to the Relative Mutagenic Activity, according to Bartsch et al. (loc cit).

[b]Vinyl chloride was used as an appropriate positive control substance which also produces reactive alkylating metabolites by microsomal activation, 50% (v/v) atmospheric concentration was the level commonly used in the Laboratories, and 5% (v/v) provided an exact parallel to the concentration of the test substance which was found most useful for the mutagenicity testing.

[c]The atmospheric control tests were negative “controls” used.

1.4.1.10 [Bronzetti (1981)]

Study reference:

Bronzetti G., Bauer C., Corsi C., Leporini C., Nieri R. And Del Carratore R. (1981): Genetic Activity Of Vinylidene Chloride In Yeast. Mutation Research, 89 (1981) 179-185

Detailed study summary and results:

Test type

Gene conversion and reverse mutation in Saccharomyces cereuisiae (D7 strain) both in vitro with and without metabolic activation

Test substance

Vinylidene chloride (> 99.57% containing 0.02% 4-methoxyphenol as stabilizer) was purchased from Fluka. It was dissolved in DMSO for use.

Administration/exposure

Into 50-ml erlenmeyer flasks were placed 1.0 ml cell suspension (6 x 10^8/ ml in buffer), 2.0 ml of 10000X g liver supernatant, 3.0 ml activation cofactors (5 mg KCl, 4.8 mg glucose 6-PO4, 3.4 mg MgCl2 6H2O and 2.5 mg NADPH per ml of 0.1 M phosphate buffer, pH 7.4), and 0.1 ml of VDC in DMSO. In experiments without metabolic activation, 5.0 ml of 0.1 M PO4 buffer (pH 7.4) was substituted for the liver homogenate and cofactors. 5 tubes were used per dose. The tubes were incubated at 37°C for 2 h on a roller drum, after which the mixture was plated on selective media for enumeration of *trp* convertants and *ilv* revertants which were expressed as numbers of cells per 10^5 and 10^6 survivors, resp., and on complete medium for survivor counts.

Results and discussion

Table 12: Induction of gene conversion and point mutation in S. cerevisiae strain D7 by VDC in suspension with and without metabolic activation

Results are given as means of 5 independent Expts ± S.D. Treatment, 2h.

VDC (mM)	Survival (%)		Trp^+ convertants/10^5 survivors		Ilv^+ revertants/10^6 survivors	
	-S10	+S10	-S10	+S10	-S10	+S10
0 (control)	100	100	0.63 ± 0.12	0.65 ± 0.11	0.40 ± 0.08	0.43 ± 0.12
10	90.5 ± 2.1	92.5 ± 1.5	0.64 ± 0.12	0.56 ± 0.10	0.73 ± 0.10	0.51 ± 0.08
20	92.4 ± 2.1	92.0 ± 2.1	0.54 ± 0.05	0.55 ± 0.06	0.63 ± 0.15	0.60 ± 0.10
30	83 ± 9.1	82.4 ± 3.6	0.72 ± 0.14	1.41 ± 0.12	0.43 ± 0.07	2.3 ± 0.25
40	69.3 ± 2.5	73.5 ± 2.8	0.64 ± 0.10	1.8 ± 0.18	0.70 ± 0.07	2.8 ± 0.20
50	56.3 ± 4.3	63.6 ± 4.3	0.62 ± 0.09	3.5 ± 0.15	0.65 ± 0.12	3.5 ± 0.20

1.4.1.11 [Bronzetti et al. (1981)]

Study reference:

Bronzetti G., Bauer C., Corsi C., Leporini C., Nieri R. And Del Carratore R. (1981): Genetic Activity Of Vinylidene Chloride In Yeast. Mutation Research, 89 (1981) 179-185

Detailed study summary and results:

Test type

Intrasanguineous host mediated assay

Test substance

Vinylidene chloride (> 99.57% containing 0.02% 4-methoxyphenol as stabilizer) was purchased from Fluka. It was dissolved in DMSO for use.

Administration/exposure

A culture (0.2 ml) of $2x10^9$ yeast cells/ml of 0.9% saline was injected into the retro-orbital sinus of the mice. For the acute study, VDC at 400 mg/kg (in 0.5 ml corn oil), was administered by gavage immediately after administration of the yeast. In the sub-acute study, VDC (100 mg/kg in 0.5 ml corn oil) was administered daily by gavage for 5 days a week (23 administrations) followed by a dose of 200 mg/kg on the day of the assay (total dose, 2500 mg/kg). Again, the administration of the yeast culture (0.2 ml) immediately preceded the administration of the VDC (200 mg/kg in 0.5 ml corn oil) on the day of assay. 4 h after injection of the yeast cells the mice were killed by cervical dislocation; the liver, lungs and kidneys were aseptically removed, pooled from 3 animals, placed in 12.0 ml of 0.10 M Na+/K' phosphate buffer (pH 7.4) and homogenized in a Potter-Elvehjem homogenizer. The homogenates were centrifuged at 2000 rpm. The pellet was resuspended in 12.0 ml buffer, and plated as described for the suspension test.

Results and discussion

Table 13: induction of mitotic gene conversion and point mutation in S. cerevisiae strain d7 in the intrasanguineous host-mediated assay in mice after repeated oral dose of 2500 mg/kg

Organ	Number of Expts.[a]	Trp^+ convertants x 10^{-5} ± S.D.	Ilv^+ revertants x 10^{-6} ± S.D.
Liver	4	1.0 ± 0.4	0.29 ± 0.03
Control DCE	4	13.0 ± 0.3	5.2 ± 0.4
Lungs	4	1.07 ± 0.50	0.30 ± 0.03
Control DCE	4	1.09 ± 0.40	0.45 ± 0.03
Kidneys	4	3.0 ± 1.0	0.2 ± 0.03
Control DCE	4	18.3 ± 1.9	6.7 ± 0.3

[a] Each Expt. was performed on pooled organs from 3 mice. Treatment, 4 h.

1.4.1.12 [Baden et al., 1982]

Study reference:

Baden J.M., Kelley M., Mazze R.I. (1982) Mutagenicity of experimental inhalational anesthetic agents: Sevoflurane, synthane, dioxychlorane, and dioxyflurane. Anesthesiology Volume 56, Issue 6, Pages 462 – 463

Detailed study summary and results:

Test type

Ames test modified for volatile compound.

Test substance

Described as Vinylidene Chloride (3%) without further specification (used as the positive control).

Administration/exposure

Two assay procedures were used. In the first bacteria on petri plates were exposed to test anesthetic vapors (0.1-30.0 %) for 8h in desiccators. A direct plate assay was also performed in which liquid anesthetic was added to soft agar and bacteria, and the mixture was spread on histidine deficient culture medium. Tests were run in the presence of metabolic activation system prepared from the livers of enzyme-induced rats.

Results and discussion

Table 14: number of revertants per plates (±SD)*

	Strain	Control Air	Vapor Per Cent (v/v) (Desiccator)						µl/Plate (Direct plate)			Vinylidene Chloride 3 Per Cent
			0.1	1	2	10	20	30	1	10	30	
Sevoflurane (n=6)	TA1535	23 ± 3	27 ± 3	26 ± 7	20 ± 2	23 ± 3	16 ± 1	15 ± 2	15 ± 1	13 ± 4	11 ± 3	69 ± 5
	TA100	128 ± 7	141 ± 9	145 ± 11	124 ± 3	124 ± 23	130 ± 33	150 ± 12	100 ± 17	121± 15	100 ± 10	415 ± 25
Synthane (n=6)	TA1535	18 ± 2	18 ± 3	16 ± 3	16 ± 2	18 ± 2	8 ± 1	6 ± 0	25 ± 6	13 ± 3	15 ± 3	56 ± 7
	TA100	107 ± 3	98 ± 4	97 ± 11	116 ± 17	128 ± 3	18 ± 0	1 ± 19	122 ± 17	100 ± 13	129 ± 13	380 ± 31
Dioxychlorane (n=3)	TA1535	45 ± 9							41 ± 5	41 ± 6	33 ± 5	408 ± 48
	TA100	141 ± 7							143 ± 17	134 ± 13	146 ± 7	985 ± 35
Dioxyflurane	TA1535	20 ± 5							16 ± 5	26 ± 3	15 ± 3	155 ± 5

(n=3)	TA100	97 ± 15							114 ± 10	117 ± 13	101 ± 1	611 ± 7

*With liver metabolic activation system (S9)

1.4.1.13 [Oesch (1983)]

Study reference:

Oesch F., Protic-Sabljic M., Friedberg T., Klimisch H.-J.and Giatt H.R. (1983) Vinylidene chloride: changes in drug-metabolizing enzymes, mutagenicity and relation to its targets for carcinogenesis. Carcinogenesis Vol.4 No.8 pp.1031 -1038

Detailed study summary and results:

Test type

The mutagenicity experiments with his^- S. typhimurium were performed with minor modification of the methods described by Ames et al. and, in case of the volatile VDC, Bartsch et al. Experiments with trp^- E. coli were conducted analogously.

Test substance

VDC (gas chromatographic purity: 99.996% VDC; 27 ppm methylchloride + vinylchloride + chloroacetylene; 6 ppm dichloroacetylene; 4 ppm /ra/ts-l,2-dichloroethylene), stabilized with 200 ppm 4-methoxyphenol (monomethyl ether of hydroquinone), and 4-methoxyphenol were obtained from BASF AG, Ludwigshafen, FRG.

Administration/exposure

A volume (500 - 767 µl) of S-9 mix (or 500 µl 150 mM KCI), 100 µl of the bacterial suspension and 2000 µl top agar (which consisted of 0.55% agar, 0.55% NaCl, 50 µM histidine, 50 µM biotin, 50µM tryptophan and 25 mM sodium phosphate buffer pH 7.4, 45°C) were mixed in a test tube and poured onto a Petri dish with minimal agar consisting of 1.5% agar and Vogel-Bonner E medium with 2% glucose. In the case of solid test compounds, the mixture also contained 10 – 30 µl of a solution of the compound in dimethyl sulfoxide. In the case of VDC, the plates were placed for 4 h in a desiccator with VDC. After a further incubation for 2 - 3 days in the absence of VDC at 37°C in the dark, the colonies (his^+ or trp^+ revertants) were counted.

Results and discussion

Table I shows that metabolically activated VDC was mutagenic in all six bacterial strains used: S. typhimurium TA1535, TA100 and TA92, three DNA repair variant strains derived from the substitution mutant his G46; S. typhimurium TA98 and TA1537, two different frameshift mutants; and E. coli WP2

uvrA, which possesses a substitution mutation in a gene required for tryptophan synthesis. The stabilizer 4-methoxyphenol could not account for any of these mutations, as it did not show mutagenic effects (Table I). In the absence of an activating system, VDC and 4-methoxyphenol were not mutagenic.

Table 15: Investigation for liver enzyme-mediated mutagenicity and toxicity of VDC and of its stabilizer 4-methoxyphenol in various bacterial strains

		Revertant colonies/plate					
Test compound	Surviving fraction	TA100	TA1535	TA92	TA1537	TA98	WP2 uvrA
0 p.p.m. VDC	0	105	24	30	17	32	26
375	0.1	740	223	245	42	202	560
2250	0	880	490	319	43	247	600
4500	0	1270	500	285	52	277	690
10500	0	1080	550	295	41	274	740
22500	0	1380	660	263	46	314	680
100 mg 4-Methoxyphenol per 20 l desiccator	0	125	22	40	25	39	23
0	0	102	9	28	17	36	21
3.15-1000 µg 4-Methoxyphenol per plate	0.9-1.0	93-103	8-11	25-32	16-25	34-39	20-25

Minimal agar plates with *his*$^-$ *S. typhimurium* or *trp*$^-$ *E. coli* strains and with NADPH-fortified liver postmitochondrial supernatant fraction (corresponding to 50 mg tissue/plate) from untreated male adult Swiss Webster mice were kept at 37°C for 4 h in a desiccator with VDC. After further incubation for 2 days at 37°C, colonies (revertants) were counted. The stabilizer 4-methoxyphenol was tested by the same method (whereby no significant sublimation in the desiccator was observed) or by adding it (at 6 different concentrations) directly to the plates. The values are means from 2 to 4 replicate incubations. The individual values deviated by < 10% from the mean values, except in some cases where the numbers of colonies were low. Toxicity was estimated by determining the surviving fraction of 600 *his*$^+$ mutants which were added as an internal standard to mutagenicity plates (with TA1537).

Activation of VDC by various metabolizing systems

The liver and the kidney are the main target organs for the toxic and carcinogenic effects of VDC. Subcellular preparations of these organs from various mammalian species were tested for their ability to activate VDC to a mutagen (Table II). Liver S-9 mix from Chinese hamsters and mice were most active. Preparations from male Swiss Webster mice did not differ appreciably from preparations from females of the same strain nor from preparations from C57BL females. Preparations from C57BL males were also similarly active when used in low amount (12.5 mg tissue equivalents/plate), whilst they were less active than those from Swiss Webster mice when used in higher amounts (25 and 50 mg tissue equivalents/plate). Rat liver S-9 mix caused a clearly weaker activation than that from Swiss Webster mouse. Two preparations from human liver were also used. Both had similar activity. When large amounts of postmitochondrial fraction were used, they led to marked mutagenic effects, whereas at smaller concentrations they were much less

active than liver preparations from other species. Preparations from kidney were substantially less active than those from liver. Preparations from Chinese hamsters and from male mice of both strains regularly led to a weak activation, whereas those from female mice and from rats were mostly inactive. In a few experiments, kidney S-9 mix from rats and female mice weakly activated VDC. The reasons for the discrepancies among experiments are not clear, but may, at least in part, be due to the weakness of the mutagenicity. To investigate the possible roles of microsomal epoxide hydrolase and conjugation with glutathione in the activation and inactivation of VDC, epoxide hydrolase (purified from rat liver microsomes) or glutathione to S-9 mix from liver or kidney of Swiss Webster mice were added. Fifty units of enzyme, a > 20-fold excess over the endogenous activity in the S-9 mix, did not affect the mutagenicity of VDC. The epoxide hydrolase inhibitor, 1,1,1-trichloropropene 2,3-oxide, also had no effect on the activation of VDC by S-9 mix (this experiment was performed with TA98 in presence of 1 mM trichloropropene oxide). Glutathione usually reduced the mutagenicity of VDC by between 20 and 50%. However, glutathione increased the mutagenicity of VDC in some experiments, especially when weakly active preparations from kidney or when small amounts of liver preparations were used (data not shown). Kidney S-9 mix from female mice regularly activated VDC to a mutagen in the presence of glutathione, whereas this was usually not the case in its absence. The effect was always weak, maximally 100 colonies above solvent controls of -150 colonies.

Table 16: Activation of VDC to a mutagen by liver and kidney postmitochondrial supernatant fractions from various species

					Number of induced revertants/plate				
					Postmitochondrial fraction (tissue equivalents)				
Experiment	Organ	Species	Sex	p.p.m. VDC in mutagenicity experiments	6.25 mg	12.5 mg	25 mg	50 mg	100 mg
I	Liver	Swiss Webster mouse	male	4500	_ a	389	688	613	_a
			female	4500	_ a	384	683	719	_ a
		C57BL mouse	male	4500	_ a	381	215	296	_ a
			female	4500	_ a	362	598	570	_ a
		Chinese hamster	male	4500	_ a	312	619	654	_a
		Sprague Dawley rat	male	4500	_ a	162	243	345	_a
II	Liver	Human	male	2250	22	87	142	278	584
				4500	41	72	153	305	529

III	Liver	Human	male	2250	4	77	152	345	605
		Swiss Webster mouse	male	2250	133	310	354	475	559
IV	Kidney	Swiss Webster mouse	male	4500	_ a	_ a	93	67	85
		Sprague Dawley rat	male	4500	_ a	_ a	19	41	15
		Chinese hamster	male	4500	_ a	_ a	171	105	158
V	Kidney	C57BL mouse	male	450	_ a	_ a	_ a	126	_ a
				1250	_ a	_ a	_ a	106	_ a
				4500	_ a	_ a	_ a	108	_ a
				12 500	_ a	_ a	_ a	139	_ a
VI	Kidney	C57BL mouse	male	90	_ a	_ a	_ a	31	_ a
				450	_ a	_ a	_ a	70	_ a
				1250	_ a	_ a	_ a	113	_ a
VII	Kidney	C57BL mouse	female	90	11	_ a	18	7	_ a
				450	13	_ a	6	3	_ a
				1250	7	_ a	5	-3	_ a
				4500	6	_ a	10	- 1 2	_ a

Histidine-poor agar plates containing *his*⁻ *S. typhimurium* TA100 and NADPH-fortified postmitochondrial fraction from 6.25 to 100 mg liver or kidney were exposed to VDC for 4 h at 37°C. After a further incubation for 2 days at 37°C, his^+ revertant colonies were counted. Values are means from 2 (Experiment II, V, VI, VII) or 3 (other experiments) replicate incubation minus the corresponding number of mutants in the absence of VDC (98 —153). The variation coefficients of the number of colonies on replicate plates were < 10%. The two human liver samples were taken during surgical treatment, transported on ice and used for mutagenicity experiments, within 3 h after they had been taken. [a]Not determined.

1.4.1.14 [Drevon and Kuroki (1979)]

Study reference:

Drevon C. and Kuroki T. 1979: Mutagenicity Of Vinyl Chloride, Vinylidene Chloride And Chloroprene In V79 Chinese Hamster Cells. Mutation Research, 67 173-182

Detailed study summary and results:

Test type

In this publication, 34 chemicals were tested in a mammalian mutagenicity assay conducted on V79 Chinese hamster cells in the presence of metabolic activation system.

Test substance

The test substance specified in the publication is "vinylidene chloride", containing 0.3% 4-methoxyphenol as antioxidant.

Administration/exposure

Three concentrations of VDC were tested as vapour at 2, 10 and 20% in air.

V79 cells were incubated 18-24h after plating in the presence of post-mitochondrial fraction of mouse liver, co-factors and the test substance for 1 to 15h. Then cells were washed, incubated for 2-3h in fresh culture medium and plated for toxicity and mutagenicity. Mutations were determined by resistance to 8-azaguanine (AZA^r) and to ouabin (OUA^r). The selective media were added 48h after plating and changed once 5-7 days later. The cultures were fixed and stained with Giemsa after 12 days for AZA^r and 14 days for OUA^r. Mutations frequency was calculated for 10^5 survivors. The post-mitochondrial fractions were obtained after centrifugation of liver homogenates from mice pre-treated with phenobarbitone.

Results and discussion

No significant increase in the number of mutants colonies were observed.

Table 17: Mutagenicity of VDC in mammalian mutagenicity assay conducted on V79 Chinese hamster

Conc. in air (%)	Metabolic activation system [a]	Cytotoxicity	Number of colonies per 10^5 survivors	
			AZA^r	OUA^r
0	**complete, with rat S15**	63.0	4.5	0.4
2		15.3	4.2	0
10		2.3	0	0
0	**complete, with mouse $15**	68.3	6.4	0.4
2		61.3	3.1	0
10		64.4	3.9	0.2
0	**minus $15**	62.8	1.0	0.4
2		49.3	3.8	0.3
10		60.3	2.1	0.6

[a] Post-mitochondrial fractions obtained after centrifugation at 9000 or 15 000 × g of liver homogenates from mice pretreated with phenobarbitone (0.1% in drinking water for 1 week).

The authors concluded that no mutation was induced on the 8-azaguanine and ouabin loci when vinylidene chloride was assayed with V79 cells as target cells.

1.4.1.15 [Mc Gregor (1991)]

Study reference:

Mc Gregor D., Brown A.G., Cattanach P., Edwards I., McBride D., Riach C., Shepherd W. and Caspary W.J. 1991: Responses of the L5178Y Mouse Lymphoma Forward Mutation Assay: V. Gases and Vapors (publication), Environmental and Molecular Mutagenesis, 17:122-129.

Detailed study summary and results:

Test type

The substance VDC was tested in the L5178Y mouse lymphoma assay based on the OECD guideline 490 but adapted for testing vapors and gases. Cultures were exposed to the substance, which was delivered as vapors for 4 hr, with and without metabolic activation, then cultured for 2 days before plating in soft agar for cloning efficiency and mutant selection.

This study is adapted from the OECD guideline 490.

Test substance

VDC was supplied by the National Toxicology Program Chemical Repository, Radian Corporation, Austin, TX. No more information is available.

Administration/exposure

The study was conducted on the L5178Y tk+/tk- mouse lymphoma cells obtained from Dr. D. Clive, Burroughs Wellcome Co., Research Triangle Park, NC. Air control (negative control) and positive control groups were incubated in parallel with the test groups. The positive controls 3-Methylcholanthrene (MCA) in presence of metabolic activation and methyl ethanesulphonate (MMS) in absence of metabolic activation were supplied by Radian Corporation.

The first experiment was a toxicity test (measure of cell population expansion). This test was followed by three main experiments in the absence of S9 mix and two experiments in presence of S9 mix (S9 mix obtained from rat liver). The main experiments were conducted with at least five test concentrations with two cultures per concentrations.

Vapor of VDC was prepared in a glass flask plugged with a Suba-seal cap. The flask was securely anchored in a 37°C water batch in a fume hood. After the air extracted, volume of volatile liquid were injected into the flask until some liquid phase persisted. Then, a volume of air equal to the volume of test vapor to be introduced was removed from each culture tube, and the vapour was delivered into the culture flask.

Each tube was incubated for 4 hr on a horizontal axis roller drum rotating at 10 rpm. At the end of the incubation time, the exposed cultures were washed and resuspended in medium, and incubated for a 2 day expression period. Petri plates for cloning efficiency and mutant selections were prepared, and incubated for 11-14 days in 5% C02 at 37°C. Colonies were counted using an Artek 880 Automated Colony Counter. Toxicity was expressed as either a reduction of cell population growth during the expression period or a reduction in cloning efficiency. A measure of the overall toxicity was the relative total growth (RTG). Mutant fraction (MF) was calculated as follows: MF = 200 X mutant clones per plate (usually a mean of 3) divided by total clones per plate (usually a mean of 3) = mutants/10^6 clonable cells.

The statistical analysis consisted of a dose trend test [Barlow et al., 1972, p. 2151] and a variance analysis of

pairwise comparisons of each dose against the vehicle control.

Results and discussion

In the first trial without S9 mix, there was a significant response, only at 4% (v/v) vinylidene chloride. This result was not supported by similar responses at two higher concentrations (6% and 8%) where cell survival was similar to that found in the control cultures. Then, two further trials were conducted without S9 mix at higher concentrations. In the first one, the highest concentration was 15% and no significant mutagenic response was observed. In the second one, a significant response was observed at 30% without reaching the factor 2, with a RTG reduced to 21%. In the presence of S9 mix, toxicity was greatly enhanced and mutagenicity was observed at all dose levels with acceptable survivals. The lowest dose where there was a 2-fold increase in mutant frequency was 0.16%, with a RTG value of 70%.

Table 18: Results of VDC tested in L5178Y MLA (Mouse Lymphoma Assay)

Without S9 mix / Trial 1					
Conc. %	**CE**	**RTG**	**MC**	**MF**	**AVG MF**
0 (air)	52 63 81 62	80 103 101 117	20 48 41 57	13 26 17 31	22
1	71 78	134 127	38 72	18 31	24
2	0 R 65	0 108	58 64	0 33	
4	69 69	98 91	70 81	34 39	**37**
6	70 58	117 92	53 59	25 34	29
8	71 76	107 128	74 76	35 33	34
MMS 15 µg/mL	24 27	89 27	57 75	81 93	**87**

Without S9 mix / Trial 2					
Conc. %	**CE**	**RTG**	**MC**	**MF**	**AVG MF**
0 (air)	85 78 76 79	100 99 93 109	63 40 52 68	25 17 27 29	25
2	65 68	79 94	46 32	24 16	20
6	74 72	92 101	39 28	18 13	15
9	61 67	88 98	30 31	16 15	16
12	66 68	91 83	22 28	11 14	12
15	71 67	74 59	40 27	19 13	16
MMS 15 µg/mL	35 33	21 21	159 154	152 158	**15 5**

Without S9 mix / Trial 3					
Conc. %	**CE**	**RTG**	**MC**	**MF**	**AVG MF**
0 (air)	73 79 71 79	130 103 78 89	31 66 133 52	14 28 62 22	32
10	63 60	88 77	92 61	49 34	41
14	60 82	30 69	60 66	33 27	30
20	56 65	81 94	70 47	42 24	33
25	63 64	66 93	53 60	28 331	30
30	32 43	19 22	66 63	69 49	**59**
MMS 15 µg/mL	27 16	18 18	202 137	252 282	**26 7**

With S9 mix / Trial 1					
Conc. %	**CE**	**RTG**	**MC**	**MF**	**AVG MF**
0 (air)	73 67 77 68	100 97 115 88	107 97 94 92	49 48 41 45	46
0.16	68 81	69 70	201 207	99 86	**92**
0.31	70 46	79 42	213 210	102 153	**127**
0.63	52 47	37 37	293 299	188 211	**200**
1.25	31 36	22 25	308 294	337 271	**304**
2.5	41 45	16 22	359 305	290 227	**258**
MCA 2.5	33 34	10 15	393 458	399 456	**427**

With S9 mix / Trial 2					
Conc. %	**CE**	**RTG**	**MC**	**MF**	**AVG MF**
0 (air)	83 81 60 79	100 87 86 127	140 115 118 124	56 48 66 52	56
1	35 57	13 25	251 293	237 171	**204**
1.5	23 44	6 12	174 297	249 227	**238**
2	33 446	10 11	226 264	228 193	**211**
2.5	22 43	8 13	134 251	205 195	**200**
3	8 R Lethal R	2	136	591	R
3.5	8 R 4 R	1 1	184 87	783 757	R

µg/mL					

MCA	24	9	495	697	**600**
2.5	28	11	422	502	
µg/mL					

CONC = concentration / CE: Cloning efficiency / RTG = relative total growth / MC = mutant colony count
MF = mutant frequency (mutant colonies per $l0^6$ clonable cells)
AVE MF = average of mutant frequencies; underline when P < 5% (statistically significant)
R: Rejected CE<50% or CE > 120% or RTG < 1% / MMS = methyl methanesulfonate / MCA = methylcholanthrene

In conclusion, vinylidene chloride induced increases of the mutant frequency in the mouse lymphoma assay with metabolic activation. A mutant frequency increased up to 6-fold the negative control was observed with a dose-related trend and in presence of acceptable cytotoxicity (24% of survivals). In absence of metabolic activation, results were ambiguous. A statistically significant increase less than two-fold the negative control (factor 1.8) was observed at the limit of acceptable cytotoxicity (21% of survival) in one trial.

1.4.1.16 [Costa (1984)]

Study reference:

Costa A.K. and Ivanetich K.M. 1984: Chlorinated ethylenes: their metabolism and effect on DNA repair in rat hepatocytes (publication), Carcinogenesis, 5(12):1629-1636.

Detailed study summary and results:

Test type

This study was conducted on the basis of the OECD Guideline 482 (Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells In Vitro). The ability of VDC to induce unscheduled DNA synthesis was assessed in freshly isolated rat hepatocytes.

Test substance

The test substance was described as being vinylidene chloride purchased from Merck Chemicals, Darmstadt, FRG. No other information is available.

Administration/exposure

Viable hepatocytes were freshly isolated by perfusion from male Long-Evans rats (180-220g) pre-treated with phenobarbital. The viability of hepatocytes exceeded 90% at start of the experiment.

The study was assessed by the method of Andrae and Schwarz (1981): hepatocytes were incubated for 2.5 h in fresh medium containing 1,1-dichloroethene (2.1 mM) dissolved in ethanol and deoxy-[5-3H]cytidine (20 µCi, specific activity = 24 Ci/mmol). The reaction was terminated with ice-cold buffer. After centrifugation and washing, the pellet was frozen at -20 °C. Then it was resuspended and washed, diluted. An aliquot was used for measurement of the absorbance at 260 nm (parental DNA). Another aliquot was precipitated with trichloroacetic acid and collected on filters. The filters were counted in a Beckman LS 9000 liquid scintillation counter. Unscheduled DNA synthesis was identified by the radioactive peak banding with

parental DNA.

The concentration tested in the test was 2.1 mM and was the maximum VDC concentration which did not significantly affect the viability of isolated hepatocytes after 120 min. DMSO (0.01%) was used as negative control, and ethanol (0.001%) which was the vehicle did not stimulate the unscheduled DNA synthetis. The positive control was benzo[a]-pyrene (20 µM).

The Student's test was utilised to evaluate the difference between means. A difference was considered significant with $p < 0.01$.

Results and discussion

The authors concluded that VDC stimulated unscheduled DNA synthesis in isolated hepatocytes.

1.4.1.17 [Koch (1988)]

Study reference:

Koch R., Schlegelmich R. and Wolf H.U. 1988: Genetic effects of chlorinated ethylenes in the yeast Saccharomyces cerevisiae (publication), Mutation Research, 206:209-216.

Detailed study summary and results:

Test type

The induction of mitotic recombination (gene conversion or crossing-over) as well as mitotic aneuploidy of VDC was tested in the Saccharomyces cerevisae assay, similarly to the OECD guideline 481 (deleted in 2014).

The test was conducted on two strains D7 and D61.M. The strain D7 was used to detect mitotic gene conversion at the trp5 locus and reverse mutation at the ilvI-92 locus. The strain D61.M was used to study the induction of chromosomal malsegregation or aneuploidy, induced by interaction of the test substance with tubulin assembly. This strain forms red cycloheximide-sensitive colonies which do not require leucine. The loss of chromosome VII results in monosomic colonies which are white, cycloheximide-resistant and require leucine.

Test substance

The test substance described in the publication is 1,1-dichloroethylene (vinylidene chloride, CAS 75-35-4) obtained from EGC Chemie (Steinheim) and was of analytical grade.

Administration/exposure

The test was conducted on the strains D7 and D61.M provided by Dr. F. K. Zimmerman, Technische Hochschule Darmstadt (F.R.G.), in a liquid test procedure.

The substance was directly added into the cells suspension without dilution or preparation. Each experiment was repeated twice. Ethyl methanesulfonate (EMS) and Ethyl acetate were used as positive controls. The metabolic activation system was obtained from post-mitochondrial supernatant of liver homogenate from mouse pre-treated with Aroclor 1254. The S9 mix was prepared according the recommendations of Ames.

From strain D7, logarithmic-phase cells grown in 20% glucose liquid medium, containing a high level of cytochrome P-450, as well as stationary-phase cells combined with an exogenous metabolic activation, were used. After incubation for 2 h at 30°C, the mixtures of cells, test substance and S9 mix (with metabolic activation) or buffer (in absence of metabolic activation) were plated on each of 3 trp- and ile- selective plates for determination of convertants and revertants, and also on 3 plates containing complete medium for determination of survival.

From strain D61.M, growing cells in presence of metabolic activation were used. The mixtures of cells, test substance and S9 mix were stored overnight on ice bath. After a further incubation for 2.5 h at 30°C, they were plated onto selective medium containing cycloheximide, and also onto complete medium for determination of survival. After 5 days of incubation at 30°C, cycloheximide-resistant colonies were transferred to a medium lacking leucine. Only resistant and leucine-requiring colonies, indicating chromosome loss, were regarded as monosomics.

Results and discussion

The results obtained in the assay conducted on stationary phase cells from strain D7 without metabolic activation showed a low increase in the number of convertants or revertants (the increase did not exceed 50%). A remarkable increase in the number of revertants by 400-700% was noted using stationary-phase cells in the presence of metabolic activation as well as using log-phase cells. The results obtained in the assay conducted on D61.M cells showed that VDC increased the aneuploidy rate considerably, with no marked differences with or without metablic activation.

Table 19: Induction of mitotic gene conversion by VDC in Saccharomyces cerevisiae, strain D7

Substance	Stationnary phase cells without S9 mix		Stationnary phase cells with S9 mix		Logarithmic phase cells			
					Test 1		Test 2	
	Survivors (%)	Convertants per 10^5 survivors	Survivors (%)	Convertants per 10^5 survivors	Survivors (%)	Convertants per 10^5 survivors	Survivors (%)	Convertants per 10^5 survivors
Negative control	**100**	**2.30**	**100**	**2.16**	**100**	**1.97**	**100**	**2.01**
Tested doses								
25.1 mM	86.8	1.94	88.9	2.28	93.8	2.80	101.6	2.59
50.3 mM	91.8	2.07	80.8	2.75	89.9	3.46	47.7	3.93
75.4 mM	74.4	2.81	73.0	3.01	45.6	4.71	28.0	3.76
100.5 mM	65.3	2.25	62.1	3.51	19.3	4.64	11.8	5.00
Positive control EMS (24.4 mM)	58.3	28.21	63.7	25.43	/	/	/	/

The given values are from one representative test, with the exception for the assay on logarithmic phase cells where 2 independent results are documented.

Table 20: Induction of reverse mutation by VDC in Saccharomyces cerevisiae, strain D7

Substance	Stationnary phase cells without S9 mix		Stationnary phase cells with S9 mix		Logarithmic phase cells			
					Test 1		Test 2	
	Survivors (%)	Revertants per 10^6 survivors	Survivors (%)	Revertants per 10^6 survivors	Survivors (%)	Revertants per 10^6 survivors	Survivors (%)	Revertants per 10^6 survivors
Negative control	**100**	**1.40**	**100**	**1.09**	**100**	**1.75**	**100**	**1.32**
Tested doses								
25.1 mM	86.8	1.77	88.9	2.94	93.8	1.87	101.6	2.27
50.3 mM	91.8	1.22	80.8	6.74	89.9	2.44	47.7	5.52
75.4 mM	74.4	1.51	73.0	8.66	45.6	4.81	28.0	7.06
100.5 mM	65.3	1.93	62.1	9.12	19.3	9.09	11.8	11.11
Positive control EMS (24.4 mM)	58.3	315.17	63.7	283.20	/	/	/	/

Data come from the same experiment as described in the previous table regarding the mitotic gene conversion results.
The given values are from one representative test, with he exception for the assay on logarithmic phase cells where 2 independent results are documented.

Table 21: Induction of mitotic aneuploidy by VDC in Saccharomyces cerevisiae, strain D61.M

Substance	Survivors (%)		Total resistants (to cycloheximide) per 10^6 survivors [a]		White resistant colonies [a]				White, leu – and resistant colonies per 10^6 survivors [c]	
					Total		Leu - [b]			
	-S9	+ S9	-S9	+ S9	-S9	+ S9	-S9	+ S9	-S9	+ S9
Negative control	100	100	1.92	2.01	32	26	13	12	0.90	0.81
VDC										
25.1 mM	100.6	88.1	2.42	2.67	53	39	26	21	1.72	1.62
50.3 mM	86.7	69.3	4.27	3.84	48	50	29	33	2.26	2.28
75.4 mM	45.7	22.6	4.69	5.39	57	26	36	18	5.27	5.33
100.5 mM	15.4	7.7	4.65	4.34	34	22	27	18	11.76	15.59
Positive control Ethyl acetate (204.08 mM)	40.3	/	3.49	/	97	/	68	/	11.40	/

a The frequencies of resistants are based on colonies resistant to cycloheximide
b Leucine-requiring colonies
c For the determination of monosomics, white, resistant and leucine-requiring colonies were scored.

In conclusion, under the test conditions, VDC induced increases of the number of revertants and convertants in presence of metabolic, and elevated the rate of aneuploidy considerably.

1.4.1.18 [Waskell (1978)]

Study reference:

Waskell Lucy 1978: A study of the mutagenicity of anesthetics and their metabolites (publication), Mutation Research, 57:141-153.

Detailed study summary and results:

Test type

No guideline is available for this type of test. One single-strand break in the double-stranded circular phage PM2 DNA molecule can be detected by a sensitive assay described by Kiihnlein and his colleagues. The phage DNA molecule contains 18 000 bases and, according to the authors, a single break between any two

bases in the DNA can be detected with this very sensitive assay.

Test substance

The study was conducted on vinylidene chloride given by Dow Chemical Compagny. No more information was available.

Administration/exposure

The study was conducted on double-stranded circular radioactive phage PM2 DNA, given by Drs. U. Kuhnlein and S. Linn. The study was conducted in closed containers because of volatility of VDC. The phage PM2 DNA was exposed to VDC. An aqueous phase was saturated with VDC in a sealed vial. PM2 DNA, at a final concentration of 0.05 mM nucleotide and 3092 cpm/mM nucleotide, was added to this salt saturated solution. The reaction mix was incubated at 37 °C for 62h without shaking. The DNA was partially depurinated by heating for 3 min at 70 °C and exposed to alkali to generate single-strand breaks at apurinic sites, but do not denature covalently closed circular PM2 DNA molecules. The solution was filtered through a nitrocellulose filter, which selectively retains single-stranded DNA. The filters were dried and counted. The percentage of molecules with a single-strand break was determined from the amount of radioactivity retained by the filter.

Results and discussion

The DNA exposed to VDC had a greater number of single-strand breaks than the control. In the case of untreated DNA, 35 % of the DNA was found to have single-strand breaks, while VDC treatment resulted in 69 % single-strand breaks.

1.4.1.19 [Crebelli (1992)]

Study reference:

Crebelli R., Andreoli C., Carere A., Conti G., Conti L., Cotta Ramusino M. and Benigni R. 1992: The induction of mitotic chromosome malsegregation in Aspergillus nidulans. Quantitative structure activity relationship (QSAR) analysis with chlorinated aliphatic hydrocarbons (publication), Mutation Research, 266:117-134.

Detailed study summary and results:

Test type

The ability to induce chromosome malsegregation, to arrest conidial germination (and mitosis) and lethality (cytotoxicity) has been experimentally studied for 24 chlorinated aliphatic hydrocarbons, including VDC.

A liquid exposure protocol was routinely used in the mold Aspergillus nidulans. This procedure is used for treatment of mononucleate conidia during early germination, in order to study the first mitotic division(s).

Test substance

The substance tested in this study was identided as "1,1-dichloroethylene, CAS 75-35-4", with a purity of 99%, from Aldrich. DMSO was used as solvent.

Administration/exposure

The test was conducted on the mold diploid strain P1 Aspergillus nidulans as it was showed it has the hability to carry out the metabolic conversion of several promutagenic and procarcinogenic compounds (Bignami *et al.,* 1981). Euploid yellow segregants in the diploid strain Pl were detected as homozygous (yA2/yA2) or hemizygous (yA2/0) yellow sectors or patches in heterozygous (yA2/yA2 +) pale green colonies growing on complete medium. Mitotic segregants were isolated, often streaked for purification, and analyzed for their nutritional requirements in order to distinguish yellow whole chromosome segregants (which are p-fluorophenylalanine-resistant and require aneurine and p-aminobenzoic acid) from yellow cross-overs (which are p-fluorophenylalanine-sensitive and may require paminobenzoic acid) (Morpurgo *et al.,* 1979).

Conidia were pre-incubated in a semi-liquid agar complete medium at 37°C with gentle shaking. After 3.5 h, samples of pregerminating conidia were treated in sealed capped glass tubes with VDC until the emergence of germ tubes (usually 3-3.5 h). A range of concentrations was applied to obtain dose-effect relationships as well as to determine the lowest concentration arresting conidial germination (ARR) or inducing one lethal hit per cell (37% of survivors, D37). Treatments were interrupted by serial dilutions with sterile water and the conidia were plated on an agarized complete medium. Only plates with fewer than 20 colonies were used for the detection of mitotic segregants to prevent normal colonies from encroaching on abnormal, slow-growing aneuploids.

VDC was tested at the concentrations of 0.025, 0.05, 0.1, 0.125, 0.15, 0.175 and 0.2% (v/v). The test concentrations were selected in a pilot experiment. Doses which produced a delay or reversible arrest of conidial germination were selected for the main test: the lowest concentration assayed slighly delayed the emergence of germ tubes, and the highest one induced severe delay or arrest of conidial germination.

If the substance was unable to induce reproducible, significant increases in segregation rates at any of the concentrations assayed, it was evaluated as negative, i.e., ineffective in malsegregation induction in Aspergillus.

Results and discussion

1,1-dichloroethylene produced reproducible increases only in the frequency of euploid whole chromosome segregants (non-disjunctional diploids and haploids) in a range of doses where mitotic growth was affected, but not arrested. The arrest of conidial germination occurred at 0.2 %. No increase of cross-over rates was observed.

Table 22: Effects of VDC in mitotic segregation assay in A. nidulans diploid strain P1

Concentrations (% v/v)	Survival (%)	Scored colonies	Yellow segregants[a] in abnormal colonies				Yellow segregants[a] in normal colonies			
			Whole chromosome		Cross-over		Whole chromosome		Cross-over	
			n	%	n	%	n	%	n	%
0.025	100	260	2	0.77	0	-	0	-	4	1.54
0.05	93	815	9	**1.10****	0	-	0	-	10	1.23
0.1	85	392	8	**2.04*****	0	-	0	-	2	0.51
0.125	70	334	8	**2.39*****	1	0.30	1	0.30	2	0.60
0.15	50	249	5	**2.40*****	0	-	0	-	3	1.20
0.175	41	440	10	**2.27*****	1	0.23	0	-	0	-
0.2	20	233	2	0.86	0	-	1	0.43	1	0.43
Untreated[b]	100	2 553	6	0.24	1	0.04	2	0.08	16	0.63
Positive control[c]	30	240	33	12.50***	1	0.42	3	1.25	5	2.08

* p<0.05 / ** p<0.01 / *** p<0.001 (χ^2 test).

[a] Yellow segregants were classified as whole choromosome segregants (non-disjunctional diploids and haploids) when segregation of genetic markers on both arms of chromosome 1 was observed, and as cross-overs (not distinguished from terminal deletions) when segregation of markers on the right arm only was observed.

[b] Cumulative value: all the experiments shown in the Table were carried out using the same conidial suspension..

[c] Thiabendazole, 40 µg/mL

VDC was considered as positive for chromosome malsegregation induction.

1.4.1.20 [Sawada (1987)]

Study reference:

Sawada M., Sofuni T. and Ishidate Jr. M. 1987: Cytogenetic studies on 1,1-dichloroethylene and its two isomers in mammalian cells in vitro and in vivo (publication), Mutation Research, 187:157-163.

Detailed study summary and results:

Test type

This assay was conducted similarly to OECD guideline 473 (In Vitro Mammalian Chromosome Aberration Test), in Chinese hamster cell line (CHL).

Several deviations were identified. Description of the test conditions, preparation of the treatment formulations, details on the dosing phase, number of replicates and individual results are not available. In addition, the number of metaphases analysed per dose levels was insufficient (only 100 well-spread metaphases), and no positive control are available.

Test substance

The test substance was identified as being 1,1-DCE (CAS number 75-35-4) with a purity of 99%, and was purchased from Aldrich Chemical Compagny, Inc. This test substance contained 200 ppm of p-methoxyethanol as a stabilizer.

Administration/exposure

The test was conducted on Chinese hamster lung fibroblast cell line (CHL) from Ishidate and Odashima (1977), both in the presence and in the absence of metabolic activation (S9 mix). The test item, diluted in DMSO, was tested up to 2.0 mg/mL, limit of toxicity of the substance. The S9 fraction was prepared from the liver homogenate of male F344 rats pre-treated with PCB (KC-400). The bottles were sealed tightly with silicon stoppers, because of the very low boiling point of VDC. After a 6-h treatment period, the cells were rinsed and recultured with fresh medium for another 18 h period. The frequency of the cells with chromosomal aberrations was scored in 100 well-spread metaphases for each concentration group.

Results and discussion

In absence of metabolic activation, no significant increase of chromosomal aberrations were observed up to 2 mg/mL. In presence of metablic activation, an obvious dose-related induction of chromosomal aberrations was observed: 14% aberrant cells at 0.25 mg/ml and 54% (maximum) at 1.5 mg/ml. Almost all aberrations were chromatid-type, and exchanges were more predominant than gaps and breaks.

Although VDC contained 200 ppm of p-methoxyphenol as a stabilizer, the authors indicated that 20 µg/ml of p-methoxyphenol induced no chromosomal aberrations in the presence of S9 mix.

Metyrapone (an inhibitor of the P-450 activity) or GSH (which plays an important role in the detoxification of the active metabolites, Jones and Hathway, 1978) was added to the culture at several concentrations together with S9 mix, and then VDC was added at a final concentration of 1.5 mg/ml. The frequencies of aberrant cells decreased as the concentration of metyrapone or GSH increased (0.1-1.0 mM), and was only 8% at 1.0 mM of metyrapone (detailed results not available). This finding confirms that cytochrome P-450 in the liver microsome participates in the activation of VDC, and that GSH inhibited the induction of chromosomal aberrations.

Table 23: Frequency of cells with chromosomal aberrations in CHL cells treated with 1,1-dichloroethylene

Dose (mg/mL)	S9 mix	Cells with aberrations [a] (%)				
		ctg	ctb	cte	dic	Total
0	+	1	1	0	0	2
0.125	+	2	0	6	0	8
0.25	+	5	4	6	1	14
0.5	+	7	16	20	0	29
1.0	+	12	20	32	1	41
1.5	+	16	27	35	0	54
2.0	+	Toxic				
0	-	2	0	0	0	2
0.125	-	1	0	0	0	1
0.25	-	1	0	0	0	1
0.5	-	0	0	0	0	0
1.0	-	0	1	1	0	2
1.5	-	1	0	0	0	1
2.0	-	1	1	0	0	2

[a] ctg, chromatid gaps / ctb, chromatid breaks / cte, chromatid exchanges / dic, dicentric chromosomes.

Based on the results, VDC induced an obvious dose-related increase of chromosomal aberrations in presence of metabolic activation. The induction of chromosomal aberrations was inhibited by the addition of metyrapone, indicating that cytochrome P-450 participates in the activation of VDC.

1.4.1.21 [Sawada (1987)]

Study reference:

Sawada M., Sofuni T. and Ishidate Jr. M. 1987: Cytogenetic studies on 1,1-dichloroethylene and its two isomers in mammalian cells in vitro and in vivo (publication), Mutation Research, 187:157-163.

Detailed study summary and results:

Test type

This study was conducted similarly to OECD Guideline 479 (Genetic Toxicology: In Vitro Sister Chromatid Exchange Assay in Mammalian Cells) deleted in 2014, in Chinese hamster cell line (or CHL).

Several deviations were identified. Description of the test conditions, preparation of the treatment formulations, details on the dosing phase, number of replicates and individual results are not available. In addition, no positive control were concurrently tested.

Test substance

The test substance was identified as being 1,1-DCE (CAS number 75-35-4) with a purity of 99%, and was purchased from Aldrich Chemical Compagny, Inc. This test substance contained 200 ppm of p-methoxyethanol as a stabilizer.

Administration/exposure

The test was conducted on Chinese hamster lung fibroblast cell line (CHL) from Ishidate and Odashima (1977), both in the presence and in the absence of metabolic activation (S9 mix). The test item, diluted in DMSO, was tested up to 0.1 mg/mL. No information on the selection criteria of the maximum dose level is available. The bottles were sealed tightly with silicon stoppers, because of the very low boiling point of VDC. The S9 fraction was prepared from the liver homogenate of male F344 rats pre-treated with PCB (KC-400). After a 6-hour treatment period, cells were rinsed with PBS and recultured for another 24 h period. BUdR was present at a final concentration of 7 µM throughout treatment and during the subsequent incubation period. The sister-chromatid differentiation was made according to the method of Sakanishi- and Takayama (1977). The number of Sister Chromatid Exchange (SCEs) was counted in 50 metaphases for each concentration group.

Results and discussion

A stastistically significant increase was observed in the presence of metabolic activation at the two highest tested dose levels, without reaching twice the negative control value (increase factor of 1.6 and 1.8 over the control group). The SCE-inducing potential of VDC was relatively weak at the highest tested dose of 0.1 mg/mL.

Table 24: Number of sister chromatod exchanges (SCEs) in CHL cells treated with 1,1-dichloroethylene

Dose (mg/mL)	S9 mix	SCEs/cell	
		Range	Mean ± SD
0	+	2 – 19	10.0 ± 3.5
0.025	+	4 – 32	12.4 ± 5.3
0.05	+	3 – 23	13.3 ± 4.4
0.075	+	5 – 26	16.4 ± 5.0*
0.1	+	9 - 37	18.7 ± 5.9*
0	-	1 – 20	10.8 ± 4.1
0.025	-	3 - 22	11.2 ± 4.1
0.05	-	6 – 20	11.5 ± 3.0
0.075	-	5 – 20	11.2 ± 3.4
0.1	-	4 - 20	11.3 ± 3.6

* Significantly different from control (t-test, P < 0.01).

This assay showed that VDC significantly increased the number of SCEs in the presence of S9 mix at the maximum tested dose level of 0.1 mg/mL, although its induction was relatively weak. The lack of information regarding the cytotoxicity level doesn't allow to demonstrate that the test was conducted at the highest achievable dose level.

1.4.2 Animal data

Type of test	Description	Results	Reference
Micronucleus test (somatic cells)	Micronucleus in fetal liver and blood in mice (IP) No guideline available	Negative	Sawada M. *et al.*, 1987
	Micronucleus in bone marrow in mice (oral) Similar to OECD guideline 474	Negative	Sawada M. *et al.*, 1987
	Micronucleus in peripheral blood in mice exposed by inhalation According to OECD guideline 474	Negative	NTP, 2015
Chromosome aberrations	Chromosome aberrations in bone marrow in rats exposed by inhalation Similar to OECD guideline 475	Negative	Quast J.F., 1986
Comet assay (somatic cells)	In lung, liver, kidney and bone marrow from rats exposed by inhalation According to OECD guideline 489	Positive in lung, kidney and liver Negative in bone marrow	Anonymous, 2016
DNA synthesis and DNA repair (somatic cells)	In liver and kidneys from rats and mice exposed by inhalation No guideline followed	Few alkylated nucleotides recovered and DNA repair synthesis only modestly elevated	Reitz R.H., et al., 1980
Dominant Lethal Test	In rats exposed by inhalation Similar to OECD guideline 478	Negative	Short R.D. et al., 1977

(germ cells)	In mice exposed by inhalation Similar to OECD guideline 478	Negative	Anderson D. et al., 1977
Sex-linked recessive lethal assay (germ cells)	In Drosophila melanogaster (oral) Similar to OECD Guideline 477 (deleted in 2014)	Negative	Foureman P., et al., 1994

[1] The studies are described just after in the next sections 3.8.2.1 to 3.8.2.9

1.4.2.1 [Sawada (1987)]

Study reference:

Sawada M., Sofuni T. and Ishidate Jr. M. 1987: Cytogenetic studies on 1,1-dichloroethylene and its two isomers in mammalian cells in vitro and in vivo (publication), Mutation Research, 187:157-163.

Detailed study summary and results:

Test type

The authors assayed the ability of 1,1-dichloroethylene (VDC) to induce micronucleus in fetal liver and blood of exposed pregnant mice. No guideline is available for this type of study. The test was conducted according to the transplacental micronucleus test described by Cole *et al*., 1981. Pregnant mice were injected with VDC intraperitoneally on the 18th day of gestation. At 24 h after the injection, the liver and blood cells from fetuses were prepared for analysis of micronuclei from erythrocytes.

The test conditions are insufficiently described, the preparation of the treatment formulations and individual results are not available. In addition, no positive control was tested.

Test substance

The test substance was identified as being 1,1-DCE (VDC, CAS number 75-35-4) with a purity of 99%, and was purchased from Aldrich Chemical Compagny, Inc. This test substance contained 200 ppm of p-methoxyethanol as a stabilizer.

Test animals

The test was conducted on pregnant ICR mice purchaised from Clea Japan Inc. The number of pregnant mice per group was not available.

Administration/exposure

Pregnant mice were dosed with VDC intraperitoneally on the 18th day of gestation, at the dose levels of 25, 50 and 100 mg/kg bw. An additional group treated with the vehicle (olive oil) was used as negative control. No positive control were added. At 24h after the injection, the fetal liver and fetal blood cells suspended in fetal calf serum were fixed with methanol, and stained with Acridine Orange to distinguish micronuclei from RNA containing basophilic stippling that occasionally appeared in the fetal blood cells. 1000 erythrocytes emitting red fluorescence (which correspond to polychromatic erythrocytes or PCE in Giemsa staining) were

observed per fetus and erythrocytes with micronuclei which emitted yellowish green fluorescence were scored. Four fetuses per group in the negative control group and at 25 mg/kg and six fetuses per group at 50 and 100 mg/kg were analysed.

Results and discussion

No significant increase of micronucleated polychromatic erythrocytes (MNPCE) either in liver or blood of fetuses exposed from 25 to 100 mg/kg were observed. The ratio PCE / (PCE + NCE) did not show any sign of cytotoxicity.

Table 25: Micronucleated erythrocytes in mouse foetuses after treatment of parental females with VDC

Dose (mg/kg)	Number of fetuses analyzed	Tissue	MNPCE[a] (%)		PCE/(PCE + NCE)[c] (%)
			Range	Mean ± SD	Mean ± SD
0[b]	4	Liver	0.1 – 0.4	0.28 ± 0.15	74.3 ± 3.9
		Blood	0.0 – 0.3	0.13 ± 0.13	69.9 ± 7.1
25	4	Liver	0.2 – 0.5	0.35 ± 0.13	75.0 ± 6.2
		Blood	0.1 – 0.3	0.23 ± 0.10	68.3 ± 6.4
50	6	Liver	0.1 – 0.4	0.27 ± 0.12	75.7 ± 6.3
		Blood	0.1 – 0.3	0.22 ± 0.10	72.2 ± 3.9
100	6	Liver	0.1 – 0.4	0.25 ± 0.12	78.7 ± 2.8
		Blood	0.0 – 0.4	0.22 ± 0.15	74.2 ± 5.5

[a] MNCE = micronucleated polychromatic erythrocytes
[b] Vehicle control (olive oil, 10 mL/kg)
[c] NCE = normochromatic erythrocytes / PCE = polychromatic erythrocytes

In the conditions of the study, VDC did not induce significant increase of micronucleated erythrocytes either in the liver or the blood of fetuses from mice exposed intraperitoneally on the 18th day of gestation. The absence of cytotoxicity on the PCE/NCE ratios does not allow to confirm that the maximum dose level was tested and/or that the test item reached the fetuses.

1.4.2.2 [Sawada (1987)]

Study reference:

Sawada M., Sofuni T. and Ishidate Jr. M. 1987: Cytogenetic studies on 1,1-dichloroethylene and its two isomers in mammalian cells in vitro and in vivo (publication), Mutation Research, 187:157-163.

Detailed study summary and results:

Test type

This test, equivalent to OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test), was conducted on mice exposed to 1,1-dichloroethylene (VDC) by gavage once or 4 times at 24h intervals. Micronucleated PCE (MNPCE) were examined in the bone marrow.

Test substance

The test substance was identified as being 1,1-DCE (VDC, CAS number 75-35-4) with a purity of 99%, and was purchased from Aldrich Chemical Compagny, Inc. This test substance contained 200 ppm of p-methoxyethanol as a stabilizer.

Test animals

The study was conducted on 8-week-old male ddY mice (body weight: 31-34 g) purchased from Shizuoka Agricultural Co-op. Assoc. Six mice were used in each tested dose.

Administration/exposure

VDC was administered by gavage in olive oil to mice at 25, 50, 100 and 200 mg/kg for single administration or 25, 50 and 100 mg/kg for 4 administrations at 24 h intervals. Animals were killed 24 h after the last treatment, and femoral marrow cells were fixed and stained with Giemsa. The sampling time and the maximum dose were determined by the pilot experiment according to Hayashi *et al.*, 1984 in which preparations were made at 5 different points between 18 h and 72 h after administration. An additional group treated with only vehicle (olive oil) was used as negative control, and another group dosed once by intraperitoneal injection with 2 mg/kg Mitomycine C was used as positive control. 1000 polychromatic erythrocytes (PCE) were examined per mouse, and the number of micronucleated PCE (MNPCE) was recorded. The number of micronucleated normochromatic erythrocytes (MNNCE) among 1000 NCEs (normochromatic erythrocytes) was also recorded.

Results and discussion

Three out of 6 animals died after a single treatment with 200 mg/kg, and 1 animal died after 4 treatments with 100 mg/kg. The frequencies of MNPCE and MNNCE at each dose in both treatments were not significantly different from those in the control groups. No significant changes were observed in the ratio of PCE to total erythrocytes. This result confirms the absence of cytotoxicity to bone marow.

Table 26: Frequency of micronucleated erythrocytes in bone marrow of mice treated with 1,1-DCE

Dose (mg/kg)	Number of treatments	Surviving / treated animals	MNPCE[a] (%)		MNNCE[b] (%)		PCE/(PCE + NCE) (%)
			Range	Mean ± SD	Range	Mean ± SD	Mean ± SD
0[c]	**1**	6/6	0.1 – 0.4	0.22 ± 0.12	N.D.[e]	-	55.4 ± 4.4
25		6/6	0.0 – 0.4	0.15 ± 0.16	N.D.	-	52.7 ± 3.2
50		6/6	0.1 – 0.3	0.22 ± 0.10	N.D.	-	51.3 ± 6.7
100		6/6	0.0 – 0.3	0.17 ± 0.10	N.D.	-	57.1 ± 5.2
200		3/6	0.2 – 0.3	0.23 ± 0.06	N.D.	-	42.6 ± 10.9
0[c]	**4 at 24 h intervals**	6/6	0.1 – 0.4	0.22 ± 0.10	0.0 – 0.3	0.10 ± 0.11	50.6 ± 3.2
25		6/6	0.0 – 0.1	0.03 ± 0.05	0.0 – 0.2	0.08 ± 0.08	53.0 ± 5.6
50		6/6	0.0 – 0.3	0.17 ± 0.12	0.0 – 0.3	0.10 ± 0.13	56.7 ± 6.9

100		5/6	0.0 – 0.2	0.12 ± 0.08	0.0 – 0.2	0.12 ± 0.08	46.4 ± 7.9
Mitomycine C[d] 2 mg/kg	**1**	3/6	5.5 – 8.9	6.88 ± 1.35	N.D.	-	34.0 ± 6.7

[a] MNCE = micronucleated polychromatic erythrocytes / [b] MNNCE = micronucleated normochromatic erythrocytes
[c] Vehicle control (olive oil, 10 mL/kg) / [d] Positive control (in saline, by intraperitoneal injection)
[e] N.D. = not determined

Under the conditions of this study, VDC did not induce significant increase of MNPCE in mice either after once administration or after 4 administrations at 24 h intervals. Mortality for 3/6 animals were observed after one treatment at the dose level of 200 mg/kg. A slight decrease in the PCE/NCE ratio was observed at the highest tested dose levels (200 mg/kg after one administration or 100 mg/kg after 4 administrations), but without being statistically significant.

1.4.2.3 [Quast (1986)]

Study reference:

Quast J.F., McKenna M.J., Rampy L.W. and Norris J.M. 1986: Chronic toxicity and oncogenicity study on inhaled vinylidene chloride in rats (publication), Fundamental and applied toxicology 1986, 6:105-144.

Detailed study summary and results:

Test type

As a add-on to a 18-month cancer study, 4 male and 4 female rats were exposed by inhalation during 6 months to VDC. The test was similar to OECD guideline 475 (Mammalian Bone Marrow Chromosomal Aberration Test). Four hours prior to sacrifice, the animals were injected colchicine, and after sacrifice, femoral bone marrow cells were harvested and investigated for cytogenetic abnormalities.

The test conditions are insufficiently described. The preparation of the treatment formulations and the individual results are not available. Only 4 animals were used and 2 doses were tested. No positive control was concurrently added. Only 50 metaphases (instead of 200 as required in the guideline) were analysed per animal and no details on the type of aberrations were observed. No mitotic index were calculated to assay the cytotoxicity to bone marrow and justify of its exposure.

Test substance

The test item as described in the publication was VDC obtained from Dow Chemical company. The maximum concentration of the impurity MEHQ (mono methyl ether of hydroquinone) in test item vapour was estimated at 121 ppb. The authors concluded that this level was very low and it was unlikely that it contributes significantly to any toxicity seen in the study.

Test animals

The study was conducted on male and female Sprague-Dawley rats, 6 to 7 weeks old. Food and water were made available to the animals ad libitum except during the exposure (including controls). Three groups of 4 male and 4 female rats, one control group and two treated groups, were exposed for 18 months.

Administration/exposure

Rats were exposed for 6 months to VDC vapour at 0, 25 or 75 ppm (equivalent 100 or 300 mg/m^3), 6 hours/day, 5 days/week. Exposures were carried out in 3.7 m^3 stainless steel chambers under dynamic flow conditions. The VDC vapour concentrations were generated by metering liquid VDC into a temperature controlled vaporization flask. Chamber concentrations were monitored by infrared spectrophotometry. The mean measured concentrations in chambers were 25.4 ± 4.6 and 72.6 ± 7.5 ppm (or 101.6 ± 18.4 and 290.4 ± 30 mg/m3) for the target concentrations of 25 and 75 ppm (or 99 and 298 mg/m3) respectively. At the end of the exposure period and 4h before sacrifice, the animals were injected intraperitoneally with 4 mg/kg colchicine. Femoral bone marrow from each rat were harvested and fixed. The cells were scored for chromosomal aberrations according to standardised OECD guideline 475.

Results and discussion

The results, as they were reported, did not mentioned indication of toxicity. Fifty acceptable metaphase spreads were not found on all the slides prepared for all the animals. Cytogenetic evaluation revealed no chromatid or chromosomal aberrations in either the control or the VDC-exposed groups.

Table 27: Cytogenetic evaluation of femoral bone marrow cells from mice exposed to VDC

Exposure group	Number of rats	Sex	Number of cytogenetic abnormalities/number of cells scored
Control	4	Male	0/34 0/23 0/40 0/38
	4	Female	0/0 0/50 0 0/40
25 ppm	3[a]	Male	0/50 0/29 0/50
	4	Female	0/39 0/35 0/50 0/28
75 ppm	4	Male	0/50 0/50 0/50 0/50
	4	Female	0/50 0/32 0/50 0/50

[a] One rat died spontaneously prior to the time of evaluation

No increase in chromosome aberrations were observed in bone marrow of rats exposed for 6 months to VDC up to the dose level of 75 ppm (equivalent to 298 mg/m3). No toxicity (clinical signs or mortality) was observed, cytotoxicity to target cells was not investigated and the number of analysed metaphases was much lower than the requirement.

1.4.2.4 [NTP (2015)]

Study reference:

National Toxicology Program 2015: Technical Report NTP TR 582; Toxicology and carcinogenesis of vinylidene chloride (CAS No. 75-35-4) in F344/N rats and B6C3F1/N mice (inhalation studies); National Institutes of Health, Public Health Service, U.S. Department on Health and Human Services.

Detailed study summary and results:

Test type

This test was similar to OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test), on mice treated for 3 months by 1,1-dichloroethylene vapour. The frequency of micronuclei was determinated in 2 000 normochromatic erythrocytes (NCE) from peripheral blood.

Test substance

Vinylidene chloride, manufactured by Dow Chemical Company (Freeport, TX), was obtained in one lot from Sigma-Aldrich. The material was identified as lot SB20019301. Analysis by GC/FID indicated that the test article was stabilized with approximately 300 ppm monomethyl ether hydroquinone (MEHQ) and that the overall purity was greater than 99.9%.

Test animals

The study was conducted on male and female B6C3F1 mice, obtained from the NTP colony maintained at Taconic Farms, Inc. (Germantown, NY). On receipt, the mice were 3 or 4 weeks old. They were quarantined for 12 or 13 days and were 5 to 6 weeks old on the first day of the studies. Mice were housed individually.

Administration/exposure

Groups of 5 male and female mice were exposed by whole body to vinylidene chloride vapor for 6 hours plus 10 minutes per day, 5 days per week for 14 weeks, at the concentrations of 6.25, 12.5, 25, 50 (male and female) and 100 ppm (female only) in air. An additional group received only air as negative control.

Concentrations of vinylidene chloride were monitored by an on-line gas chromatograph. Samples were drawn from each exposure chamber approximately three per hour during each 6-hour exposure period. The average measured concentrations obtained were 6.28 ± 0.12, 12.6 ± 0.2, 25.1 ± 0.5, 50.4 ± 1.0 and 100 ± 2.2 (for the target concentrations of 6.25, 12.5, 25, 50 and 100 ppm, respectively).

At the end of the 3-month toxicity study, peripheral blood samples were obtained from mice and smears were immediately prepared, fixed and stained with acridine orange. Slides were scanned to determine the frequency of micronuclei in 2000 normochromatic erythrocytes (NCEs) per animal. The percentage of polychromatic erythrocytes (PCEs or reticulocytes) among the total erythrocyte population in the peripheral blood was scored for each dose group as a measure of bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a group with the standard error of the mean. The frequency of micronucleated cells among NCEs (MNNCE) was analyzed by a statistical software that tested for increasing trend over exposure groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposed group and the control group.

Results and discussion

Two 50 ppm males and four 100 ppm females died during the first week of the study. The mean body weights of all exposed groups of females and of males exposed to 12.5 ppm or greater were significantly less than those of the chamber control groups. Exposure concentration-related decreases in the erythrocyte counts, hemoglobin concentrations, and hematocrit values occurred at the end of the study in 12.5, 25, and 50 ppm male mice. No increase in the frequency of MNNCE was observed in peripheral blood of male or female mice exposed to vinylidene chloride, and no change in the percentage of PCE was seen, suggesting the absence of chemical-induced bone marrow toxicity.

Table 28: Micronuclei in peripheral blood erythrocytes of mice exposed to VDC by inhalation for 3 months

	Dose (ppm)	Number of mice with erythrocytes scored	Micronucleated NCEs[a]/1000 NCEs	P value[e]	PCEs[b, c] (%)
MALE					
Negative control (air)	0	5	2.40 ± 0.33		2.90 ± 0.34
Vinylidene chloride	6.25	5	2.00 ± 0.32	0.7270	2.84 ± 0.45
	12.5	5	1.40 ± 0.40	0.9478	2.58 ± 0.32
	25	5	3.20 ± 0.70	0.1422	3.08 ± 0.14
	50	5	2.10 ± 0.58	0.6728	2.96 ± 0.22
			P = 0.363[d]		
FEMALE					
Negative control (air)	0	5	1.20 ± 0.30		3.10 ± 0.38
Vinylidene chloride	6.25	5	0.90 ± 0.43	0.6917	2.88 ± 0.41
	12.5	5	1.40 ± 0.56	0.3821	2.74 ± 0.31
	25	5	1.10 ± 0.43	0.5634	2.56 ± 0.46
	50	5	1.80 ± 0.44	0.2010	3.14 ± 0.49
	100	5	1.00 ± 0.50	0.6278	2.80 ± 0.31
			P = 0.481[d]		

[a] NCE=normochromatic erythrocyte / [b] PCE=polychromatic erythrocyte / [c] Mean values ± standard error
[d] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at P≤0.025
[e] Pairwise comparison with the control group; exposed group values are significant at P≤0.006 for males and P≤0.005 for females.

The Authors concluded that VDC did not induce increase of micronucleated erythrocyte frequency in

peripheral blood of mice exposed by inhalation for 3 months.

1.4.2.5 [Anonymous (2016)]

Study reference:

See confidential Annex

Detailed study summary and results:

Test type

An alkaline in vivo comet assay was conducted according to the OECD guideline 489 (In Vivo Mammalian Alkaline Comet Assay) on rats after inhalation exposure to 1,1-dichloroethylene. Lung, liver, kidney and bone marrow were examined for DNA damage.

This study was conducted according to OECD and GLP guidelines.

Test substance

The study was conducted on 1,1-dichloroethylene (VDC) (CAS 75-35-4), from Solvay, batch 5D235, with a purity > 99.9%. A certificate of analysis is available in the study report.

Test animals

The test was conducted on Wistar rat (strain Crl:WI(Han)), 7 to 8 weeks old.

Administration/exposure

Four groups of five males were exposed to vapour of VDC, a negative control group was exposed to air and another group was exposed to EMS as positive control. Lung, liver, kidney and bone marrow were examined for DNA damage by using the alkaline in vivo Comet Assay.

Animals were exposed by nose only inhalation for 4 hours on three consecutive days at target concentrations of 25, 3, 1 and 0.1 mg/L (6350, 750, 250 and 25 ppm or 25000, 3000, 1000 and 100 mg/m3 respectively). Animals were placed in restraining tubes, which were connected to the exposure chamber. The chamber consisted of animal sections with eight animal ports each. Each animal port had its own test atmosphere inlet and exhaust outlet.

The target concentrations and sex(es) to be used were based on a MTD study, in which no substantial differences between sexes in toxicity was found, and the MTD was found to be between 40 and 80 mg/L. The main study was started with the target concentration of 25 mg/L. Since significant DNA damage and histopathological findings were observed, the next group was exposed to 3 mg/L and then 1 mg/L in order to decrease the histopathological findings. In order to obtain information about the dose response and to have an exposure level without adverse histopathological findings, the last group was exposed to 0.1 mg/L.

Samples were drawn from the test atmosphere at least ten times during each exposure period each day and

were immediatly analysed by a validated Gas Chromatographic method. The actual concentrations were slightly above the target concentrations (up to 10% at 25, 3 and 1 mg/L and up to 20% at 0.1 mg/L). The concentration measurements were equally distributed over each exposure period and stable. The variations were considered not to have affected the exposure level.

The negative control group was similarly exposed to air, the positive control group was dosed orally with 200 mg/kg EMS for two consecutive days.

For all animals, clinical signs and body weights were recorded once daily. Within 4 hours after the last inhalation exposure or within 2 and 6 hours after the last oral dosing, animals were subjected to macroscopic examination. Histopathology was performed on lung, liver and kidney. Lung, liver, kidney and bone marrow were examined for DNA damage according to the alkaline in vivo Comet Assay standard guideline.

Results and discussion

There were no unscheduled deaths. Hunched posture and piloerection were observed on day 1 only, in all animals dosed at 25 and 1 mg/L. No clinical signs were observed in animals exposed to 3 and 0.1 mg/L. At 25 mg/L exposure level, body weight loss between 12 and 16% was seen compared to day 1 values. At 3 and 1 mg/L exposure level, some slight to moderate body weight loss (between 1 and 3%) was observed.

Test substance related macroscopic findings consisted of reddish discoloration of the lungs and dark red discoloration of the liver found in one animal exposed to 25 mg/L and red brown discoloration of the kidneys of one animal exposed to a dose of 3 mg/L.

Histopathology showed adverse test substance-related microscopic findings at exposure levels of 25, 3 and 1 mg/L. At the highest dose (25 mg/L), the test item readily causes degeneration of cells in the lung, liver and kidneys. At the following dose (3 mg/L) the degeneration is primarily seen in the liver, with hepatocellular necrosis. The lungs show regeneration indicating there initially was cell damage but the cells are already recovering from that insult. The kidneys do not show any indicators of test item-related cell damage. At 1 mg/L there was only overt cell damage in the liver. At 0.1 mg/L the cells in the liver show signs of centrilobular enzyme induction in response to the test item indicated by the centrilobular hypertrophy and cytoplasmic alteration. This response, in the absence of hepatocellular necrosis, was considered to be non-adverse.

All experiments passed the acceptance criteria (negative controls, positive controls). The results obtained for each organ are listed in the table below.

- Kidney: a statistically significant increase ($p<0.05$) of the Tail Intensity (%) was observed at all test concentrations. DNA damage expressed as percentage Tail Intensity increased from 4.37% to 68.72%, 11.57% to 57.25%, 26.58% to 35.59% and 13.75% to 54.95% at 25, 3, 1 and 0.1 mg/L, respectively. The Tail intensities (%) after treatment were clearly above the historical control data range (2.31% – 24.02%) in all tested cncentrations and the increases are therefore considered

biologically relevant.

- Liver: a statistically significant increase ($p<0.05$) of the Tail Intensity (%) was observed at 25, 3 and 1 mg/L. DNA damage expressed as percentage Tail Intensity increased from 3.65% to 19.96%, 15.38% to 33.29%, 16.35% to 44.39 at 25, 3, and 1 mg/L, respectively. The Tail intensities (%) after treatment at 3 and 1 mg/L were above the historical control data range (0.13% – 21.13%) and the increases are therefore considered biologically relevant. The percentage DNA damage observed at the highest test concentration of 25 mg/L was 19.96%, just within the historical control data range (0.13% – 21.13%). Although just within the historical control data range the induction was considered biological relevant because of a significant 5.5-fold induction. At the lowest test concentration of 0.1 mg/L no statistically significant increase of DNA damage was observed.
- Lung: a statistically significant increase ($p<0.05$) of the Tail Intensity (%) was observed at 25, 3 and 1 mg/L. DNA damage expressed as percentage Tail Intensity increased from 4.61% to 32.04%, 19.72% to 50.04%, 15.05% to 81.64 at 25, 3, and 1 mg/L, respectively. The Tail intensities (%) after treatment at 25, 3 and 1 mg/L were above the historical control data range (0.69% – 18.05%) and the increases are therefore considered biologically relevant. At the lowest test concentration of 0.1 mg/L no statistically significant increase of DNA damage was observed.
- Bone marrow: no statistically significant increase in the mean Tail Intensity (%) was observed in any treated groups. At a dose of 3 mg/L, DNA damage expressed as percentage Tail Intensity increased 2.2-fold from 5.31% in the negative controls to 11.64% in VDC-treated animals (statistically significant induction; $p<0.05$). Since this finding was within the historical control data range of 1.92% – 17.26%, the biological relevance of the increase was considered questionable.

Statistical significant and biological relevant DNA damage without adverse histopathological findings was observed in lung cells at 1 mg/L, and kidney cells at 3, 1 and 0.1 mg/L, indicating that VDC induced DNA damage in these cells. Therefore the increased DNA damage observed at these conditions is caused by a genotoxic effect. At the other exposure conditions (25 mg/L in lung, liver and kidney, 3 mg/L in lung and liver, and 1 mg/L in liver) histopathological adverse findings were observed concomitantly to statistically significant increase in DNA damage. However, this does not preclude to conclude that the increased DNA damage observed are biologically relevant and caused by a genotoxic effect.

Table 29: Results of the histopathology and the Comet Assay on the tissues of rats exposed to 1,1-DCE

Organ	Dose (mg/L)	Histopathology findings		Comet assay (DNA damage)			
				Conclusion on relevance	Tail intensity % (SD)		
		Severity	Description		Treated group	Negative control	Positive control

Lung	**25**	Severe	Degeneration/regeneration bronchiolar epithelium Lymphoid depletion BALT Inflammatory cell infiltrate peribronchial	Relevant	32.04 (24.40)*	4.61 (0.69)	86.99 (12.24)***
	3	Minimal	Regeneration bronchiolar epithelium Inflammatory cell infiltrate peribronchial	Relevant	50.04 (7.39)***	19.72 (9.75)	93.42 (1.80)***
	1	-	-	Relevant	81.64 (3.31)***	15.05 (7.08)	90.60 (2.81)***
	0.1	-	-	No relevant	5.65 (1.38)	4.73 (1.01)	4.73 (1.01)***
Liver	**25**	Severe	Single cell necrosis, centribular/bridging	Relevant	19.96 (7.94)***	3.65 (3.34)	92.71 (4.80)***
	3	Severe	Single cell necrosis, centribular/bridging Hepatocellular hypertrophy, centrilobular	Relevant	33.29 (8.93)***	15.38 (4.30)	99.04 (0.21)***
	1	Severe	Single cell necrosis, centribular Hepatocellular hypertrophy, centrilobular	Relevant	44.39 (16.94)*	16.35 (14.71)	94.53 (1.62)***
	0.1	No adverse	Hepatocellular hypertrophy, centrilobular Cytoplasmic alteration centrilobular	No relevant	16.87(4.08)	14.45 (5.50)	86.75 (2.86)***
Kidney	**25**	Severe	Tubular degeneration	Relevant	68.72 (13.85)***	4.37 (0.76)	97.05 (1.55)***
	3	-	-	Relevant	57.25 (2.56)***	11.57 (2.56)	96.62 (1.89)***
	1	-	-	Relevant	35.59 (7.90)*	26.58 (5.07)	92.93 (1.09)***
	0.1	-	-	Relevant	54.95 (11.02)***	13.75 (3.52)	87.24 (3.24)***
Bone marrow	**25**	-	-	No relevant	4.00 (0.95)	4.32 (2.08)	80.27 (10.03)***
	3	-	-	Questionable	11.64 (3.18)**	5.31 (1.30)	85.40 (2.81)***
	1	-	-	No relevant	11.88 (4.42)	10.23 (1.99)	82.22 (2.56)***
	0.1	-	-	No relevant	12.80 (7.10)	10.07 (3.02)	78.24 (3.16)***

* p<0.05; ** p<0.01; *** p<0.001 (Student t test)

It was concluded that VDC caused DNA damage in lungs, liver and kidneys of male Wistar rats after 3-days of exposure by inhalation.

1.4.2.6 [Reitz (1980)]

Study reference:

Reitz R.H., Watanabe P.G., McKenna M.J., Quast J.F., Gehring P.J. 1980: Effects of Vinylidene Chloride on DNA Synthesis and DNA Repair in the Rat and Mouse: A Comparative Study with Dimethylnitrosamine (publication), Toxicology and Applied Pharmacology (1980), 52, 357-370.

Detailed study summary and results:

Test type

Effects of Vinylidene Chloride on DNA synthesis and DNA repair were tested in the Rat and Mouse. The potential of VDC to induce DNA alkylation, DNA repair, and DNA replication was investigated in the liver and kidneys.

Test substance

Radioactive [^{14}C]VDC (0.443 mCi/mmol) was synthesized by New England Nuclear. This material was labeled at both C-l and C-2, and had greater than 99% radiochemical purity by gas chromatographic analysis. Non-radioactive VDC (redistilled, 99.95% pure with residual inhibitor content l-5 ppm monomethylether of hydroquinone) was obtained from the Saran and Converted Products Research Laboratory, 1603 Building, Dow Chemical Company, Midland, Michigan.

Test animals

The study was conducted on male mice, CD-strain and male rats, Sprague Dawler strain, both obtained from Charles River Laboratories. The animals were 18-20 g (mice) or 200-250 g (rats) when received, and were acclimated for at least 1 week before use. Both control and exposed animals were deprived of food and water during the exposure; otherwise these were available ad libitum.

Administration/exposure

Animals were exposed to VDC vapors for 6 hours at 10 and 50 ppm (40 or 200 mg/m3) under dynamic conditions. The concentration of VDC was measured by periodically sampling the chamber atmosphere and determining the VDC concentration by gas chromatography.

For comparative purpose, the carcinogen Dimethylnitrosamine (DMN) was also studied in parallel by one intraperitoneal injection at 10 and/or 3 mg/kg. The doses of DMN and VDC (10 mg/kg ip, and 50 ppm inhalation for 6 hr, respectively) are about the highest levels that can be administered to CD-1 male mice without causing some delayed mortality, and are at least roughly equivalent in terms of their acute toxicity.

DNA synthesis was measured 48 hr after exposure. A population was divided into a control and treated group. Each group received an ip injection of radioactive [^{3}H]thymidine. Four hours after the injection, the animals were sacrificed and DNA was isolated from kidney and/or liver. Increased DNA replication, calculated as a "replicative index" was obtained by dividing the mean specific radioactivity of DNA preparations from treated groups by the mean specific radioactivity of the DNA preparations from the concurrent control group. A value of 1.0 or less indicates that the treatment did not stimulate DNA replication, while numbers larger than 1.0 show stimulation of DNA replication, indicating regeneration following cellular injury.

DNA repair was measured immediately after removing animals from exposure. A population was divided into a control and treated group. Each group was further divided into two subgroups (three to six

animals/subgroup). One subgroup received radioactive [3H]thymidine by ip, and the other subgroup received an identical injection of [3H]thymidine dissolved in 40 mg/ml hydroxyurea. The injection of hydroxyurea simultaneously with [3H]thymidine suppresses normal replicative DNA synthesis, but does not inhibit DNA repair systems. A treated and control group injected with [3H]thymidine but not hydroxyurea were included in each experiment. Four hours later, the animals were sacrificed by cervical dislocation, and the DNA was prepared from kidney and/or liver.

The repair ratio was calculated as follows: $RR = (T)_{HU} / [(C)_{HU} \times CF]$

with:

$(T)_{HU}$ = mean specific radioactivity from the treated subgroup injected with [3H]thymidine plus hydroxyurea

$(C)_{HU}$= mean specific radioactivity from the control subgroup injected with [3H]thymidine plus hydroxyurea

CF = correction factor for the effect of the test chemical on normal replicative DNA synthesis, calculated by dividing the mean specific radioactivity of the DNA in the treated group by that of the control group.

Samples of kidney and liver were removed at necropsy and prepared for histopathological assessment.

Statistical Analysis

- DNA synthesis: Each treatment/tissue group was then analyzed for a significant increase over the appropriate control by use of Student's t test. Results were considered significant at a p value < 0.05.
- [^{14}C]VDC alkylated DNA: the logarithm of the specific radioactivity was subjected to linear regression analysis versus time (hr after the termination of the 6 hr exposure to VDC).
- DNA Repair: Increases are considered significant at the $p < 0.05$ level.
- DNA prelabeled with [3H]thymidine: Data from control and VDC-50 ppm exposed groups at 0, 48, and 120 hr post-exposure were subjected to a two-way analysis of variance with $p = 0.05$ as the level of significance.

Results and discussion

DNA damage

The levels of radioactivity which were incorporated into DNA from the tissues of animals exposed to radioactive VDC are summarized in the table below. VDC alkylation of DNA was very low in kidney and liver of both rats and mice. The highest level of alkylation was observed in the kidneys of mice exposed to 50 ppm VDC and corresponded to only 30 alkylations per million nucleotide residues. After exposure to either 50 or 10 ppm VDC, the DNA from the liver of mice contained 5- to 10-fold less radioactivity than the DNA from the kidneys. In rats exposed to 10 ppm VDC, the DNA of kidney contained about two fold greater activity than the DNA of liver. Compared to mice exposed to 10 ppm VDC, about six fold less radioactivity was found in the DNA of rat kidneys, while similar amounts were found in the DNA from the liver of both species.

Table 30: DNA damage produced by VDC in rats and mice

Species	Treatment	No (pooled)	Tissue	Dpm/mg DNA [a]	Number of determinations	Alkylations/nucleotide (x 10^6)
Mouse	**50 ppm**	**Pool of 5 mice**	**Kidney**	88	1	30
		Pool of 5 mice	**Liver**	18	1	6.1
	10 ppm	**Pool of 7 mice**	**Kidney**	34.0 ± 13.4	2	11
		Pool of 7 mice	**Liver**	2.8 ± 0.0	2	0.94
Rat	**10 ppm**	**Pool of 2 rats**	**Kidney**	5.9 ± 1.9	3	2
		Pool of 2 rats	**Liver**	2.6 ± 1.9	6	0.87
	10 mg/kg DMN	**-**	**Liver**	-	from Pegg and Hui (1978)	3000 - 4000

[a] average of multiple determinations except for the data of 50 ppm VDC in mouse.
DMN = Dimethylnitrosamine

DNA repair

DNA repair was measured directly in animals exposed to VDC (50 and 10 ppm) or injected with DMN (20, 10, and 3 mg/kg, ip). DMN treatment caused a high increase of DNA repair. The relative increase in repair synthesis caused by DMN was more than seven fold, and this increase was dose related. In contrast, a much smaller increase in the repair ratio (1.00-1.38) occurred after exposure of mice to VDC. The only statistically significant increase caused by VDC was that occuring in the kidneys of the mice exposed to 50 ppm. DNA repair was not significantly altered in the livers of the mice exposed to 50 ppm VDC, or in the kidneys or livers of mice exposed to 10 ppm.

Table 31: DNA repair in tissues of mice exposed to VDC

Species	Substance	Treatment	Tissue	RR Repair Ratio ± 95 % confidence limit
Mouse	**VDC**	**50 ppm**	**Kidney**	**1.38 ± 0.09 ****
			Liver	1.16 ± 0.363
		10 ppm	**Kidney**	1.16 ± 0.255
			Liver	0.764 ± 0.222
	DMN	**20 mg/kg**	**Liver**	7.37 ± 2.16
		10 mg/kg		3.04 ± 0.896
		3 mg/kg		1.60 ± 1.14

DMN = Dimethylnitrosamine
**: Ratio significantly different than 1.0, t test with $p < 0.05$.

Epigenetic effect

The procedure used to evaluate non-genetic effects differs from that employed to measure DNA repair in two important respects: hydroxyurea is not used to suppress normal DNA replication and [^{3}H]thymidine is injected approximately 48h after the event suspected of causing tissue damage.

Exposure to 50 ppm VDC caused about a 25-fold increase in incorporation of [^{3}H]thymidine into DNA of mouse kidneys, while exposure to 10 ppm VDC produced about an 8-fold increase in [^{3}H]thymidine incorporation in this tissue. These effects probably reflect increased levels of DNA replication rather than changes in the DNA precursor pool sizes, since a large number of mitotic figures were seen in treated, but

not control, tissues during microscopic examination. Effects on DNA replication in the mouse liver were much smaller (2.4 and 1.2 times control at 50 and 10 ppm VDC, respectively).

DMN treatment also produced only a small increase in replication of DNA in the livers of treated mice.

Rats exposed to 10 ppm VDC exhibited much smaller effects on DNA replication than mice exposed to the same concentration. In the rat kidney DNA replication was only elevated 2.2-fold while DNA replication in the liver was slightly decreased.

Table 32: DNA synthesis in tissues from rats and mice exposed to VDC

Species	Substance	Treatment	Tissue	[3H]thymidine incorporation relative to control
Mouse	**VDC**	**50 ppm**	**Kidney** **Liver**	27.7 2.45
		10 ppm	**Kidney** **Liver**	7.72 1.2
Rat		**10 ppm**	**Kidney** **Liver**	2.2 0.88
Mouse	**DMN**	**10 mg/kg** **3 mg/kg**	**Liver**	1.97 1.14

DMN = Dimethylnitrosamine

Histopathologic findings

Table 33: Summary of histopathologic findings in tissues from mice treated with VDC

Exposure	Post exposure period (hours)	Histologic findings	
		Kidney	**Liver**
VDC, 50 ppm	**0** **8** **24** **48** **96** **192**	Toxic nephrosis Progressing nephrosis Progressing nephrosis Increased mitotic figures Regeneration apparent Regeneration continuing	Slight centribullar swelling No effect
VDC, 10 ppm	**0** **96**	Slight dilatation, swelling Nephrosis-variable 0-20% affected	-
DMN, 10 mg/kg	**4**	-	Centribullar swelling Hyaline degeneration
	52	-	Accentuated lobular pattern, necrosis minimal or absent
DMN, 10 mg/kg	**4**	-	Centribullar swelling
	52	-	No effect

The authors conluded that an important distinction between DMN and VDC has been demonstrated. Tumorigenic doses of DMN produced relatively little tissue damage, but were associated with a high degree of DNA alkylation and DNA repair synthesis at doses where there is little or no cytotoxicity. In contrast, exposure to tumorigenic doses of VDC induced minimal DNA alkylation or DNA repair synthesis, but can cause extensive cytotoxicity with concomitant cell regeneration and DNA replication.

1.4.2.7 [Short (1977)]

Study reference:

Short R.D., Minor J.L., Winston J.M. and Lee C-C. 1977: A dominant lethal study in male rats after repeated exposures to vinyl chloride or vinylidene chloride (publication), Journal of Toxicology and Environmental Health, 3:965-968.

Green S., Auletta A., Fabricant J, Kapp R., Manandhar M., Sheu C-J., Springer J. and Whitfield B. 1985: Current status of bioassays in genetic toxicology - the dominant lethal assay (publication), Mutation Research, 154:49-67.

Detailed study summary and results:

Test type

This study, incorporated into a carcinogenic study with VDC, was similar to OECD Guideline 478 (Genetic Toxicology: Rodent Dominant Lethal Test) and was conducted on male rats exposed to VDC by inhalation. Some deviations were noted: test conditions were not sufficiently detailed, purity of test substance was not reported, no positive control group was present, only one dose was tested (instead of 3) and the number of animals per group was not sufficient (at least a total of 400 implants is recommended).

Test substance

The test substance described in the publication was vinylidene chloride (VDC). No more information was available.

Test animals

The study was conducted on CD male rats from Charles River Breeding Laboratories, North Wilmington, Massachusetts), weighting 180-200 g at study initiation. Rats were given free access to feed and water (e.g. ad libitum), except during the daily exposure period.

Administration/exposure

Male rats were exposed 6 hours/day for 5 days/week to 55 ppm (or 220 mg/m3) of VDC. A negative control group was exposed only to air. The dominant lethal study was modified according to Green et al (1977). During week 11 of exposure, each male was housed with two unexposed virgin females for at least seven successive evenings or until the male mated with two females. During the day, the males continued to be exposed to VDC. The females were examined in the morning for evidence of mating (presence of sperm in vaginal smears or presence of vaginal plug). On gestational day 13, females were sacrified and the number of lutea and implants (viable and dead) was determined.

Results and discussion

A reduced ratio of pregnant to mated females in the group mated with males exposed to VDC was observed. The mating period ranged from 7 days for the control group to 8-9 days for the treated group. Pregnancies that resulted from the mating of exposed males with unexposed females were normal in terms of the number of corpora lutea and implants. In these females, there was no evidence of pre-implantation loss, as measured by the ratio of implants to corpora lutea, or post-implantation loss, as measured by the ratio of viable implants to total implants. However, the occurrence of a pre-implantation loss was suggested by the increased number of non pregnant females in the group mated with treated males. But the relevance of this finding is questionable since there is no indication of pre-implantation loss in pregnant females, and a post-implantation loss is more indicative of dominant lethality than a pre-implantation loss.

Table 34: Mating and pregnancy in VDC-exposed male rats and unexposed females in the dominant lethal test.

	Results	
	Negative control	**Exposed to VDC at 55 ppm**
Exposed male rats		
Number of tested males	12	11
Number of mated males with 2 females	12	9
Number of mated males with 1 female	0	2
Number of mated males with no female	0	0
Number of fertile males[c]	12	10
Unexposed female rats		
Number of mated females	24	20
Number of pregnant females	24	13[b]
With 1 dead implant	7	3
With 2 dead implants	3	1
With > 3 dead implants	2	2
Mean[a] of corpora lutea per dam	15.8 ± 0.6	15.1 ± 0.5
Mean[a] of implants per dam	13.7 ± 0.8	14.2 ± 0.5
Implants per corpora lutea x 100 (mean[a])	86 ± 3	94 ± 2
Viable/total implants x 100 (mean[a])	92 ± 2	93 ± 3

[a] Mean ± SE calculated on a per dam basis for the number of pregnant rats
[b] Significantly different from control (fisher's exact probability test, $p<0.05$)
[c] Fathered at least one litter with one or more viable implants.

The authors concluded that there was no evidence of dominant lethal mutations in the germinal cells of male rats exposed to VDC by inhalation.

1.4.2.8 [Anderson (1977)]

Study reference:

Anderson D, Hodge M.C.E., Purchase I.F.H. 1977: Dominant Lethal Studies with the Halogenated Olefins Vinyl Chloride and Vinylidene Dichloride in Male CD-1 Mice (publication), Environmental Health Perspectives (1977), 21, 71-78.

Detailed study summary and results:

Test type

This study was equivalent to OECD Guideline 478 (Genetic Toxicology: Rodent Dominant Lethal Test), and was conducted on male mice exposed by inhalation.

Test conditions were not sufficiently detailed, individual results were not available and purity of test substance was not reported.

Test substance

The tested substance described in the publication was VDC, obtained from ICI Ltd., Mond Division, Runcorn, Cheshire, U.K.

Test animals

The study was conducted on CD mice from Charles River (Manston, Kent). Undosed females were 8-10 weeks old when mated and male mice immediatly after dosing were 10-12 weeks old.

Administration/exposure

Male mice were exposed by inhalation to VDC for 6 hours/day for 5 days at 2 exposure levels 10, 30 and 50 ppm selected on the basis of a preliminary experiment. Males were exposed to air as negative control group. A positive control group was treated with Cyclophosphamide (200 mg/kg) by intraperitoneal injection once on day 5 of exposure. Males were caged individually and females in pairs. They received food and water ad libitum. The required concentrations of VDC were generated by a controlled fluid-feed/atomizer technique. The method involves continuously passing a known volume of the compound through a concentric jet atomizer, where it is vaporized. During the dosing the mice were housed individually in chambers made of stainless steel and glass. During the mating period, one male mice were caged with 2 female mice for 5 days. After, females were removed and rehoused in pairs. Females were killed 15 days after the first day of mating and examined for pregnancies (implantations, early deaths and late deaths).

Results and discussion

The mating frequency was high in the two groups exposed to the lowest doses of VDC in all weeks by comparison with the negative control group, and it was statistically significantly lower in the high exposure group in weeks 0-8 and the positive control (weeks 0, 1, 7). Pregnancy frequency was significantly different in the highest VDC exposure group at weeks 0-6 and the positive control group (weeks 0, 1, 7). According to the authors, this effect was probably due to infertility of the males. No reduction on fertility and pregnancy frequencies was observed in groups exposed to VDC at 10 and 30 ppm. Regarding the total number of implantations per pregnant female, the results of the positive control group was statistically significantly different from the negative control group (weeks 1, 2, 3, 6, 7). Only the VDC group exposed to 10 ppm showed a significant difference from the negative control group in the week 0 (pre-treatment). There is no

indication of pre-implantation loss. The positive control group showed significant differences in the number of pregnant females with one or more early deaths in weeks 1, 2 and 3. The results of late deaths were not reported in the publication. However, the authors concluded that late deaths were distributed throughout all the groups and did no appear to be treatment related.

Table 35: Number and percentage of male mice mating each week (which survived treatment)

Mating week after treatment	Group 1		Group 2		Group 2		Group 4		Group 5	
	Negative control		VDC, 10 ppm		VDC, 30 ppm		VDC, 50 ppm		Positive control[a]	
	Nb of mice	%	Nb of mice	%	Nb of mice	%	Nb of mice	%	Nb of mice	%
0[b]	50	100	20	100	18	100	**8*** **	**38**	**17****	**76**
1	50	98	20	95	18	100	**8*** **	**75**	**17****	**65**
2	50	96	20	100	18	94	**7*** **	**86**	16	94
3	50	94	20	100	18	89	**7*** **	**43**	17	94
4	49	98	20	100	18	89	**7*** **	**43**	15	100
5	17	94	20	100	17	82	**7*** **	**57**	17	100
6	49	88	20	100	17	100	**7*** **	**43**	17	88
7	49	92	20	90	17	100	**6***	**50**	**16****	**75**
8	48	92	19	95	17	88	**7***	**57**	19	86

* p<0.05 / ** p< 0.01 / *** p<0.001 / [a] Cyclophosphamide at 200 mg/kg by intraperitoneal injection
[b] The mating before treatment is represented by week 0.

Table 36: Number of mated females becoming pregnant

Mating week after treatment	Negative control		VDC, 10 ppm		VDC, 30 ppm		VDC, 50 ppm		Positive control[a]	
	pregnant	mated	pregnant	mated	pregnant	mated	pregnant	mated	pregnant	mated
0	73	100	30	40	25	36	**5****	**16**	**17*****	**34**
1	82	100	32	40	33	36	**7****	**16**	**20*****	**34**
2	75	100	36	40	32	36	**7****	**14**	25	33
3	82	100	34	40	26	36	**5****	**14**	28	34
4	85	98	36	40	27	36	**6*****	**14**	26	32
5	80	94	34	40	26	34	**6****	**14**	29	34
6	75	98	33	40	32	34	**4*****	**14**	25	34
7	77	98	33	40	28	34	7	13	**19***	**33**
8	73	96	28	39	26	34	7	13	28	39

Only females which survived to treatment were included in this table. / * p<0.05 / ** p< 0.01 / *** p<0.001
[a] Cyclophosphamide at 200 mg/kg by intraperitoneal injection

Table 37: Mean total implants per pregnant female

Mating week after treatment	Negative control	VDC, 10 ppm	VDC, 30 ppm	VDC, 50 ppm	Positive control[a]
0	12.2	**11.0****	12.0	11.4	11.5
1	12.3	13.1	12.8	11.7	**9.2*****
2	12.3	11.7	12.1	11.0	**9.5*****

3	12.0	12.1	13.2	13.4	**11.0***
4	12.0	12.1	13.0	12.7	12.1
5	11.8	11.7	12.5	10.7	12.0
6	12.5	12.4	12.1	12.6	**11.1****
7	12.6	13.0	12.5	12.7	**11.7***
8	12.3	13.6	12.4	12.6	11.6

* p<0.05 / ** p< 0.01 / *** p<0.001 / [a] Cyclophosphamide at 200 mg/kg by intraperitoneal injection

Table 38: Number of pregnant females with one or more early deaths (ED)

Mating week after treatment	Negative control		VDC, 10 ppm		VDC, 30 ppm		VDC, 50 ppm		Positive control[a]	
	0	**≥ 1 ED**	**0**	**≥ 1 ED**	**0**	**≥ 1 ED**	**0**	**≥ 1 ED**	**0**	**≥ 1 ED**
0	40	33	14	16	10	15	1	4	9	8
1	41	41	18	14	19	14	6	1	**0*** **	**20**
2	39	36	18	18	17	15	3	4	**2*** **	**23**
3	45	37	18	16	15	11	4	1	**7****	**21**
4	40	445	17	19	11	16	2	4	12	14
5	41	39	15	19	11	15	3	3	12	17
6	40	35	14	19	15	17	2	3	12	13
7	43	34	15	18	15	13	5	2	10	9
8	37	36	13	15	13	13	3	4	15	5

** p< 0.01 / *** p<0.001 / [a] Cyclophosphamide at 200 mg/kg by intraperitoneal injection

Table 39: Mean number and percentage of early deaths (ED) per pregnancy

Mating week after treatment	Group 1		Group 2		Group 3		Group 4		Group 5	
	Negative control		**VDC, 10 ppm**		**VDC, 30 ppm**		**VDC, 50 ppm**		**Positive control[a]**	
	ED Number[b]	**ED %[c]**	**ED Number[b]**	**ED %[c]**	**ED Number[b]**	**ED %[c]**	**ED Number[b]**	**ED %[c]**	**ED Number[b]**	**ED %[c]**
0	0.59	4.8	0.80	10.1	0.84	7.3	2.00	17.3	1.06	9.1
1	0.72	5.7	0.72	5..3	0.61	5.1	0.29	2.2	**4.00*** **	**45.6*** **
2	0.69	5.5	0.75	6.9	0.56	4.4	0.71	5.7	**4.04*** **	**41.4*** **
3	0.76	6.4	0.68	6.3	0.46	3.6	0.80	5.3	**2.32*** **	**21.8*** **
4	0.79	6.9	0.83	7.1	1.00	7.4	1.00	10.3	0.85	6.9
5	0.78	6.6	0.68	6.1	0.73	6.2	0.67	5.6	0.86	6.9
6	0.88	7.0	0.91	7.6	0.94	8.3	1.00	7.5	0.64	5.6
7	0.65	4.9	0.85	6.6	0.57	4.5	0.57	4.3	0.74	6.1
8	0.85	7.0	0.75	5.4	0.85	7.1	0.71	5.8	0.30	2.4

*** p<0.001 / [a] Cyclophosphamide at 200 mg/kg by intraperitoneal injection
[b] Mean number of early deaths (ED) per pregnancy / [c] Early deaths (ED) as a percentage of total implants per pregnant female

The best indication of mutagenic activity of a substance in the dominant lethal test is an increase of the post-implantation foetal early death. Based on the data obtained with VDC, there was no evidence of mutagenic effects at the tested doses and in the conditions of the test. The authors concluded that vinylidene dichloride did not cause lethal mutations in germ cells of male CD-1 mice exposed at 10, 30 or 50 ppm.

1.5 Carcinogenicity

1.5.1 Animal data by oral route

1.5.1.1 [Maltoni (1977)]

Study reference:

Maltoni C., Cotti G., Morisi L. and Chieco P. 1977: Carcinogenicity bioassays of vinylidene chloride Research plan and early results (publication), Medicina Del Lavoro, 68(4) 241-262.

Maltoni C., Cotti G., and Chieco P. 1984: Chronic toxicity and carcinogenicity bioassays of vinylidene chloride, Acta oncologica 5 (2).

Roberts S.M., Jordan K.E., Warren D.A., Britt J.K. and James R.C. 2002: Evaluation of the carcinogenicity of 1,1-dichloroethylene (vinylidene chloride) (review article or handbook), Regulatory Toxicology and Pharmacology, 35:44-55.

United States Environmental Protection Agency 1985: Health assessment document for vinylidene chloride; Final report EPA/600/8-83/031F

Detailed study summary and results:

Test type

A carcinogenicity study on VDC was conducted on rats by gavage for 12 months.
Important deviations are noted: only the early results are available; no data on statistical analyses are available; the exposure period was for 1 year and gavage was only 4-5 days/week; all the animals are kept under observation until spontaneous death. Several shortcomings of the study design and reporting lead to difficulties in the interpretability of the study results.

Test substance

The test item as described in the publication was 1,1-dichloroethylene obtained from the Central Laboratory of Solvay (Bruxelles). The purity of the sample used in the study was > 99.9% (999.5 g/kg). The impurities identified were 1,2 dichloroethylene trans (0.4 g/kg), acetone (0.1 g/kg), methylene chloride (0.05 g/kg), mono and dichloroacetylene (0.02 g/kg) and paramethoxyphenol (as stabilizer at 200 ppm).

Test animals

The study was conducted on male and female Sprague-Dawley rats, 9-10 weeks old at start, supplied by Institute of Oncology and Tumour Centre, Bologna (Italy). They were housed 5/cage in polycarbonate cages with tops made of stainless steel wire.

Administration/exposure

In a first step, three groups (5, 10 and 20 mg/kg bw) of 50 male and 50 female Sprague-Dawley rats were exposed, with a concurrent control group of 100 male and 100 female rats. In a second step, a lower dose group (0.5 mg/kg bw) of 50 male and 50 female Sprague-Dawley rats was added with its specific concurrent control group of 82 males and 77 females. The treatment was conducted 4-5 days/week for 52 weeks for all the groups. All the animals are kept under observation until spontaneous death; the end of the experiment was 147 weeks after the start of the 52-week treatment period.

Clinical evaluation and pathology

The animals are controlled weekly and weighted every 2 weeks during the period of treatment and monthly after the treatment is over. All the detectable gross pathological changes are recorded during the control. A complete autopsy is carried out on each animal. Histological examinations are performed on the Zymbal glands, interscapular brown fat, salivary glands, tongue, lungs, river, kidneys, spleen, stomach, different segments of the intestine, bladder, brain, bone marrow (sternum) and any other organs with pathological lesions. Furthermore, cytological examinations are carried out on the bone marrow of the femur.
No information either on other examinations or statistical methods are available.

Results and discussion

No biologically significant changes were observed in mortality or body weight, and no biologically significant non-cancer or cancer effects were found in any organ. The results regarding the incidence of the tumors 147 weeks after the start of the study (including 52 weeks of treatment) are presented in the table below. Different kinds of tumours were observed in treated rats and in the control group. The mammary tumour was the most frequent. But no increase was observed in treated rats compared to the control group.

Table 40: Incidence of tumors in rats exposed by ingestion to VDC for 52 weeks

	Control group 1 [e]	**Control group 2** [e]	**0.5 mg/kg bw**	**5 mg/kg bw**	**10 mg/kg bw**	**20 mg/kg bw**

Results after 147 weeks						
Number of animals at start	100M + 100F	82M + 77F	50M + 50F	50M + 50F	50M + 50F	50M + 50F
Corrected number [a]	100M + 99F	68M + 74F	47M + 47F	49M + 50F	48M + 50F	49M + 48F
Total tumours [d]						
Total males with tumours (%) [c]	29 (29.0)	13 (19.1)	10 (21.3)	11 (22.4)	9 (18.8)	9 (18.4)
Total females with tumours (%) [c]	63 (63.6)	38 (51.4)	23 (48.9)	32 (64.0)	28 (56.0)	25 (52.1)
Total animals with tumours (%) [c]	92 (46.2)	51 (35.9)	33 (35.1)	43 (43.4)	37 (37.8)	34 (35.0)
Mammary tumours [b]						
Males with tumours (%) [c]	10 (10.0)	2 (2.9)	2 (4.2)	3 (6.1)	2 (4.2)	2 (4.1)
Females with tumours (%) [c]	57 (57.6)	34 (45.9)	22 (46.8)	28 (56.0)	24 (48.0)	23 (47.9)
Total animals with tumours (%) [c]	67 (33.7)	36 (25.3)	24 (25.5)	31 (31.3)	26 (26.5)	25 (25.8)
Zymbal gland carcinomas						
Males with tumours (%) [c]	2 (2.0)	0	0	0	2 (4.2)	0
Females with tumours (%) [c]	0	1 (1.3)	0	0	0	0
Total animals with tumours (%) [c]	2 (1.0)	1 (0.7)	0	0	2 (2.0)	0
Leukemias						
Males with tumours (%) [c]	9 (9.0)	4 (5.9)	3 (6.4)	3 (6.1)	1 (2.1)	2 (4.1)
Females with tumours (%) [c]	2 (2.0)	3 (4.0)	1 (2.1)	1 (2.0)	1 (2.0)	0
Total animals with tumours (%) [c]	11 (5.5)	7 (4.9)	4 (4.2)	4 (5.5)	2 (2.0)	2 (2.1)
Other tumours (total)						
Males with tumours	17	7	5	6	12	8
Males with B tumours / M tumours	(11B / 6M)	(5B / 2M)	(1B / 4M)	(2B / 4M)	(6B / 6M)	(6B / 2M)
Females with tumours	22	12	4	7	8	5
Females with B tumours / M tumours	(11B / 11M)	(5B / 7M)	(0B / 4M)	(2B / 5M)	(4B / 4M)	(3B / 2M)
Total animals with tumours	39	19	9	13	20	13
Total animals with B tumours / M tumours	(22B / 17M)	(10B / 9M)	(1B / 8M)	(4B / 9M)	(10B / 10M)	(9B / 4M)

[a] Alive animals after 14 weeks when the first tumour was observed (a mammary carcinoma)
[b] Two or more tumours of the same and/or the different types may be present in the same animal (fibroadenomas, carcinomas, sarcomas, carcinosarcomas)
[c] The percentages are referred to the corrected number.
[d] Several animals have two or more tumours
[e] Control group 1 (in the step 1) for 5, 10 and 2 mg/kg bw/dau treated groups / Control group 2 (in the step 2) for 0.5 mg/kg bw/day treated group

The authors concluded that there was no increase in tumour incidence in any of the treated group of rats exposed to VDC by gavage for 1 year. The tumours observed seem to be age correlated (spontaneous tumours).

1.5.1.2 [Ponomarkov (1980)]

Study reference:

Ponomarkov V., Tomatis L. 1980: Long-Term Testing of Vinylidene chloride and Chloroprene for Carcinogenicity in Rats (publication), Oncology 1980, 37, 136-141.

Detailed study summary and results:

Test type

A carcinogenicity study on VDC was conducted on rats by oral exposure.

Test substance

The test item as described in the publication was VDC from Merck-Schuchardt (Darmstadt, FRG) with a purity of 99%, containing 0.03% 4-methoxyphenol.

Test animals

The study was conducted on inbred BDIV rats, originally provided by Prof. H. Druckrey (Freiburg, Germany). Animals were housed in Makrolon N111 cages under normal laboratory conditions. They were maintained on Charles River food pellets until January 1976, from then on Aliment Extralabo biscuits (Pietrement). Water was available ad libitum. Samples of feed were analysed twice for the presence of nitrosamines, dimethylnitrosamine and diethylnitosamine were present at levels of 0.2-0.6 ppb.

Administration/exposure

VDC was dissolved in olive oil and administered to animals by stomach tube. Twenty four pregnant females were given a single dose of 150 mg/kg bw VDC on the 17th day of gestation and their progeny (89 males and 90 females) were treated weekly with 50 mg/kg oral treatment (in 0.3 mL olive corn oil) from time of weaning for life span (age of 120 weeks). A control group (14 females) received 0.3 mL of corn oil on the 17th day of pregnancy and their progeny (53 males and 53 females) were given 0.3 mL olive oil weekly for life beginning at weaning. All survivors were killed at 120 weeks or when moribund.

Clinical evaluation and pathology

All animals were autopsied and major internal organs as well as those that showed gross abnormalities were examined histologically.

Results and discussion

Survival rates were similar in the treated group and in the control group. Litter sizes and pre-weaning mortality were similar in VDC-treated and control groups, and there is no difference in body weights.

In rats weekly treated that died up to 30 weeks after the start of treatment, congestion of lungs and kidney was detected. Later, up to 80-90 weeks, haemorrhages and lobular necrosis of the liver were noted. The animals which died after 90 weeks and survivors killed at the age of 120 weeks showed degenerative lesions of liver.

Table 41: Number of survivors of females rats treated orally with VDC on day 17 of pregnancy and of their progeny treated for life

Groups	Initial number of animals	Number of survivors according the treatement duration (weeks)											
		10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**	**110**	**120**
Females: treated	24	24	24	24	24	24	24	24	23	23	22	19	14
Females: control	14	14	14	14	14	14	14	14	14	14	12	11	11
Progeny: treated weekly													

Males	89	85	85	82	81	80	78	76	75	71	65	54	41
Females	90	86	85	83	81	81	80	80	79	75	72	61	56
Progeny: control													
Males	53	53	53	53	53	53	53	52	52	49	44	41	27
Females	53	52	52	52	52	52	51	50	49	47	40	34	26

There were only minor differences in the tumour incidence between treated and control animals. A few tumours were observed in treated males that were not seen in the control group, notably one squamous-cell carcinoma of the stomach, one liver carcinoma, one seminoma and one rectal adenomatous polyp. Meningiomas are more frequent in treated males progeny than in controls (6/81 vs 1/49) but the difference was not statistically significant. In treated females progeny, two liver cell carcinomas, one liver cell adenoma, and one carcinoma and one adenoma of the salivary gland were observed, which were not seen in the control group. In addition, hyperplastic liver nodules were found in 2/23 females given a single VDC administration during pregnancy, and 2/81 males and 6/80 females among the progeny. This type of nodules was not found in controls, and this incidence is statistically significant.

Table 42: Tumor incidences in of females rats treated orally with VDC on day 17 of pregnancy and of their progeny treated for life.

Group	**Nb of rats [1]**	**Nb of rats with tu-mours (%)**	**Total nb of tumours (per rat)**	**Rats with more than 1 tumour Nb (%)**	**Distribution of tumours Number of tumours (%)**								**Liver hyper-plastic nodules Nb (%)**
					Menin-ge	Oral cavity	Sto-mach	Liver	Soft tissue	Mam-mary gland	Ovary	Other	
Females: treated	23	11 (47.8)	14 (0.6)	3 (13)	0	2 (8.7)	1 (4.3)	0	0	8 (34.8)	2 (8.7)	1 [2] (4.3)	2 (8.7)
Females: control	14	5 (35.7)	7 (0.5)	2 (14.3)	0	1 (7.1)	0	0	1 (7.1)	4 (28.6)	0	1 [5] (7.1)	0
Progeny, treated Males	81	31 (38.3)	35 (0.4)	4 (4.9)	6 (7)	5 (6.2)	1 (1.2)	1 (1.2)	9 (11.1)	1 (1.2)	-	12 [3] (14.8)	2 (2.5)
Females	80	53 (66.3)	64 (0.8)	11 (13.8)	0	1 (1.3)	2 (2.5)	3 (3.8)	7 (8.8)	39 (48.8)	6 (7.5)	6 [4] (7.5)	6 (7.5)
Progeny, control Males	49	16 (32.7)	16 (0.3)	0	1 (2)	2 (4.1)	0	0	4 (8.2)	0	-	9 [6] (18.4)	0
Females	47	24 (51.1)	29 (0.6)	5 (10.6)	0	1 (2.1)	1 (2.1)	0	0	22 (46.8)	3 (6.4)	2 [7] (4.3)	0

Nb: number
[1] Survivors at the time the first tumours were observed
[2] Urinary bladder papilloma
[3] 1 lymphoma, 4 pituitary adenomas, 3 adrenal cortical adenomas, 1 spleen haemangioma, 1 lung sarcoma, 1 skin squamous cell carcinoma, 1 seminoma
[4] 1 salivary gland carcinoma, 1 salivary gland adenoma, 1 lymphoma, 1 pituitary adenoma, 1 rectal adenomatous plyp, 1 uterine adenoma
[5] 1 adrenal cortical adenoma
[6] 1 osteosarcoma, 1 mediastinal sarcoma, 1 lung epidermoid carcinoma, 2 lymphomas, 1 spleen haemangioma, 2 pituitary adenomas, 1 adrenal cortical adenoma
[7] 1 lymphoma, 1 uterine adenoma

Globally, the oral administration of VDC to rats did not induce a statistically significant increase in the total number of tumours, although an increased incidence was observed at certain sites.

1.5.1.3 [Quast (1983)]

Study reference:

Rampy L. W., Quast J. F., Humiston C. G., Balmer M. F. and Schwetz B. A. 1977: Interim Results of Two-Year Toxicological Studies in Rats of Vinylidene Chloride Incorporated in the Drinking Water or Administered by Repeated Inhalation (publication), Environmental Health Perspectives, 21:33-43.

Quast J.F., Humiston C.G., Wade C.E., Ballard J., Beyer J.E., Schwetz R.W. and Norris J.M 1983: A chronic toxicity and oncogenicity study in rats and subchronic toxicity study in dogs on ingested vinylidene chloride (publication), Fundamental and applied toxicology 1983, 3:55-62.

Detailed study summary and results:

Test type

A carcinogenicity study was conducted on rat dosed by oral route (in drinking water) for up to 2 years. The design was similar to OECD 451.

Test substance

The test item as described in the publication was VDC or vinylidene chloride or 1,1 dichloroethylene with a mimimum purity of 99.5%. It was obtained from Dow Chemical company U.S.A. The samples used in the studies were distilled to bring the residual MEHQ (mono methyl ether of hydroquinone) into the range of 1 to 5 ppm. The stability of VDC in water was verified before the initiation of the studies. Samples were analysed by gas chromatography, and the following impurities concentrations were obtained: vinyl bromide 4 ppm; vinyl chloride 3-50 ppm; trans-1,2-dichloroethene 138-1700 ppm; cis-1,2-dichchloroethene 24-680 ppm; 1,1,1-trichloroethane 2-60 ppm; 1,1,2-trichloroethane 48 ppm; Hydroquinone monomethyl ether (inhibitor) 2 ppm.

Test animals

The study was conducted on male and female Sprague-Dawley rats, 6 to 7 weeks old, and randomly placed (2/cage) into suspended wire bottom cages. Food and water were made available to the animals ad libitum.
Groups of 48 rats/sex were exposed to different concentrations to the test item, and a group of 80 rats/sex supplied drinking water without VDC was used as controls. For the interim kill at 90 days, ten rats/sex/group were used.

Administration/exposure

The animals were exposed up to 2 years to the target concentrations of 50, 100 or 200 ppm in drinking water. Drinking water containing the test item was prepared daily and analyzed at periodic intervals during the study. The actual mean concentrations determined by gas chromatographic analysis, were 68 ± 21, 99 ± 22 and 206 ± 33 ppm in drinking water for the respective nominal concentrations of 50, 100 and 200 ppm. Based on the mean analyzed concentrations in the drinking water over the 2-year period, the animals exposure corresponds to the ingested dose of 7, 10 and 20 mg/kg bw/day for the males and 9, 14 and 30 mg/kg bw/day for the females. The control groups were supplied drinking water without test item.

Clinical evaluation and pathology

The rats were observed at least twice weekly. Food consumption and water consumption were determined at periodic intervals. Body weights were measured weekly at first and then monthly until the end of the two-year study. Hematologic determinations, urinalysis and clinical chemistry analysis were conducted. All rats that were found moribund or dead during the study were subjected to gross pathological examination. At terminal necropsy, the brain, heart, liver, kidneys, and testes were weighed.

Microscopic examinations were generally conducted on the followings organs from controls rats and 200 ppm rats: esophagus, salivary glands, stomach, large intestine, pancreas, liver, kidney, urinary bladder, prostate, accessory sex glands, epididymis, testes, ovaries, oviducts, uterus, mammary tissue, brain (cerebrum, cerebellum, brain stem), pituitary gland, spinal cord, peripheral nerve, trachea, lungs: nasal turbinates, sternum and sternal bone marrow, spleen, mediastinal lymphoid tissue, lymph nodes (mesenteric), heart, aorta, skeletal muscle, adrenal glands, thyroid and parathyroid glands, adipose tissue, skin, eye and any gross lesions suggestive of a significant pathological alteration or neoplasm formation. Selected target organs and grossly recognized neoplastic changes in the rats receiving 100 or 50 ppm were also examined microscopically.

Statistical evaluation of body weight and organ weight data, food and water consumptions, hematology and clinical chemistry determinations were made using an analysis of variance and Dunnett's test (Steel and Torrie, 1960). All mortality and neoplasm incidence data were analyzed using the Fisher Exact Probability Test (Siegel, 1956). The level of significance in all cases was $p < 0.05$.

Results and discussion

No clinical signs were observed throughout the 2-year study. Except for occasional statistically significant differences in the cumulative mortality percentages, the mortality in the treated groups was comparable to the controls. Mean body weights of the rats over the 2-year period were similar for all groups. Occasionally occurring statistically significant differences (increases and decreases) in food and water consumption data were neither dose-related nor time-related and were not considered to be related to the substance. The hematological determinations and urinalyses were within the normal range for all the groups. No consistent

or dose-related differences were observed in the clinical chemistry parameters or sulfhydryl levels between VDC-treated and control rats. Mean organ weights and organ to body weight ratios were not significantly different when compared to the control group.

The only pathological findings considered to be related to the ingestion of VDC were observed in the liver.

Increase of hepatocellular fatty change was observed in both sexes. In males, those receiving the highest concentration showed a statistically significance, and this group also showed an increased incidence of hepatocellular swelling. A trend towards an increased incidence of hepatic changes was noted in the male rats at 100 ppm. Minimal hepatocellular fatty change and hepatocellular swelling were also detected in female rats at all dose levels. No significant hepatocellular necrosis considered exposure-related was evident in either male or female rats at any of the dose levels. In addition, there was no change in liver weight, no change in clinical chemistry measurements diagnostic for liver damage, and no other indication of abnormal liver function. The authors considered that hepatocellular swelling was not biologically significant and was not an adverse effect. The statistically significant hepatocellular mid-zonal fatty change, however, was considered a minimal adverse effect in this study.

Regarding tumor pathology, the only histopathological findings was an increase of mammary gland fibroadenomas/ adenofibromas in the females ingesting drinking water containing 50 ppm VDC. However, the incidence in control animals was so high that the slightly higher incidence in treated rats was not considered statistically or toxicologically significant. In addition, the incidence was within the normal range of the historical control data from this laboratory and was not dose related.

No other tumor types were observed which were significantly increased or decreased in any tissues. The total neoplasm incidence in the male and female rats in the treated groups was not different than the control groups.

Table 43: Incidence of neoplasms in rats ingesting VDC in the drinking water

Concentration of VDC in water (ppm)	Total number rats in Group		Total number of Neoplasms		Average number of Neoplams/rat in each group	
	Male	Female	Male	Female	Male	Female
0	80	80	100	187	1.3	2.3
50	48	48	53	132	1.1	2.8
100	48	48	43	120	0.9	2.5
200	47	48	53	124	1.1	2.6

Table 44: Incidence of mammary gland tumours in rats ingesting VDC in the drinking dater (1)

Sex	Male	Female

Concentration in drinking water	0 ppm	50 ppm	100 ppm	200 pm	0 ppm	50 ppm	100 ppm	200 ppm
Mammary gland [2]								
Total number of rats	80	48	48	47	80	48	48	48
Fibroadenoma/adenofibroma	2/2	1/1	4/4	0	**94/53**	**67/40** [3]	**64/36**	**64/35**
Adenoma	0	0	0	0	0	0	1/1	0
Carcinoma without metastasis	0	1/1	0	0	4/4	1/1	2/2	0
Papillary duct adenoma	0	0	0	1/1	0	0	0	0

[1] Only organs with a result significantly different from control data when analyzed using Fischer's Exact Probability Test ($p < 0.05$) are presented.
[2] Data listed as number of neoplasms/number of rats with neoplasm. All gross masses were examined microscopically.
[3] Significantly different from control data when analyzed using Fischer's Exact Probability test, $p < 0.05$.

There were no significant differences between the groups of rats ingesting VDC and the control groups. The only treatment-related observation in the rats was histopathological changes in the liver. The observation was characterized by midzonal fatty change which occurred in the females at all dose levels and in the males only at the 200 ppm level. No exposure-related neoplastic changes occurred in the rats in any of the test groups.

1.5.1.4 [NTP (1982)]

Study reference:

National Toxicology Program 1982; Technical Report Series No. 228; Carcinogenesis Bioassay of Vinylidene Chloride (CAS No. 75-35-4) in F344 Rats and B6C3F1 Mice (Gavage Study); U.S. Department of health and human services. Public Health Service. National Institute of Health (1982).

Detailed study summary and results:

Nota: Information below related to study in rats

Test type

A carcinogenicity study was conducted on vinylidene chloride. Rats were exposed to the substance by oral route (gavage) at dose levels of 1 or 5 mg/kg and for 2 years (104 weeks).

Test substance

The test substance as cited in the report was vinylidene chloride, manufactured by Dow Chemical company (Freeport, TX). The lot identification used in this study was V83848 with a purity of 99%. In this lot, two impurities were identified by vapor-phase chromatography: transdichloroethylene (0.15%) and MEHQ (0.02%). This lot was analyzed monthly and did not change in composition over the course of the study.

Test animals

The test was conducted on male and female F344/N rats obtained from NCI Fredrick Cancer Research

Center (Frederick, MD). Animals were 4 weeks old at the receipt and they were observed for 5 weeks because of the presence of parasites or other diseases. Then, they were assigned to control or treated groups according to a table of random numbers. They were housed five per cage (polybocarbonate cages), and all in the same room. No other chemicals were on test in that room. Diet and water were available ad libidum.

Administration/exposure

Groups of 50 male and 50 female animals were exposed to 1 or 5 mg/kg, 5 times/week and during 104 weeks. An additional control animals (group at 0 mg/kg) received corn oil alone. The choice of the tested dose levels was based on subchronic toxicity study (90-day duration).

Clinical evaluation and pathology

Mortality was observed twice daily. Body weights were recorded every 2 weeks. Gross examination and histopathology were performed on major tissues, major organs, and all gross lesions from killed animals and from animals found dead (unless precluded in whole or in part by autolysis or cannibalization). The tissues prepared for microscopic examination were: skin, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, heart, salivary gland, liver, pancreas, stomach, small intestine, large intestine, kidneys, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate and seminal vesicles or uterus, testis or ovary, brain, thymus, larynx, and esophagus.

Probabilities of survival were estimated by the procedure of Kaplan and Meier (1958). Animals were statistically censored as of the time they died of other than natural causes. Animals dying from natural causes were not statistically censored. The incidence of neoplastic or non-neolastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific site to the number of animals in which that site is examined. The one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each level, and the Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971). If tumors showed a significant increase with this “unadjusted analysis” which does not take into account the survival differences between the groups, life table analyses of primary tumor incidence were carried out.

Results and discussion

No significant differences in probability of survival were observed in any group of either sex of rats. But 12 control and 10 low-dose males were accidentally killed during week 82 of the study as a result of a 5-hour temperature excursion to 37°C and 1 low-dose male was accidentally killed during the week 42. These animals were censored in the survival analysis but were examined histopathologically. In male rats, 20/50 (40%) of the vehicle controls, 24/50 (48%) of the low-dose, and 37/50 (74%) of the high dose group lived to the end of the study at 104 weeks. In female rats, 27/50 (54%) of the vehicle controls, 28/50 (56%) of the low-dose, and 29/50 (58%) of the high-dose group lived to the end of the study at 104 weeks. The large

number of accidental deaths in the control group and low-dose male rats may have influenced the incidence of late appearing tumors in these groups.

No compound-related clinical signs were observed. Mean body weights of dosed and control rats of either sex were comparable throughout the study.

Table 45: Mean body weight change of Rats administered VDC by gavage for 2 years

	Week No.	Mean body weight change (g)			Weight change relative to Control group (%)	
		Control group	1 mg/kg	5 mg/kg	1 mg/kg	5 mg/kg
MALE	0	191[a]	190 [a]	193 [a]		
	1	20	23	24	+15	+20
	20	150	160	157	+7	+5
	40	210	214	210	+2	0
	60	230	239	233	+4	+1
	80	236	232	240	-2	+2
	100	218	222	223	+2	+2
FEMALE	0	139 [a]	140 [a]	139 [a]		
	1	10	12	12	+20	+20
	20	56	82	66	+46	+18
	40	79	84	83	+6	+5
	60	114	117	118	+3	+4
	80	143	147	150	+3	+5
	100	170	166	176	-2	+4

[a] Initial weight
[b] Weight change relative to controls =
{[Weight change (dosed group) – Weight change (Control group]) / Weight change (Control group)} * 100

Several non-neoplastic lesions of possible significance were observed. The incidence of chronic inflammation of the kidney in both male and female rats was higher in high-dose animals than in controls (males: 52%, 50% and 90% in the controls, low-dose and high-dose respectively; females: 6%, 12% and 20% in the controls, low dose and high-dose respectively). Although the occurrence of chronic nephritis appears to be dose related, this lesion is common in aging rats.

Table 46: Incidence of non-neoplatics lesions in rats administered VDC by gavage for 2 years

	MALE			FEMALE		
	Vehicle control	Low dose 1 mg/kg	High dose 5 mg/kg	Vehicle control	Low dose 1 mg/kg	High dose 5 mg/kg
Kidney [(a)]	50	48	48	49	49	44
Mineralization		1 (2%)			1 (2%)	
Cyst, NOS			1 (2%)			
Glomerulonephritis, NOS		1 (2%)				
Inflammation, NOS				1 (2%)		
Inflammation, chronic	**26 (52%)**	**24 (50%)**	**43 (90%)**	**3 (6%)**	**6 (12%)**	**9 (20%)**
Pigmentation, NOS	1 (2%)					
Calcinosis, NOS					1 (2%)	
Kidney / Tubule [(a)]	50	48	48			
Necrosis,Focal			1 (2%)			

Urinary bladder [(a)]	44	42	43	45	45	41
Hemorrhage		1 (2%)				
Hyperplasia, epithelial					3 (7%)	1 (2%)

[a] Number of animals with tissue examined microscopically

Histopathological findings related to neoplasms are summarized in the table below. The table contains the results of Fisher's exact tests and Cochran-Armitage trend tests for those primary tumors whose incidence was 5% or greater in at least one of the three groups. Because of the many early deaths in the control and low-dose male rat groups, life table analyses of primary tumor incidence were also carried out for tumors showing a significant increase by the "Unadjusted analysis".

Table 47: Incidence of primary tumors in rats administred VDC by gavage for 2 years

	MALE			**FEMALE**		
	Control group	**1 mg/kg**	**5 mg/kg**	**Control group**	**1 mg/kg**	**5 mg/kg**
SUBCUTANEOUS TISSUE						
Fibroma [a]	0/50 (0)	1/48 (2)	4/48 (8)			
P values (Unadjusted analysis) [b, c]	**P=0.024**	N.S.	N.S.			
P values (Adjusted analysis) [d, c]	**P=0.114**	**P=0.508**	**P=0.162**			
Week to first observed tumor	-	99	94			
HEMATOPOIETIC SYSTEM						
Leukemia, NOS [a]	7/50 (14)	4/48 (8)	8/48 (17)	10/49 (20)	4/48 (8)	4/45 (9)
P values (Unadjusted analysis) [b, c]	N.S.	N.S.	N.S.	N.S.	**P=0.033 N**	N.S.
Week to first observed tumor	82	73	100	94	101	91
All leukemia [a]				10/49 (20)	6/50 (12)	5/45 (11)
P values (Unadjusted analysis) [b, c]				N.S.	N.S.	N.S.
Week to first observed tumor				94	88	74
All Lymphoma [a]				3/49 (6)	1/50 (2)	0/45 (0)
P values (Unadjusted analysis) [b, c]				N.S.	N.S.	N.S.
Week to first observed tumor				82	102	-
All Lymphoma or Leukemia [a]				13/49 (27)	7/50 (14)	5/45 (11)
P values (Unadjusted analysis) [b, c]				N.S.	N.S.	**P=0.05 N**
Week to first observed tumor				82	88	74
LIVER						
Neoplastic Nodule [a]				4/49 (8)	0/50 (0	0/45 (0
P values (Unadjusted analysis) [b, c]				**P=0.045 N**	N.S.	N.S.
Week to first observed tumor				85	-	-
PITUITARY						
Adenoma, NOS [a]	7/49 (14)	10/47 (21)	10/44 (23)	16/48 (33)	20/49 (41)	24/43 (56)
P values (Unadjusted analysis) [b, c]	N.S.	N.S.	N.S.	**P=0.017**	N.S.	**P=0.026**
P values (Adjusted analysis) [d, c]				**P=0.114**	**P=0.108**	**P=0.162**
Week to first observed tumor	75	66	102	65	78	71
ADRENAL						
Pheochromocytoma [a]	6/50 (12)	5/48 (10)	13/47 (28)	1/48 (2)	2/50 (4)	3/43 (7)
P values (Unadjusted analysis) [b, c]	**P=0.010**	N.S.	**P=0.045**	N.S.	N.S.	N.S.
P values (Adjusted analysis) [d, c]	**P=0.247**	**P=0.396 N**	**P=0.422**			
Week to first observed tumor	96	104	73	82	102	85
PANCREATIC ISLETS						
Islet-cell Carcinoma [a]	4/49 (8)	1/47 (2)	6/48 (13)			
P values (Unadjusted analysis) [b, c]	N.S.	N.S.	N.S.			

Week to first observed tumor	96	104	104			
Islet-cell, Adenoma or Carcinoma [a]	4/49 (8)	1/47 (2)	8/48 (17)			
P values (Unadjusted analysis) [b, c]	**P=0.025**	N.S.	N.S.			
P values (Adjusted analysis) [d, c]	**P=0.249**	**P=0.149 N**	**P=0.588**			
Week to first observed tumor	96	104	104			
TESTIS						
Intertitial-Cell Tumor [a]	43/50 (86)	39/47 (83)	47/48 (98)			
P values (Unadjusted analysis) [b, c]	**P=0.013**	N.S.	**P=0.034**			
Week to first observed tumor	78	55	73			

N.S. Not Significant
N Negative trend which indicates a lower incidence in a dosed group than in a control group.
[a] Number of animals with tumors / number of animals examined at site (percent).
[b] Probability when "Unajusted analysis" (refer to Cochran-Armitage test and the Fisher exact test, which do not account for survival differences among groups):
Control group: probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) / Dosed group: probability level for the Fisher exact test for the comparison of that dosed group with the vehicle-control group when P is less than 0.05; otherwise, not significant (N.S.).
[c] A negative trend (**N**) indicates a lower incidence in a dosed group than in a control group.
[d] Probability when "Adjusted analysis" (refer to Life Table Analysis which adjusts for intercurrent mortality) calculated for tumors showing a significant increase by the "unadjusted" analyses.

Pheochromocytomas of the adrenal in male rats were observed in increased incidence in the high-dose group compared with the control group. The Cochran-Armitage test for linear trend was statistically significant in the positive direction. The Fisher exact test between the high-dose group and the control group indicated a value of P=0.045. Analyses of these data by life table methods produced no statistically significant results. The historical incidence of this tumor at this laboratory in untreated male F344 rats is 32/400 (8%), while the incidence in vehicle control (corn oil) male rats across the bioassay program is 13/125 (10.4%). In female rats, this tumor was not observed in statistically significant proportions.

Islet-call adenomas or carcinomas of the pancreatic islets in male rats were observed in increased incidence in the high-dose group. The Cochran-Armitage test for linear trend was statistically significant in the positive direction, but the Fisher exact tests were not significant. Analyses of these data by life table methods revealed no statistically significant results. These tumors have been observed in 18/400 (4.5%) of the untreated male rats at this laboratory and in 6/125 (4.8%) of the male vehicle control (corn oil) rats in the Bioassay Program. In female rats, this tumor was not observed in statistically significant proportions.

Interstitial-cell tumors of the testis were observed in increased incidence in the high-dose group compared with the control group. The Cochran-Armitage test for linear trend was statistically significant in the positive direction. The Fisher exact test between the high-dose group and the control group indicated a value of P=0.034, but this high-dose effect is not statistically significant if age-adjusted analyses are used which eliminate those animals that died before 52 weeks (life table analyses are not appropriate for this tumor, since this lesion is not generally regarded as life-threatening). This tumor has been observed at incidences as high as 100% in untreated control groups at this laboratory.

Adenomas of the pituitary were observed in increased incidence in the high-dose female rats compared with the controls. The Cochran-Armitage test for linear trend was significant and the Fisher exact test between the high-dose and control groups was significant. The analysis of these data by life table methods revealed no statistically significant results. No significant differences are observed in the incidence of female rats with

either adenomas or carcinomas of the pituitary. These tumors were not observed in statistically significant proportions in male rats.

Fibromas of the subcutaneous tissue were observed in increased incidence in the dosed groups of male rats compared with controls. The Cochran-Armitage test for linear trend was statistically significant in the positive direction, but the Fisher exact tests were not significant. Analyses of these data by life table methods revealed no statistically significant results. Fibromas of the skin have been observed in 2.6% of the untreated male F344 rats and in 4% of the male vehicle control F344 rats receiving corn oil by gavage in the bioassay program.

Leukemia (morphology unspecified) in female rats was observed in decreased incidence in the dosed groups compared with the controls. The Fisher exact test between the low-dose and control groups was significant in the negative direction. The Cochran-Armitage test for linear trend was not significant. Female rats with either lymphomas or leukemias of the hematopoietic system were observed in decreased proportions in the dosed groups compared with the controls. The Fisher exact test between the high-dose and control groups is significant in the negative direction. These tumors were not observed in statistically significant proportions in male rats.

Neoplastic nodules of the liver in female rats were observed in decreased incidence in dosed groups, but the Fisher exact tests were not significant. In male rats, liver tumors were not observed in statistically significant proportions.

In brief, all of the increased tumor incidences that were statistically significant by the Fisher exact test or by the Cochran-Armitage linear trend test were not significant when life table analyses were used. This difference occurs because life table analyses adjust for intercurrent mortality and thus they minimize the impact of animals dying before the onset of late appearing tumors. This adjustment was particularly critical for the analyses of tumor incidences in male rats because 12 controls and 10 low-dose animals were accidentally killed during week 82 of the study. The tumors encountered were commonly found in aging rats of this strain. They occurred in comparable numbers in test animals and controls and were not considered to be related to administration of the test compound (Goodman et al., 1979).

NTP concluded that vinylidene chloride administered by gavage to F344 rats was not carcinogenic under the conditions of the study. An increased incidence of chronic renal inflammation was observed for either sex, but without clear evidence. This lesion commonly occurs in aged male rats and the biologically significance is questionable. The NOAEL in this study was established at 5 mg/kg-day (the highest exposure tested) for carcinogenicity.

1.5.1.5 [NTP (1982)]

Study reference:

National Toxicology Program 1982; Technical Report Series No. 228; Carcinogenesis Bioassay of Vinylidene Chloride (CAS No. 75-35-4) in F344 Rats and B6C3F1 Mice (Gavage Study); U.S. Department of health and human services. Public Health Service. National Institute of Health (1982).

Detailed study summary and results:

Nota: Information below related to study in mice

Test type

A carcinogenicity study was conducted on vinylidene chloride. Mice were exposed to the substance by oral route (gavage) at dose levels of 2 or 10 mg/kg and for 2 years (104 weeks).

Test substance

The test substance as cited in the report was vinylidene chloride, manufactured by Dow Chemical company (Freeport, TX). The lot identification used in this study was V83848 with a purity of 99%. In this lot, two impurities were identified by vapor-phase chromatography: transdichloroethylene (0.15%) and MEHQ (0.02%). This lot was analyzed monthly and did not change in composition over the course of the study.

Test animals

The test was conducted on male and female B6C3F1/N mice obtained from NCI Fredrick Cancer Research Center (Frederick, MD). Animals were 4 weeks old at the receipt and they were observed for 5 weeks because of the presence of parasites or other diseases. Then, they were assigned to control or treated groups according to a table of random numbers. They were housed five per cage (polybocarbonate cages), and all in the same room. No other chemicals were on test in that room. Diet and water were available ad libidum.

Administration/exposure

Groups of 50 male and 50 female animals were exposed to 2 or 10 mg/kg, 5 times/week and during 104 weeks. An additional control animals (group at 0 mg/kg) received corn oil alone. The choice of the tested dose levels was based on subchronic toxicity study (90-day duration).

Clinical evaluation and pathology

Mortality was observed twice daily. Body weights were recorded every 2 weeks. Gross examination and histopathology were performed on major tissues, major organs, and all gross lesions from killed animals and from animals found dead (unless precluded in whole or in part by autolysis or cannibalization). The tissues prepared for microscopic examination were: skin, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, heart, salivary gland, liver, pancreas, stomach, small intestine, large intestine, kidneys, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate and seminal vesicles or uterus, testis or ovary, brain, thymus, larynx, and esophagus.

Probabilities of survival were estimated by the procedure of Kaplan and Meier (1958). Animals were statistically censored as of the time they died of other than natural causes. Animals dying from natural causes were not statistically censored. The incidence of neoplastic or non-neolastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific site to the number of animals in which that site is examined. The one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each level, and the Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971). If tumors showed a significant increase with this "unadjusted analysis" which does not take into account the survival differences between the groups, life table analyses of primary tumor incidence were carried out.

Results and discussion

No significant differences in probability of survival were observed in any group of male mice. A significant reduction in the survival of the low-dose group of female mice was observed when compared with the high-dose group. In male mice, 33/50 (66%) of the vehicle controls, 35/50 (70%) of the low-dose, and 36/50 (72%) of the high dose group lived to the end of the study at 104 weeks. In female mice, 40/50 (80%) of the vehicle controls, 32/50 (64%) of the low-dose, and 42/50 (84%) of the high-dose group lived to the end of the study at 104 weeks.

No compound-related clinical signs were observed.

Throughout most of the study, the mean body weights of dosed male mice were lower than those of the vehicle controls. Mean body weights of high-dose and control female mice were comparable. Mean body weights of low-dose mice of either sex were lower than those of the high-dose or vehicle-control mice.

Table 48: Mean body weight change of Mice administered VDC by gavage for 2 years

	Week No.	Mean body weight change (g)			Weight change relative to Control group (%)	
		Control group	2 mg/kg	10 mg/kg	2 mg/kg	10 mg/kg
MALE	**0**	28 [a]	190 [a]	193 [a]		
	1	1	2	2	+100	+100
	20	13	11	13	-15	0
	40	19	17	19	-20	0
	60	22	21	23	-5	+5
	80	21	20	22	-5	+5
	100	20	21	21	+5	+5
FEMALE	**0**	19 [a]	18 [a]	18 [a]		
	1	2	2	3	0	+50
	20	9	8	9	-11	0
	40	12	12	13	0	+8
	60	17	15	18	-12	+6
	80	19	18	14	-5	-35
	100	24	21	24	-13	0

[a] Initial weight

[b] Weight change relative to controls

= {[Weight change (dosed group) – Weight change (Control group]) / Weight change (Control group)} * 100

Regarding non-neoplatics findings, necrosis of the liver (focal, multifocal or diffuse) was observed more frequently in dosed mice than in controls, mainly in high-dose male and low-dose female mice (male: 2 %, 7% and 14% in the controls, low-dose and high dose respectively; female: 0 %, 8% and 2% in the controls, low-dose and high dose respectively). This effect was not statistically significant at either exposure.

Table 49: Incidence of non-neoplatics lesions in Mice administered VDC by gavage for 2 years

	MALE			FEMALE		
	Vehicle control	**Low dose 2 mg/kg**	**High dose 10 mg/kg**	**Vehicle control**	**Low dose 1 mg/kg**	**High dose 5 mg/kg**
Liver [(a)]	46	46	49	47	49	49
Hemorrhage		1 (2%)		1 (2%)		
Inflammation, NOS						1 (2%)
Inflammation, focal						1 (2%)
Inflammation, multifocal	1 (2%)		3 (6%)		3 (6%)	
Inflammation, diffuse		2 (4%)				
Inflammation, granulomatous					1 (2%)	
Inflammation, focal granulomatous	1 (2%)			1 (2%)	1 (2%)	
Necrosis, NOS			**3 (6%)**			
Necrosis, focal		**1 (2%)**	**3 (6%)**		**2 (4%)**	
Necrosis, diffuse	**1 (2%)**	**2 (4%)**	**1 (2%)**		**2 (4%)**	1 (2%)
Metamorphosis fatty	4 (9%)	4 (9%)	5 (10%)	5 (11%)	2 (4%)	3 (6%)
Clear-cell change	2 (4%)		3 (6%)			
Cytologic alteration, NOS	3 (7%)					
Basophilic cyto change				1 (2%)		

[a] Number of animals with tissue examined microscopically

Histopathological findings related to neoplasms in mice are summarized in the table below. The only increased incidence was in the hematopoietic system in female mice.

An increased incidence of lymphomas was observed in the low-dose group of female mice compared with the other two groups (4%, 18% and 12% in the controls, low-dose and high dose respectively). The Cochran-Armitage test for linear trend was not significant. The Fisher exact test between the low-dose group and the vehicle control group was significant (P=0.028). No significant incidence was observed in the high-dose group. When these data were analyzed by life table methods, the increased incidence of lymphomas in the low-dose group was statistically significant (P=0.012) but the incidence in the high-dose group and the dose-response trend were not significant. The incidence of lymphomas in female mice observed in the control group (4%) is less than the historical incidence from all laboratories (31/315, 9.8%). Data was not yet available at this laboratory for other gavage studies in which corn oil was used as the vehicle. In male mice, this tumor was not observed in statistically significant proportions.

An increased incidence of lymphomas or leukemia in female mice were observed in the low-dose group compared with the controls (15%, 31% and 14% in the controls, low-dose and high-dose respectively). The Cochran-Armitage test for linear trend was not significant. The Fisher exact test between the low-dose group and the vehicle controls was significant (P=0.05). No significant incidence was observed in the high-dose group. Life table analyses, using the death of an animal as the time point of examination for tumors, and

time-adjusted tests, eliminating those animals that died before 52 weeks, did not materially affect the results. These neoplastic lesions observed in the low-dose female group were considered to be unrelated to administration of VDC because similar effects were not found in the high-dose group and because they were within the usual incidence range seen in B6C3F1 control mice.

Table 50: Incidence of primary tumors in Mice administred VDC by gavage for 2 years

	MALE			FEMALE		
	Control group	**2 mg/kg**	**10 mg/kg**	**Control group**	**2 mg/kg**	**10 mg/kg**
HEMATOPOIETIC SYSTEM						
Lymphoma, Malignant, NOS [a]	6/47 (13)	3/47 (6)	7/50 (14)			
P values (Unadjusted analysis) [b, c]	N.S.	N.S.	N.S.			
Week to first observed tumor	85	99	91			
All Malignant Lymphomas [a]	6/47 (13)	3/47 (6)	8/50 (16)	2/48 (4)	9/49 (18)	6/50 (12)
P values (Unadjusted analysis) [b, c]	N.S.	N.S.	N.S.	N.S.	**P=0.028**	N.S.
P values (Adjusted analysis) [e, c]				**P=0.449**	**P=0.012**	**P=0.150**
Week to first observed tumor	85	99	92	104	100	104
All Leukemias [a]	0/47 (0)	3/47 (6)	0/50 (0)	5/48 (10)	7/49 (14)	1/50 (2)
P values (Unadjusted analysis) [b, c]	N.S.	N.S.	N.S.	**P=0.031 (N)**	N.S.	N.S.
Week to first observed tumor	-	82	-	70	91	74
All Lymphoma or Leukemia [a]	6/47 (13)	6/47 (13)	8/50 (16)	7/48 (15)	15/49 (31)	7/50 (14)
P values (Unadjusted analysis) [b, c]	N.S.	N.S.	N.S.	N.S.	**P=0.050**	N.S.
P values (Adjusted analysis) [e, c]				**P=0.231 N**	**P=0.037**	**P=0.581 N**
Week to first observed tumor	85	82	92	70	91	74

N.S. Not Significant

N **N**egative trend which indicates a lower incidence in a dosed group than in a control group.

[a] Number of animals with tumors / number of animals examined at site (percent).

[b] Probability when "Unajusted analysis" (refer to Cochran-Armitage test and the Fisher exact test, which do not account for survival differences among groups):
Control group: probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) / Dosed group: probability level for the Fisher exact test for the comparison of that dosed group with the vehicle-control group when P is less than 0.05; otherwise, not significant (N.S.).

[c] A negative trend (**N**) indicates a lower incidence in a dosed group than in a control group.

[d] The 95 percent confidence interval of the relative risk between each dosed group and the control group.

[e] Probability when "Adjusted analysis" (refer to Life Table Analysis which adjusts for intercurrent mortality) calculated for tumors showing a significant increase by the "unadjusted" analyses.

NTP concluded that vinylidene chloride administered by gavage to B6C3F mice was not carcinogenic under the conditions of the study. A slight increased incidence of necrosis of the liver was observed in male mice but without clear evidence. The NOAEL in this study was established at 10 mg/kg-day (the highest exposure tested) for carcinogenicity.

1.5.2 Animal data by inhalation

1.5.2.1 [Maltoni (1977)]

Study reference:

Maltoni C., Cotti G., Morisi L. and Chieco P. 1977: Carcinogenicity bioassays of vinylidene chloride Research plan and early results (publication), Medicina Del Lavoro, 68(4) 241-262.

Maltoni C., Cotti G., and Chieco P. 1984: Chronic toxicity and carcinogenicity bioassays of vinylidene chloride, Acta oncologica 5 (2).

Roberts S.M., Jordan K.E., Warren D.A., Britt J.K. and James R.C. 2002: Evaluation of the carcinogenicity of 1,1-dichloroethylene (vinylidene chloride) (review article or handbook), Regulatory Toxicology and Pharmacology, 35:44-55.

United States Environmental Protection Agency 1985: Health assessment document for vinylidene chloride; Final report EPA/600/8-83/031F

Detailed study summary and results:

Nota*:* Information below related to study in rats

Test type

A carcinogenicity study on VDC was conducted in rats exposed by inhalation. Six groups (one control group and five treated groups) of male and female Sprague-Dawley rats were treated with VDC by vapour for 52 weeks, 4 hours daily and 4-5 days weekly.

Test substance

The test item as described in the publication was 1,1-dichloroethylene obtained from the Central Laboratory of Solvay (Bruxelles). The purity of he sample used in this study was > 99.9% (999.5 g/kg). The impurity were 1,2-dichloroethylene trans (0.4 g/kg), acetone (0.1 g/kg), methylene chloride (0.05 g/kg), mono and dichloroacetylene (0.02 g/kg) and paramethoxyphenol (as stabilizer at 200 ppm).

Test animals

The study was conducted on male and female Sprague-Dawley rats, 16 weeks old at start, supplied by Institute of Oncology and Tumour Centre, Bologna (Italy). They were housed 5/cage in polycarbonate cages with tops made of stainless steel wir.

Administration/exposure

Four groups of 30 male and 30 female rats were exposed to 10, 25, 50 and 100 ppm (equivalent to 40, 100, 200 and 400 mg/m3), and one group of 60 male and 60 female rats was exposed to 150 or 200 ppm (equivalent to 600 or 800 mg/m3). The highest dose level of 800 mg/m3 was reduced to 600 mg/m3 after 2 exposures because of high toxicity. A concurrent untreated group of 100 male and 100 female rats served as negative control group. The treatment was conducted for 4 hours daily, 4-5 days/week and for 52 weeks for all the groups. From time to time, the treatment was reduced from 5 to 4 days weekly because of high toxicity observed. Following the 52-week exposure, animals were observed until spontaneous death (total duration 137 weeks). The control of VDC concentrations is carried out by gas chromatography.

Clinical evaluation and pathology

The animals were controlled weekly and weighted every 2 weeks during the period of treatment and monthly after the treatment is over. A complete autopsy was carried out on each animal. Histological examinations were performed on the Zymbal glands, interscapular brown fat, salivary glands, tongue, lungs, river, kidneys, spleen, stomach, different segments of the intestine, bladder, brain, bone marrow (sternum) and any other organs with pathological lesions. Cytological examinations were carried out on the bone marrow of the femur. No information either on other examinations or statistical methods are available.

Results and discussion

No biologically significant non-neoplastic effects in any organ in either sex were noted. Different kinds of tumours were observed in rats treated and in the control groups. The mammary tumour was the most frequent. An increase in the incidence of mammary tumours was observed in female animals (but not when only carcinomas were considered). Among the treated groups, no clear dose relationship effect was evident.

Table 51: Incidence of tumors in rats exposed by inhalation to Vinylidene Chloride for 52 weeks. Results after 137 weeks (end of the study)

	Control group	40 mg/m3 (10 ppm)	100 mg/m3 (25 ppm)	200 mg/m3 (50 ppm)	400 mg/m3 (100 ppm)	600 mg/m3 (150 ppm)
(Results after 137 weeks)						
Number of animals at start	100M + 100F	30M + 30F	30M + 30F	30M + 30F	30M + 30F	60M + 60F
Corrected number of animals [a]	83M + 98F	29M + 30F	27M + 30F	30M + 30F	30M + 30F	59M + 59F
Animals with mammary tumours [b]						
Males with tumours (%) [c]	11 (13.2)	4 (13.8)	4 (14.3)	7 (23.3)	6 (20.0)	8 (13.5)
Females with tumours (%) [c]	61 (62.2)	28 (93.3)	21 (70.0)	23 (76.7)	25 (83.3)	44 (74.6)
Total animals with tumours (%) [c]	72 (39.8)	32 (54.2)	25 (43.8)	30 (50.0)	31 (51.7)	52 (44.1)
Histologically examined animals	11M + 56F	4M + 24F	4M + 20F	7M + 22F	5M + 23F	8M + 43F
Fibromas and fibroadenomas						
Males with tumours (%) [d]	11 (100.0)	3 (75.0)	4 (100.0)	7 (100.0)	5 (100.0)	6 (75.0)
Females with tumours (%) [d]	45 (80.3)	24 (100.0)	20 (100.0)	21 (95.4)	22 (95.7)	38 (88.4)
Total animals with tumours (%) [d]	56 (83.6)	27 (96.4)	24 (100.0)	28 (96.5)	27 (96.4)	44 (86.3)
Carcinomas						

Males with tumours (%) [d]	0	1 (25.0)	0	0	0	1 (12.5)
Females with tumours (%) [d]	16 (28.6)	5 (20.8)	4 (20.0)	1 (4.5)	3 (13.0)	9 (20.9)
Total animals with tumours (%) [d]	16 (23.9)	6 (21.4)	4 (16.7)	1 (3.4)	3 (10.7)	10 (19.6)
Sarcomas						
Total animals with tumours (%) [d]	0	0	0	0	0	0
Carcinomas						
Males with tumours (%) [d]	0	0	0	0	0	1 (12.5)
Females with tumours (%) [d]	0	0	0	0	0	1 (2.3)
Total animals with tumours (%) [d]	0	0	0	0	0	2 (3.9)

[a] Alive animals after 10 weeks when the first tumour was observed (leukemia)
[b] Two or more tumours of the same and/or the different types may be present in the same animal (fibroadenomas, carcinomas, sarcomas, carcinosarcomas)
[c] The percentages are referred to the corrected number.
[d] The percentages are referred to the total number of animals bearing mammary tumours and histologically examined.

An increase in the incidence of mammary tumours was observed in treated female rats when compared to the control group, but without apparent dose-response trend.

1.5.2.2 [Maltoni (1977)]

Maltoni C., Cotti G., Morisi L. and Chieco P. 1977: Carcinogenicity bioassays of vinylidene chloride Research plan and early results (publication), Medicina Del Lavoro, 68(4) 241-262.

Maltoni C., Cotti G., and Chieco P. 1984: Chronic toxicity and carcinogenicity bioassays of Vinylidene chloride, Acta oncologica 5 (2).

Roberts S.M., Jordan K.E., Warren D.A., Britt J.K. and James R.C. 2002: Evaluation of the carcinogenicity of 1,1-dichloroethylene (vinylidene chloride) (review article or handbook), Regulatory Toxicology and Pharmacology, 35:44-55.

United States Environmental Protectin Agency 1985: Health assessment document for Vinylidene chloride; Final report EPA/600/8-83/031F

Detailed study summary and results:

Nota*:* Information below related to study in mice

Test type

A carcinogenicity study on VDC was conducted on mice exposed by inhalation.

Test substance

The test item as described in the publication was 1,1-dichloroethylene obtained from the Central Laboratory of Solvay (Bruxelles). The purity of the sample used in this study was > 99.9% (999.5 g/kg). The impurity

were 1,2-dichloroethylene trans (0.4 g/kg), acetone (0.1 g/kg), methylene chloride (0.05 g/kg), mono and dichloroacetylene (0.02 g/kg) and paramethoxyphenol (as stabilizer at 200 ppm).

Test animals

The study was conducted on male and female Swiss mice, 16 weeks old at start, supplied by Institute of Oncology and Tumour Centre, Bologna (Italy). They were housed 5/cage in polycarbonate cages with tops made of stainless steel wir.

Administration/exposure

Four groups of 30 male and 30 female mice were exposed to 10, 25, 50 and 100 ppm (equivalent to 40, 100, 200 and 400 mg/m3), and one group of 60 male and 60 female mice was exposed to 200 ppm (equivalent to 800 mg/m3). A concurrent untreated group of 100 male and 100 female mice served as negative control group. Doses of 800, 400 for 2 days, and 200 mg/m3 for 4 days caused high mortality and severe toxicity, causing termination of these portions of the assay. The dose level of 100 mg/m3 was found to be the highest acceptable dose for Swiss mice for long-term exposure. Therefore the number of animals exposed to 100 mg/m3 was enlarged. An additionnal group of 120 male and 120 female mice exposed to 100 mg/m3 was added with its concurrent control group (90 male and 90 female mice). In the two additional groups, the mice were 9 weeks old. The treatment was conducted for 4 hours daily, 4-5 days/week and for 52 weeks for all the groups. From time to time, the treatment was reduced from 5 to 4 days weekly because of high toxicity observed. Following the 52-week exposure, animals were observed until spontaneous death (total duration 126 weeks).

The chamber for inhalation exposure was built of stainless steel and glass. The control of VDC concentrations was carried out by gas chromatography.

Clinical evaluation and pathology

The animals are controlled weekly and weighted every 2 weeks during the period of treatment and monthly after the treatment is over. All the detectable gross pathological changes are recorded during the control.

The study was completed at 121 weeks. A complete autopsy is carried out on each animal. Histological examinations are performed on the Zymbal glands, interscapular brown fat, salivary glands, tongue, lungs, river, kidneys, spleen, stomach, different segments of the intestine, bladder, brain, bone marrow (sternum) and any other organs with pathological lesions. Furthermore, cytological examinations are carried out on the bone marrow of the femur.

No information either on other examinations are available. A statistical analysis (Exact Fisher test and Logrank test) was conducted on the incidence of tumours.

Results and discussion

Groups treated at 200, 100 and 50 ppm (800, 400 and 200 mg/m3) caused the death of a high number of animals, and had to be stopped. The concentration of 25 ppm (or 100 mg/m3) was found to be the highest tolerable dose by mice.

Non-neoplastic changes, mainly typical necrosis, were found in the liver and in the kidney, and both in the control and treated animals. The most frequent findings were regressive changes (hepatocyte vacuolization, swelling, fatty degeneration and necrosis) and amyloidosis in the liver, regressive changes (swelling and necrosis of tubular cells), amyliodosis of glomureli and chronic nephritis in kidneys. The incidence of these lesions in the groups of animals treated with low concentrations (10 and 25 ppm) was compatible with a long survival. The findings in the liver did not indicate a clear relationship with exposure to VDC. Findings in the kidney suggested a dose-response relationship, a higher incidence of more pronounced regressive changes were found in kidneys with renal adenocarcinoma. Degeneration and necrotic changes in kidneys, especially in the tubular region, were noted in male mice that died from exposure to 200 ppm VDC (4 days daily, for 2 days).

Table 52: Incidence of non-neoplastic lesions of kidney in Mice in the 52-week inhalation study of VDC. Results after 121 weeks (end of the study)

Treatment		Animals (Swiss mice)			Number (N) of animals with non neoplastics lesions of kidney							
Groups (ppm)	Expo -sure	Sex	Number at start	Age at start	Examined animals		Regressive change		Amyloidosis		Chronic nephritis	
					N	%	N	%	N	%	N	%
200 [b] (first step)	2 days [a]	M	60	16 weeks	59	98.3	0	84.7	6	10.2	4	6.7
		F	60		49	81.7	0	-	23	46.9	22	44.9
		M + F	120		108	90.0	50	41.7	29	26.8	26	24.1
100 [b] (first step)	2 days [a]	M	30	16 weeks	27	90.0	9	22.2	19	70.4	15	55.5
		F	30		27	90.0	0	-	24	88.9	20	74.1
		M + F	60		54	90.0	6	11.1	43	79.6	35	64.8
50 [b] (first step)	1 week [a]	M	30	16 weeks	29	96.7	1	3.4	24	82.7	16	55.2
		F	30		27	90.0	0	-	18	66.7	17	63.0
		M + F	60		56	93.3	1	1.8	42	75.0	33	58.9
25 (first step)	52 week	M	30	16 weeks	28	93.3	0	-	25	89.3	18	64.3
		F	30		30	100.0	1	3.3	24	80.0	19	63.3
		M + F	60		58	96.7	1	1.7	49	84.5	37	63.9
10 (first step)	52 week	M	30	16 weeks	23	76.7	0	-	23	100.0	17	73.9
		F	30		25	83.3	0	-	19	76.0	11	44.0
		M + F	60		48	80.0	0	-	42	87.5	28	58.3
Controls (first step)	-	M	100	16 weeks	82	82.0	0	-	59	71.9	57	69.5
		F	100		89	89.0	0	-	59	66.3	61	68.5
		M + F	200		171	85.5	0	-	118	69.0	118	69.0
25	52	M	120	9 weeks	110	91.7	0	-	78	70.9	63	57.3

(second step)	**week**	**F**	120		112	93.3	0	-	77	68.7	59	52.7
		M + F	240		222	92.5	0	-	155	69.8	122	54.9
Controls (second step)	**-**	**M**	90	9 weeks	81	90.0	0	-	44	54.3	48	59.2
		F	90		63	70.0	2	3.1	32	50.8	25	39.7
		M + F	180		144	80.0	2	1.4	76	52.8	73	50.7

[a] The treatment was interrupted because of the high toxic effects and high mortality

Table 53: Incidence of non-neoplastic lesions of liver in Mice in the 52-week inhalation study of VDC. Results after 121 weeks (end of the study)

Treatment	**Animals (Swiss mice)**			**Number (N) of animals with non neoplastics lesions of liver**					
Groups (ppm)	**Sex**	**Number at start**	**Age at start**	**Examined animals**		**Regressive change**		**Amyloidosis**	
				N	**%**	**N**	**%**	**N**	**%**
25 (first step)	**M**	30	16 weeks	28	93.3	0	-	17	60.7
	F	30		30	100.0	4	13.3	7	23.3
	M + F	60		58	96.7	4	6.9	24	41.4
25 (second step)	**M**	120	9 weeks	107	89.2	8	7.5	50	46.7
	F	120		111	92.5	11	9.9	26	41.4
	M + F	240		218	90.8	19	8.7	96	44.0
10 (first step)	**M**	30	16 weeks	26	86.7	0	-	10	38.5
	F	30		27	90.0	3	11.1	6	22.2
	M + F	60		53	88.3	3	5.7	16	30.2
Controls (first step)	**M**	100	16 weeks	85	85.0	5	5.9	73	85.9
	F	100		91	91.0	3	3.3	67	73.6
	M + F	200		176	88.0	8	4.5	140	79.5

Various tumors were reported and the most important ones were kidney adenocarcinomas, mammary tumours and pulmonary adenomas.

The most important result is a statistically significant increase of kidney adenocarcinomas in the 100 mg/m3 (or 25 ppm) group. This type of tumours was also found in two of survivors of the 50 ppm. They were almost exclusively found in males (30 of the 31). Such tumours were not observed in the 10 ppm group or in the control group. The incidence was 0/120 (0%), 0/24 (0%), and 28/119 (23.5%) in male mice in the combined control, 40 mg/m3, and combined 100 mg/m3 groups, respectively.

A statistical significant increase in mammary tumours were found in treated female mice at both exposures, without a clear dose response relationship. The incidence was 3/187 (1.6%), 6/30 (20%), and 16/148 (11%) in females in the combined control, 10 ppm, and combined 25 ppm groups, respectively.

There was also a statistically significant increase in the pulmonary adenomas in both exposed groups, without a clear dose response relationship. The incidence was 13/332 (3.9%), 14/58 (24.1%), and 41/283 (14.5%) in male and female mice combined in the combined control, 10 ppm, and combined 25 ppm groups, respectively. However no pulmonary carcinomas were observed in any mice.

Table 54: Incidence of tumors in mice exposed by inhalation to Vinylidene Chloride for 52 weeks. Results after 121 weeks (end of experiment)

	Control group	40 mg/m3 (10 ppm)	100 mg/m3 (25 ppm)	200 mg/m3 (50 ppm)	400 mg/m3 (100 ppm)	600 mg/m3 (150 ppm)
STEP 1 (Results after 137 weeks)						
Number of animals at start	100M + 100F	30M + 30F	30M + 30F	30M + 30F	30M + 30F	60M + 60F
Kidney adenocarcinomas						
Corrected number of animals [a]	54M + 73F	24M + 26F	21M + 29F	18M + 16F	13M + 14F	1M + 28F
Males with tumours (%) [b]	0	0	**3 (14.3)** [c]	2 (11.1)	0	0
Females with tumours (%) [b]	0	0	0	0	0	0
Total animals with tumours (%) [b]	0	0	3 (6.4)	2 (5.9)	0	0
Mammary tumours[b]						
Corrected number of animals [a]	95M + 98F	30M + 30F	30M + 30F	27M + 29F	22M + 29F	6M + 53F
Males with tumours (%) [b]	1 (1.0)	0	0	0	0	0
Females with tumours (%) [b]	2 (2.0)	**6 (20.0)** [c, e]	**4 (13.3)** [d]	2 (6.9)	4 (13.8)	1 (1.9)
Total animals with tumours (%) [b]	3 (1.5)	6 (10.0)	4 (6.7)	2 (3.6)	4 (7.8)	1 (1.7)
Pulmonary tumours						
Corrected number of animals [a]	80M + 92F	28M + 30F	28M + 29F	26M + 27F	18M + 26F	5M + 46F
Males with tumours (%) [b]	3 (3.7)	**11 (39.3))** [c, e]	**7 (25.0))** [c, e]	2 (7.7)	2 (11.1)	0
Females with tumours (%) [b]	4 (4.3)	3 (10.0)	**7 (24.1))** [c, f]	4 (14.8)	2 (7.7)	2 (4.3)
Total animals with tumours (%) [b]	7 (4.1)	14 (24.1)	14 (24.6)	6 (11.3)	4 (9.1)	2 (3.9)
Leukemia						
Corrected number of animals [a]	99M + 98F	30M + 30F	30M + 30F	29M + 29F	23M + 30F	9M + 57F
Males with tumours (%) [b]	1 (1.0)	1 (3.3)	2 (6.7)	0	2 (8.7)	0
Females with tumours (%) [b]	8 (8.2)	6 (20.0)	1 (3.3)	3 (10.3)	3 (10.0)	3 (5.3)
Total animals with tumours (%) [b]	9 (4.6)	7 (11.7)	3 (5.0)	3 (5.2)	5 (9.4)	3 (4.5)
STEP 2 (Results after 137 weeks)						
Number of animals at start	90M + 90F	/	120M + 120F	/	/	/
Kidney adenocarcinomas						
Corrected number of animals [a]	66M + 82F		98M + 112F			
Males with tumours (%) [b]	0		**25 (25.5)** [c]			
Females with tumours (%) [b]	0		1 (0.9)			
Total animals with tumours (%) [b]	0		26 (12.4)			
Mammary tumours[b]						
Corrected number of animals [a]	85M + 89F		118M + 118F			
Males with tumours (%) [b]	0		1 (0.8)			
Females with tumours (%) [b]	1 (1.1)		**12 (10.2)** [c, f]			
Total animals with tumours (%) [b]	1 (0.6)		13 (5.5)			
Pulmonary tumours						
Corrected number of animals [a]	74M + 86F		113M + 113F			
Males with tumours (%) [b]	3 (4.0)		**16 (14.2)** [c, f]			
Females with tumours (%) [b]	3 (3.5)		**11 (9.3)** [d]			
Total animals with tumours (%) [b]	6 (3.7)		27 (11.7)			
Leukemia						
Corrected number of animals [a]	87M + 89F		120M + 119F			
Males with tumours (%) [b]	3 (3.4)		3 (2.5)			
Females with tumours (%) [b]	8 (7.9)		10 (8.4)			
Total animals with tumours (%) [b]	11 (5.7)		13 (5.4)			

[a] Alive animals when the first tumor was observed:
Kidney adenocarcinoma: 55 weeks; Mammary tumor: 25 weeks; Pulmonary adenoma: 36 weeks; Leukemia: 12 weeks
[b] The percentage are referred to the corrected number
[c] Statistically significant (exact fisher test, one-sided), $p \leq 0.05$ / [d] Statistically significant (exact fisher test, one-sided), $p \leq 0.01$
[e] Statistically significant (Logrank test), $p \leq 0.05$ / [f] Statistically significant (Logrank test), $p \leq 0.01$

1.5.2.3 [Maltoni (1977)]

Study reference:

Maltoni C., Cotti G., Morisi L. and Chieco P. 1977: Carcinogenicity bioassays of vinylidene chloride Research plan and early results (publication), Medicina Del Lavoro, 68(4) 241-262.

Maltoni C., Cotti G., and Chieco P. 1984: Chronic toxicity and carcinogenicity bioassays of Vinykidene chloride, Acta oncologica 5 (2).

Roberts S.M., Jordan K.E., Warren D.A., Britt J.K. and James R.C. 2002: Evaluation of the carcinogenicity of 1,1-dichloroethylene (vinylidene chloride) (review article or handbook), Regulatory Toxicology and Pharmacology, 35:44-55.

United States Environmental Protectin Agency 1985: Health assessment document for Vinylidene chloride; Final report EPA/600/8-83/031F

Detailed study summary and results:

Nota*:* Information below related to study in hamsters

Test type

A carcinogenicity study on VDC was conducted on hamsters exposed by inhalation.

Test substance

The test item as described in the publication was 1,1 dichloroethylene obtained from the Central Laboratory of Solvay (Bruxelles). The purity of he sample used in this study was > 99.9% (999.5 g/kg). The impurity were 1,2 dichloroethylene trans (0.4 g/kg), acetone (0.1 g/kg), methylene chloride (0.05 g/kg), mono and dichloroacetylene (0.02 g/kg) and paramethoxyphenol (as stabilizer at 200 ppm).

Test animals

The study was conducted on male and female Chinese hamsters, 28 weeks old at start. The source of the hamster is not specified in the data. They were housed 5/cage in polycarbonate cages with tops made of stainless steel wir. One group of 30 male and 30 female hamsters was exposed to 25 ppm (equivalent to 100mg/m3). A concurrent untreated group of 18 male and 17 female hamsters served as negative control group.

Administration/exposure

The chamber for inhalation exposure are built of stainless steel and glass. The control of VDC concentrations is carried out by gas chromatography. The treatment was conducted for 4 hours daily, 4-5 days/week and for 52 weeks. Following the 52-week exposure, animals were observed until spontaneous death (total duration 157 weeks).

Clinical evaluation and pathology

The animals are controlled weekly and weighted every 2 weeks during the period of treatment and monthly after the treatment is over. All the detectable gross pathological changes were recorded during the control. All the animals were kept under observation until spontaneous death. The study was completed in 157 weeks. A complete autopsy was carried out on each animal. Histological examinations were performed on the Zymbal glands, interscapular brown fat, salivary glands, tongue, lungs, river, kidneys, spleen, stomach, different segments of the intestine, bladder, brain, bone marrow (sternum) and any other organs with pathological lesions. Furthermore, cytological examinations were carried out on the bone marrow of the femur.

No information either on other examinations or statistical methods are available.

Results and discussion

No biologically significant changes were seen in mortality or body weight. There were no significant differences either in non-cancer effects in any organ or in tumor development between control and treated hamsters.

1.5.2.4 [Hong (1981)]

Study reference:

Hong C.B., Winston J.M., Thornburg L.P. and Lee C.C. 1981: Follow-up study on the carcinogenicity on vinyl chloride and vinylidene chloride in rats and mice : tumor incidence and mortality subsequent to exposure (publication), Journal of Toxicology and Environmental Health, 7:909-924.

Detailed study summary and results:

Nota*:* Information below related to study in rats

Test type

A carcinogenicity study on VDC was conducted on rats exposed by inhalation. The work reported in this publication was to evaluate the development and incidence of neoplastic changes during a 12-months post-exposure recovery period in rats exposed to VDC for various lengths of time (1, 3, 6 and 10 months).

Test substance

The test item as described in the publication was 1,1 dichloroethylene obtained from Aldrich Chemical Co. (Milwaukee, Wis.). The VDC was heated to 37°C to generate the vapour.

Test animals

The study was conducted on male and female CD rats from Charles River Laboratories (Wilmington, Mass), 12 months old at the initiation of the exposure. Pulverized or block laboratory animal food (Wayne Lab Blox) and water were given ad libidum except during exposure.

Administration/exposure

Four to sixteen rats of each sex were exposed to 55 ppm (220 mg/m3) for 6 hours daily, 5 days per week. A concurrent untreated group served as negative control group. Four exposure periods were tested: 1 month (4 rats per sex and per group), 3 and 6 months (8 animals per sex and per group) and 10 months (16 animal per sex for the control group, and 14 males and 16 females for the treated group). The inhalation exposure was in cubical stainless steel chambers. The VDC concentrations were controlled by gaz chromatography wih a flame ionization detector. At the end of the exposure period, all animals were removed from exposure chambers and maintained in their respective animal rooms for a 12-months follow-up observation period.

Clinical evaluation and pathology

All animals were observed throughout the study for adverse signs. Feed consumption was recorded weekly and body weight biweekly. Aortic blood from 4 males and 4 females of each group was collected at interim and final terminations. When moribund or at scheduled termination times, animals were sacrified and necropsied. Gross examinations were carefully performed on the entire animal, with special attention to mammary gland, lung, liver, spleen, kidney (organs showing tumor development in previous studies) and other tissues with pathological changes. These tissues were prepared for microscopic examination. All external and internal tumours were examined and identified histologically.

A one-tailed Fisher exact probability test was used to compare tumor incidence between control and exposed groups. No correction was made to ensure an overall significance level of 0.05 for the multiple simultaneous comparisons.

Results and discussion

A strong mortality was noticed in the group exposed for 10 months: 20/30 rats (67%) exposed died or were sacrified. One rat exposed to 220 mg/m3 for 1 month and one rat exposed for 6 months became moribund and were terminated. Hepatic hemangiosarcoma were observed in one male exposed to VDC for 6 month. Mammary gland fibroadenoma was noted in five females in the control group and five females exposed to VDC. The reported tumors incidence in animals exposed to 220 mg/m3 VDC was similar to that in controls.

Table 55: Deaths and early terminations of rats exposed to 220 mg/m3 of VDC vapour for up to 10 months

	Exposure for 1 month		Exposure for 3 months		Exposure for 6 months		Exposure for 10 months	
	Control group	Treated group	Control group	Treated group	Control group	Treated group	Control group	Treated group
Animals per group	**4M + 4F**	**4M + 4F**	**8M + 8F**	**8M + 8F**	**8M + 8F**	**8M + 8F**	**16M + 16F**	**14M + 16F**
Mortality during exposure period	0	0	0	0	0	0	2F	1M
Mortality during follow-up period								
0 – 3 months	0	0	0	0	0	0	1F	0
3 – 6 months	0	0	1F	0	1M	0	1M	3M/3F
6 – 9 months	0	1M	0	0	1M	0	3M/1F	7M/1F
9 – 12 months	0	0	3F	0	1F	1M	2M/3F	5F
Total	**0/8**	**1/8**	**4/16**	**0/16**	**3/16**	**1/16**	**13/32**	**20/30**

Table 56: Tumor incidences in rats exposed to 220 mg/m3 of VDC vapour for up to 10 months

	Exposure for 6 months		Exposure for 10 months		Cumulative incidence	
	Control group	Treated group	Control group	Treated group	Control group	Treated group
Animals per group	**20M + 20F**	**20M + 20F**	**16M + 16F**	**14M + 16F**	**36M + 36F**	**34M + 36F**
LIVER						
Neoplastic nodules	0	0	0	0	**0**	**0**
Hepatocellular carcinoma	0	0	1M	0	**1M**	**0**
Hemangiosarcoma	0	1M	0	0	**0**	**1M**
LUNG						
Bronchioalveolar tumor	0	0	0	0	**0**	**0**
hemangiosarcoma			0	0	**0**	**0**
MAMMARY GLAND						
Fibroadenoma	1F	1F	4F	4F	**5F**	**5F**
Adenocarcinoma/carcinoma	1F	0	0	0	**1F**	**0**
VARIOUS ORGANS						
Malignant lymphoma	0	0	0	0	**0**	**0**

The reported incidence of the tumours observed (hepatic hemangiosarcoma, mammary gland fibroadenoma) was similar to that in controls.

1.5.2.5 [Hong (1981)]

Study reference:

Hong C.B., Winston J.M., Thornburg L.P. and Lee C.C. 1981: Follow-up study on the carcinogenicity on

vinyl chloride and vinylidene chloride in rats and mice : tumor incidence and mortality subsequent to exposure (publication), Journal of Toxicology and Environmental Health, 7:909-924.

Detailed study summary and results:

Nota*:* Information below related to study in mice

Test type

A carcinogenicity study on 1,1-dichloroethylene was conducted on mice exposed by inhalation. The work reported in this publication is to evaluate the development and incidence of neoplastic changes during a 12-months post-exposure recovery period in mice exposed to VDC for various lengths of time (1, 3 and 6 months).

Test substance

The test item as described in the publication was 1,1 dichloroethylene obtained from Aldrich Chemical Co. (Milwaukee, Wis.). The VDC was heated to 37°C to generate the vapour.

Test animals

The study was conducted on male and female albino CD-1 mice from Charles River Laboratories (Wilmington, Mass), 12 months old at the initiation of the exposure. Pulverized or block laboratory animal food (Wayne Lab Blox) and water were given ad libidum except during exposure.

Administration/exposure

Eight to twelve mice of each sex were exposed to 55 ppm (220 mg/m3) for 6 hours daily, 5 days per week. A concurrent untreated group served as negative control group. Four exposure periods were tested: 1 and 3 months (16 mice per sex for the control group and 8 mice per sex for the treated group), and 6 months (28 animals per sex for the control group and 12 animals per sex for the treated group). The inhalation exposure was in cubical stainless steel chambers. The VDC concentrations were controlled by gaz chromatography wih a flame ionization detector. At the end of the exposure period, all animals were removed from exposure chambers and maintained in their respective animal rooms for a 12-months follow-up observation period.

Clinical evaluation and pathology

All animals were observed throughout the study for adverse signs. Feed consumption was recorded weekly and body weight biweekly. Aortic blood from 4 males and 4 females of each group was collected under anesthesia at interim and final terminations. When moribund or at scheduled termination times, animals were sacrified and necropsied. Gross examinations were carefully performed on the entire animal, with special attention to mammary gland, lung, liver, spleen, kidney (organs showing tumor development in previous

studies) and other tissues with pathological changes. These tissues were prepared for microscopic examination. All external and internal tumours were examined and identified histologically.

A one-tailed Fisher exact probability test was used to compare tumor incidence between control and exposed groups. No correction was made to ensure an overall significance level of 0.05 for the multiple simultaneous comparisons.

Results and discussion

A higher mortality was noticed in the group exposed for 6 months: a total of 11 of 24 (46%) mice exposed died or were sacrified before the end of the study. In the concurrent control group, the mortality represented 20% (11 of 56 mice died or were sacrified before the end of the study).

Hepatocellular tumors occurred in 4 of 28 (14 %) male mice exposed to VDC vapour (cumulative incidence) and in 10 of 60 (17%) male mice of the control group.

Bronchioloalveolar tumors were observed in 5 of 56 (9 %) male and female mice exposed to VDC vapour (cumulative incidence) and in 16 of 120 (13%) in male and female mice of the control group.

One male exposed for 3 months had hemangiosarcoma of the mesentery.

Table 57: Number of deaths of mice exposed to 220 mg/m3 of VDC vapour for up to 6 months

	Exposure for 1 month		**Exposure for 3 months**		**Exposure for 6 months**	
	Control group	**Treated group**	**Control group**	**Treated group**	**Control group**	**Treated group**
Animals per group	**16M + 16F**	**8M + 8F**	**16M + 16F**	**8M + 8F**	**28M + 28F**	**12M + 12F**
Mortality during exposure	0	0	0	0	0	0
Mortality during follow-up period						
0 – 3 months	0	0	0	1M	2M	1M/1F
3 – 6 months	0	0	2M	0	1M/1F	1M/1F
6 – 9 months	1F	1F	1M/1F	2M/1F	2M	1M
9 – 12 months	1M	0	1M/1F	1M	1M/4F	3M/3F
Total	**2/32**	**1/16**	**6/32**	**5/16**	**11/56**	**11/24**

Table 58: Tumor incidences in mice exposed to 220 mg/m3 of VDC vapour for 1, 3 and 6 months followed by a recovery period of 12 months

	Exposure for 1 month		**Exposure for 3 months**		**Exposure for 6 months**		**Cumulative incidence**	
	Control group	**Treated group**	**Control group**	**Treated group**	**Control group**	**Treated group**	**Control group**	**Treated group**
Animals per group	**16M + 16F**	**8M + 8F**	**16M + 16F**	**8M + 8F**	**28M + 28F**	**12M + 12F**	**60M + 60F**	**28M + 28F**
LIVER								
Hemangiosarcoma	0	0	0	0	1F	0	**1F**	**0**
Hepatocellular tumor	4M	3M	2M	0	4M/1F	1M	**10M/1F**	**4M**
LUNG								
Bronchioalveolar tumor	2M/1F	1M	2M	2M/1F	4M/7F	1M	**8M/8F**	**4M/1F**

Metastatic adenocarcinoma	0	0	0	0	0	0		**0**
MAMMARY GLAND								
Adenocarcinoma/carcinoma	1F	0	0	0	3F	0	**4F**	**0**
VARIOUS ORGANS								
Hemangiosarcoma of the mesentery	0	0	0	1M	0	0	**0**	**1M**

The reported incidence of the tumours observed (hepatocellular and bronchioalveolar tumors) were not higher in VDC-exposed group than in control group.

1.5.2.6 [Lee (1977)]

Study reference:

Lee C.C., Bhandari J.C., Winston J. M., House W. B., Peters P. J., Dixon R. L. and Woods J. S. 1977: Inhalation Toxicity of Vinyl Chloride and Vinylidene Chloride (publication), Environmental Health Perspectives, 21:25-32.

Lee C.C., Bhandari J.C., Winston J. M., House W. B., Peters P. J., Dixon R. L. and Woods J. S. 1978: Carcinogenicity of Vinyl Chloride and Vinylidene Chloride, Journal of Toxicology and Environmental health, 4:15-30.

Detailed study summary and results:

Nota*:* Information below related to study in rats

Test type

A carcinogenicity study on 1,1-dichloroethylene was conducted on rats exposed by inhalation.

Test substance

The test item as described in the publication was 1,1-dichloroethylene obtained from Aldrich Compagny, with a purity of 99%. VDC was heated to 37°C to generate the vapour.

Test animals

The study was conducted on male and female albino CD rats from Charles River Laboratories (Wilmington, Mass), 2 months old at the initiation of the exposure. All animals lived in the same stainless steel cages with wire bottoms during exposure and outside of the chambers. They were housed two per cage. Pulverized or block laboratory animal food (Wayne Lab Blox) and water were given ad libidum except during exposure.

Administration/exposure

Thirty six rats of each sex were exposed to 55 ppm (220 mg/m3) for 6 hours daily, 5 days per week and for 12 months. The concurrent untreated group was exposed to untreated air.

The inhalation chambers are cubical type and made of stainless steel, with a volume of 3.5 m3. One chamber was used for 55 ppm of VDC, and one chamber was used for uncontaminated air as control. Chamber air was initially sampled with a syringe and monitored using a gas chromatograph (Varian-2700) with a flame ionization detector. An automatic sampling system was later used. Periodically, a sample was directed to the gas chromatograph and the readout was processed by a Varian CDS II1 electronic integrator.

Clinical evaluation and pathology

All animals were observed throughout the study for adverse signs. Feed consumption was recorded weekly and body weight biweekly. Hematology analysis was performed on all samples. Prothrombin time, SGOT, alkaline phosphatase, bilirubin, creatinine, LDH, a-HBDH, immunoglobin IgA, IgB-A, IgB-B, and IgM, total protein, albumin, globulin, and collagen contents in liver and lung were also measured. Macrophage counts of pulmonary washings and cytogenic analysis of bone marrow cultures were performed on the control, and animals receiving VDC. Limbs from the longest exposed animals were examined for osteoporosis or malacia using a senograph x-ray machine. They were examined for the presence of any bone tumors, any changes in bone density, cortical thickness or striations within the bone cortex,any loss of bone cortex, or any unusually trabecular pattern of the bone. Gross examination was carefully performed on all tissues including the brain, pituitary, thyroids, respiratory tract, alimentary canal, urogenital organs, thymus, heart, liver, pancreas, spleen, mesenteric lymph nodes, and body cavities. The brain, liver, kidneys, spleen and gonads were removed and weighed. Tumors with adjacent normal tissues and the whole or portions of the various tissues were fixed, processed, sectioned, and stained for microscopic examination. All external and internal tumors were carefully examined and identified histologically.

Four animals of each sex, and exposure level were terminated for various laboratory tests, gross and histopathological examinations at the end of 1, 2, 3, 6, and 9 months; the surviving animals were terminated at the end of 12 months. When moribund or at scheduled termination times, animals were sacrified and necropsied.

A one-tailed Fisher exact probability test (Siegel, 1956) was used to compare tumor incidence between control and exposed groups.

Results and discussion

No remarkable adverse signs were seen in any rats during the first 7 months. One female rat exposed to VDC was terminated (rough hair coat, lost muscular tone, lethargic and lost weight. No death occurred in the control group. The body weights of the female rats exposed to VDC were generally less than that of the female controls after the 4th week. Those of the males exposed to VDC were generally less than that of the male controls after the 24th week. No persistent change was found in the following laboratory results of the

male and female rats VDC as compared to the controls: hematology, clinical blood chemistry, pulmonary macrophage count, cytogenic analysis of bone marrow culture, x-ray examination of extremities, collagen contents in liver and lung, serum ALA synthetase, urinary ALA level, and serum a-fetoprotein.
A mild to markedly severe focal, disseminated vacuolization, probably fatty change, was observed in livers of most of the rats treated with VDC. Two rats exposed to VDC developed hemangiosarcoma in the mesenteric lymph node or subcutaneous tissue. Hemangiosarcoma was not found in the liver, lung or any other organs of any control rats. A few other tumors occasionally occurred in one or several rats.

1.5.2.7 [Lee (1977)]

Study reference:

Lee C.C., Bhandari J.C., Winston J. M., House W. B., Peters P. J., Dixon R. L. and Woods J. S. 1977: Inhalation Toxicity of Vinyl Chloride and Vinylidene Chloride (publication), Environmental Health Perspectives, 21:25-32.

Lee C.C., Bhandari J.C., Winston J. M., House W. B., Peters P. J., Dixon R. L. and Woods J. S. 1978: Carcinogenicity of Vinyl Chloride and Vinylidene Chloride, Journal of Toxicology and Environmental health, 4:15-30.

Detailed study summary and results:

Nota*:* Information below related to study in mice

Test type

A carcinogenicity study on VDC was conducted on mice exposed by inhalation.

Test substance

The test item as described in the publication was 1,1-dichloroethylene obtained from Aldrich Compagny, with a purity of 99%. VDC was heated to 37°C to generate the vapour.

Test animals

The study was conducted on male and female albino CD-1 mice from Charles River Laboratories (Wilmington, Mass), 2 months old at the initiation of the exposure. All animals lived in the same stainless steel cages with wire bottoms during exposure and outside of the chambers. Mice were housed four to six per cage. Pulverized or block laboratory animal food (Wayne Lab Blox) and water were given ad libidum except during exposure.

Administration/exposure

Thirty six mice of each sex were exposed to 55 ppm (220 mg/m3) for 6 hours daily, 5 days per week and for 12 months. The concurrent untreated group was exposed to untreated air. The inhalation chambers are cubical type and made of stainless steel, with a volume of 3.5 m3. One chamber was used for 55 ppm of VDC, and one chamber was used for uncontaminated air as control. Chamber air was initially sampled with a syringe and monitored using a gas chromatograph (Varian-2700) with a flame ionization detector. An automatic sampling system was later used. Periodically, a sample was directed to the gas chromatograph and the readout was processed by a Varian CDS II1 electronic integrator.

Clinical evaluation and pathology

All animals were observed throughout the study for adverse signs. Feed consumption was recorded weekly and body weight biweekly. Hematology analysis and clinical blood chemistry (SGPT and BUN) were performed on all samples. Macrophage counts of pulmonary washings and cytogenic analysis of bone marrow cultures were performed on the control, and animals receiving VDC. Limbs from the longest exposed animals were examined for osteoporosis or malacia using a senograph x-ray machine. Gross examination, especially for any appearance of abnormal growth or other lesions, was carefully performed on all tissues including the brain, pituitary, thyroids, respiratory tract, alimentary canal, urogenital organs, thymus, heart, liver, pancreas, spleen, mesenteric lymph nodes, and body cavities. The brain, liver, kidneys, spleen and gonads were removed and weighed. Tumors with adjacent normal tissues and the whole or portions of the various tissues were fixed, processed, sectioned, and stained for microscopic examination.

All external and internal tumors were carefully examined and identified histologically. Four animals of each sex, and exposure level were terminated for various laboratory tests, gross and histopathologic examinations at the end of 1, 2, 3, 6, and 9 months; the surviving animals were terminated at the end of 12 months. When moribund or at scheduled termination times, animals were sacrified and necropsied.

A one-tailed Fisher exact probability test (Siegel, 1956) was used to compare tumor incidence between control and exposed groups.

Results and discussion

Two males exposed to VDC died on the 13th day. They were replaced with healthy mice from the same shipment for the remainder of the experiment. Microscopic examination of these unscheduled deaths revealed acute toxic hepatitis and marked tubular necrosis of the renal cortex. In the control group, two males died during the eighth and ninth months (one death was due to injury from fighting, the cause of the other death was unknown). Of the mice exposed to VDC, two males were terminated during the ninth month and one female during the 10th month. They all had tumors in the liver. The weight gains of the male and female mice exposed to VDC were comparable to those of the controls.

No persistent change was found in the laboratory results (hematology, clinical blood chemistry, cytogenic analysis of bone marrow cultures, x-ray examinations of extremities, and serum a-fetoprotein) of the male and female mice exposed to VDC as compared with those of the controls.

Several changes in the liver were noted in mice exposed for 6 to 12 months: liver enlarged and basophilic hepatocytes, enlarged nuclei with eosinophilic inclusions, mitotic figures or polyploidy, microfoci of mononuclear cells, focal degeneration and necrosis. The incidence and severity of these lesions progressed with the length of exposure. The significance of these lesions as related to hemangiosarcoma or other liver tumour may be of a "preneoplastic" nature. All the mice that died or were terminated ahead of schedule and many mice that were terminated on schedule at various times developed one or more types of tumors. A few small nodules of bronchiolo-alveolar adenoma occurred in six mice exposed to VDC. Hepatic hemangiosarcoma occurred in three mice exposed to VDC. Hepatic cell carcinoma, renal adenoma, or skin keratoacanthoma, was observed in one or two mice exposed to VDC.

1.5.2.8 [Rampy (1977); Quast (1986)]

Study reference:

Rampy L. W., Quast J. F., Humiston C. G., Balmer M. F. and Schwetz B. A. 1977: Interim Results of Two-Year Toxicological Studies in Rats of Vinylidene Chloride Incorporated in the Drinking Water or Administered by Repeated Inhalation (publication), Environmental Health Perspectives, 21:33-43.

Quast J.F., McKenna M.J., Rampy L.W. and Norris J.M. 1986: Chronic toxicity and oncogenicity study on inhaled vinylidene chloride in rats (publication), Fundamental and applied toxicology, 6:105-144.

Detailed study summary and results:

Test type

A carcinogenicity study on VDC was conducted on rats.

Test substance

The test item as described in the publication was VDC obtained from Dow Chemical company. The maximum concentration of the impurity MEHQ (mono methyl ether of hydroquinone) in test item vapour was estimated at 121 ppb. The authors concluded that this level was very low and it was unlikely that it contributes significantly to any toxicity seen in the study.

Test animals

The study was conducted on male and female Sprague-Dawley rats, 6 to 7 weeks old, and randomly placed (2/cage) into suspended wire bottom cages. Food and water were made available to the animals ad libitum.

Food and water were made available to the animals ad libitum, except during the exposure (including controls).

Administration/exposure

Three groups of 86 male and 86 female rats (one control and two treated groups) were exposed for 18 months to VDC vapour, 6 hours/day, 5 days/week and for 6, 12 and 18 months. Exposures were carried out in 3.7 m3 stainless steel chambers under dynamic flow conditions. For the first 5 weeks of exposure, the exposure levels were 10 or 40 ppm (40 or 159 mg/m3). As no effects were found in animals at interim kill, exposure levels were raised to 25 or 75 ppm (99 or 298 mg/m3) until the end of the exposure period (18 months). The surviving animals were then held without exposure until 24 months.

The VDC vapour concentrations were generated by metering liquid VDC into a temperature controlled vaporization flask. Chamber concentrations were monitored by infrared spectrophotometry. The mean measured concentration in chambers were: 38 ± 10 and 143.2 ± 24.8 mg/m3 for the first five weeks, and 101.6 ± 18.4 and 290.4 ± 30 mg/m3 for the weeks after.

Clinical evaluation and pathology

Animals were observed frequently for signs of toxicity. Food and water were not measured. Body weight were recorded weekly at first and then once per month. Analysis of hematologic parameters, clinical chemistry and urines were conducted. All rats that were found moribund or dead during the study were subjected to gross pathological examination. At terminal necropsy, the brain, heart, liver, kidneys, and testes were weighed. Microscopic examinations were generally conducted on the followings organs from controls rats and 75 ppm rats: accessory sex glands, adipose tissue, adrenals, aorta, bone marrow (sternal), brain, epididymes, esophagus, heart, intestines (large and small), kidneys, liver, lungs, lymph nodes (mesenteric, mediastinal), mammy gland, nasal turbinates, ovaries, oviduct, pancreas, parathyroid, peripheral nerve, pituitary gland, prostate, salivary glands, skeletal muscle, skin, eye, spleen, spinal cord, sternum, stomach, testes, thymus, thyroid gland, trachea, urinary bladder, uterus, and any gross lesion or mass.

Selected target organs and grossly recognized neoplastic changes in the rats receiving 25 ppm were also examined microscopically.

Statistical evaluation of body weight and organ weight data, and hematology and clinical chemistry determinations were made using an analysis of variance and Dunnett's test (Steel and Torrie, 1960).

All mortality and neoplasm incidence data were analyzed using the Fisher Exact Probability Test (Siegel, 1956). The level of significance in all cases was $p < 0.05$.

Results and discussion

No clinical signs were observed, no eye or nasal irritation or changes in appearence or behaviour were observed. The mortality was slightly higher in the 75 ppm group in the very last months of the study. For

female rats at 75 ppm, a trend towards increased cumulative percentage of mortality was noted during the 14th to the 24th months (statistically significant at months 15, 17 and 21). Mean body weights of male rats at 100 mg/m3 (25 ppm) significantly were lower than control for the first 13 months of exposure and remained lower but not systematically significant. Mean body weights of male rats at 300 mg/m3 (75 ppm) were significantly lower from 6th to 12th months. General trend toward decreased body weights from the 17th to 24th months. Mean body weights of female rats exposed to VDC were comparable to controls or slightly higher.

Some sporadic statistically significant differences in hematologic parameters or clinical chemistry between treated and control groups were observed, but they were not considered consistently enough to be related to test item.

Changes in organs weight were observed only at 12 months of the study. Male rats exposed to VDC had significantly lower absolute liver weights (Mean ± SD: 20.57 ± 1.51 g, 15.28 ± 2.10 g and 15.13 ± 2.06 g for the control, the 100 mg/m3 (25 ppm) and the 300 mg/m3 (75 ppm) groups, respectively) with a concomitant decrease in fasted body weights (Mean ± SD: 660 ± 44 g, 576 ± 48 g and 563 ± 41 g for the control, 100 mg/m3 and 300 mg/m3 groups, respectively). Higher absolute kidney weights were observed in females exposed to both levels of VDC (Mean ± SD: 2.09 ± 0.10 g, 2.44 ± 0.26 g and 2.60 ± 0.18 g for control, 100 mg/m3 and 300 mg/m3 groups, respectively). Since no similar change were observed at 6 or 24 months, the biological significance of these findings is questionable.

Table 59: Liver and Kidney weights in rats exposd to VDC by inhalation

Months on study	Exposure (ppm)	Number of rats	Body weight (g)	Liver [a]		Kidney [a]	
				g	g/100g	g	g/100
MALES							
6	0	5	553 (42)	15.22 (3.56)	2.73 (0.42)	3.77 (0.51)	0.68 (0.06)
	25	5	522 (29)	13.81 (1.86)	2.64 (0.22)	3.63 (0.36)	0.70 (0.03)
	75	5	520 (0.48)	13.23 (1.63)	2.54(0.18)	3.57 (0.52)	0.69 (0.06)
12	0	5	660 (44)	20.57 (1.51)	3.13 (0.4)	4.39 (0.51)	0.67 (0.12)
	25	5	**576 (48)***	**15.28 (2.10)***	2.65 (0.23)	3.94 (0.39)	0.69 (0.06)
	75	5	**563 (41)***	**15.13 (2.06)***	2.69 (0.29)	4.04 (0.51)	0.72 (0.09)
24	0	13	527 (78)	18.26 (1.95)	3.50 (0.37)	5.85 (1.08)	1.14 (0.30)
	25	13	495 (85)	16.92 (2.94)	3.44 (0.41)	5.33 (0.93)	1.10 (0.24)
	75	8	492 (61)	16.96 (2.54)	3.44 (0.24)	5.41 (0.78)	1.11 (0.16)
FEMALES							
6	0	5	327 (14)	7.25 (1.15)	2.22 (0.35)	2.03 (0.28)	0.62 (0.09)
	25	5	303 (50)	6.94 (1.27)	2.29 (0.20)	1.88 (0.31)	0.62 (0.05)
	75	5	327 (17)	7.47 (0.77)	2.28 (0.14)	2.06 (0.14)	0.63 (0.05)
12	0	5	329 (19)	7.45 (0.69)	2.26 (0.11)	2.09 (0.10)	0.63 (0.03)
	25	5	366 (26)	8.39 (1.51)	2.32 (0.55)	**2.44 (0.26)***	0.67 (0.11)
	75	5	340 (38)	9.04 (0.80)	2.67 (0.21)	**2.60 (0.18)***	0.77 (0.09)
24	0	21[b]	402 (95)	13.47 (4.17)	3.37 (0.76)	2.94 (0.49)	0.77 (0.23)
	25	13[b]	418 (109)	15.39 (3.77)	3.26 (0.51)	2.96 (0.63)	0.75 (0.24)
	75	16	404 (72)	13.99 (4.39)	3.47 (0.90)	3.10 (0.55)	0.80 (0.26)

[a] Mean ± SD

[b] Four female rats from either the control or 25-ppm group were unidentified at the terminal sacrifice. For completeness of the data, the organ weights of these were included two in each of the two groups.
***** Signifcantly different from control means by analysis of the variance and dunnett's test.

Gross and histologic examinations revealed intercurrent pneumonia (infection to Mycoplasma pulmonis). Although incidence of lesions of the respiratory tissues due to this infection was lower in the control than in the exposed groups, it was concluded that this infection was not the result of exposure to VDC.

Changes considered to be treatment related were limited to the liver. The histologic examination resulted in a minimal midzonal hepatocellular fatty change in both male and female at the 6th and 12th months and in female rats dying during the 18-month exposure period. In female rats, the fatty change was statistically significant ($P < 0.05$) only at the highest exposure. There was no indication of progression of this lesion in either severity or incidence. No unusual incidence of fatty changes were observed in male rats culled or dying during the study or in female or male rats at the terminal sacrifice or among the animals of the postexposure period. Since the hepatocellular mid-zonal fatty change was reversible and did not result in altered organ weight, clinical chemistry changes, or any obvious effect in liver function, this finding in liver is not considered a severe adverse effect.

Table 60: Incidence of mizonal hepatocellular fatty change in rats exposed to VDC by inhalation

Time of examination	Number of animals with fatty changes/Number of animals examined		
	Exposure levels (ppm)		
	0	25	75
At scheduled sacrifice			
6-month interim	0/5 M 0/5 F	1/5 M 2/5 F	4/5 M 4/5 F
12-month interim	0/5M 0/5 F	3/5M 5/5 F	5/5M 5/5 F
24-month interim	1/13 M 1/19 F	0/13 M 0/11 F	0/8 M 0/16 F
At death or following culling			
During 18-month exposure period	0/27 M 0/16 F	0/25 M 6/29F	1/27 M 7/20 F
During 6-month post-exposure period (but not including 24-month terminal sacrifice)	0/46 M 0/49 F	1/47 M 0/46 F	0/51 M 1/48 F

No treatement-related histopathology was observed either at the interim or terminal sarifices in the kidneys.
In males, the incidence of subcutaneous tumors of the mammary or midcervical region was similar in the three groups and within the range of the historical controls. The incidence of subcutaneous tumors in eye, ear, or head region was very similar among the groups. Subcutaneous tumors in the body or limb region was slightly higher in the treated groups but not enough to be suggestive of a treatment effect. Tumor or masse were seen in the liver in two rats at the 75 ppm level, but without a significant difference in frequency.

In females, a statistical increase in the incidence of mammary adenocarcinomas was observed at 25 ppm but not to 75 ppm. It should be noted that the incidence of these tumour in controls of this study were at the low end of the range of the historical data in the SD rats of the laboratory. The incidence of subcutaneous tumors in the body or limb region was quite comparable in all the groups. In the kidney, one tumor was noted in each of the treated groups. The total incidences of histopathological tumors, begnin or malignant were similar to the concurrent control group and the historical data.

Table 61: Incidence of tumors in female rats exposed to Vinylidene Chloride by inhalation

Tumours in female rats	0-6 months	7-12 months	13-18 months	19-24 months	Terminal kill	Cumulative results
MAMMARY GLANDS)						
Total number of animals necropsied during the period	1	1	14	49	19	84
Control	1	5	23	46	11	86
25 ppm	1	4	15	48	16	84
75 ppm						
Fibroadenoma/adenofibroma						
Control	0	0	17/10 [a]	78/39 [a]	37/15 [a]	132/64 [a]
25 ppm	0	2/2 [a]	40/20 [a]	76/38 [a]	16/8 [a]	134/68 [a]
75 ppm	1/1 [a]	6/4 [a]	17/14 [a]	100/40 [a]	33/15 [a]	157/74 [a]
Fibroma						
Control	0	0	0	0	0	0
25 ppm	0	0	0	0	0	0
75 ppm	0	0	0	1/1 [a]	1/1 [a]	2/2 [a]
Adenocarcinoma with pulmonary metastasis						
Control	0	0	0	1/1 [a]	0	1/1 [a]
25 ppm	0	0	0	0	0	0
75 ppm	0	0	0	0	0	0
Adenocarcinoma without metastasis						
Control	0	0	0	1/1 [a]	0	1/1 [a]
25 ppm	0	1/1 [a]	2/2 [a]	1/1 [a]	4/3 [a]	**8/7** [a,b]
75 ppm	0	0	2/2 [a]	0	2/2 [a]	4/4 [a]
Leimyosarcoma without metastasis						
Control	0	0	0	0	0	0
25 ppm	0	0	0	1/1 [a]	0	1/1 [a]
75 ppm	0	0	0	0	0	0
Carcinoma of the mammary gland						
Control	0	0	0	1/1 [a]	1/1 [a]	
25 ppm	0	0	0	0	0	
75 ppm	0	0	0	0	0	
Adenofibroma based on gross examination only	0	0	0	0	0	0
Control	0	0	0	0	0	0
25 ppm	0	0	0	1/1 [a]	0	1/1 [a]
75 ppm						
TOTAL INCIDENCE OF MAMMARY TUMORS IN FEMALES						2/2 [a,c]
Control						**8/7** [a,b]
25 ppm						4/4 [a]
75 ppm						
TOTAL NUMBER OF PRIMARY						

TUMORS IN FEMALES	0	1	25	149	73	248
Control	0	4	54	119	43	220
25 ppm	1	6	26	137	65	235
75 ppm						

[a] Number of tumors / number of animals with tumors
[b] Significantly different from control data when analysed using Fisher's exact probability test, $p < 0.05$
[c] Historical data of malignant tumors in Control female Sprague-Dawley rats:
Number of females rats with tumours in the control group / Number of females rats examined in the control group for each carcinogenicity study carried out in the laboratory: 1/85; 6/88; 8/86; 4/80; 13/92; 3/84; 6/80; 9/100 and 13/86.

1.5.2.9 [Cotti (1988)]

Study reference:

Cotti G., Maltoni C., And Giuseppe L. 1988: Long-Term Carcinogenicity Bioassay on Vinylidene Chloride Administered by Inhalation to Sprague-Dawley Rats. New Results. Annals of the New York Academy of Sciences 534(1), pp. 160-168.

Detailed study summary and results:

Test type

Long-term carcinogenicity bioassay of vinylidene chloride on rats. No guideline followed. GLP: not stated.

Test substance

Vinylidene chloride. Supplier: Solvay, Belgium. Puriry: 999.43 g/kg

Test animals

Male and female Sprague-Dawley rats, breeders 13 weeks old and embryos (12th day of gestation) at the start of experiment.

Administration/exposure

The animals were exposed by:

- Inhalation, 4-7 hours daily, 5 days weekly, for 104 weeks (breeders)
- Transplacental route and then inhalation, 4-7 hours daily. 5 days weekly for 104 weeks or 15 weeks (12-day embryos)

Table 62: Vinylidene Chloride: Plan of the Experiment and Inhalation Schedule in Sprague-Dawley Rats

Group No.	Concentration (ppm)	Schedule	Age	Sex	No at start
I	100	4 hd/5 dw/7 w and then 7 hd/5 dw/97 w	Breeders	F	54
II	0	/	Breeders	F	60
I	100	4 hd/5 dw/7 w and then 7 hd/5 dw/97 w	Embryos	M	62
				F	61
				M+F	123
II	100	4 hd/5 dw/7 w and then 7 hd/5 dw/8 w	Embryos	M	60
				F	60

				M+F	120
III	0	/	Embryos	M	158
				F	149
				M+F	307
Total				M	280
				F	384
				M+F	664

Clinical evaluation and pathology

All the animals were kept under observation until spontaneous death. The status and behavior of the animals were examincd 3 times daily. The animals were submitted to clinical examination for gross changes every 2 weeks. The animals were weighed every 2 weeks during treatment and then every 8 weeks. Full necropsy and histopathologic examination were performed on all the animals.

Systematic and standardized histopathologic examinations were performed in each animal on the subcutaneous lymph nodes, brain and cerebellum, Zymbal glands, interscapular brown fat, salivary glands, Harderian glands, tongue, thymus and mediastinal lymph nodes, lungs, diaphragm, liver, kidneys, adrenals, spleen, pancreas, esophagus, mesenteric lymph nodes, stomach, various segments of the intestine, bladder, uterus, gonads, sternum, and any other organ with pathologic lesions.

Statistical methods

No information

Results and discussion

Survival

The treatment with vinylidene chloride did not affect the survival rate of any of the exposed groups.

Body weight

In breeders and in male and female offspring treated with vinylidene chloride for 104 weeks, a slight decrease in body weight was observed. In offspring treated for 15 weeks, a slight body weight decrease was evident in male but not in female animals.

Table 63: Vinvlidene Chloride: Total Tumors in Rats

Group No	Sex	Percentage of animals bearing tumors		No of malignant tumors per 100 animals
		TBMT[a]	MT[b]	
I	F	68.5	20.4	22.2
II	F	58.3	15.0	16.7
I	M	62.9	32.2	33.9
	F	67.2	29.5	34.4
	M+F	65.0	30.9	34.1
II	M	58.3	21.7	23.3
	F	78.3	23.3	23.3
	M+F	68.3	22.5	23.3
III	M	44.9	17.1	18.3
	F	78.5	17.4	17.4
	M+F	61.2	17.3	17.9

[a]Total benign and malignant tumors.
[b]Malignant tumors.

Table 64: Vinylidene Chloride: Mammary Tumors, Leukemias, Pheochromocytomas and Pheochromoblastomas in Rats

Group No	Sex	Percentage of animals bearing tumors				
		Mammary tumors		Leukemias	Pheochromocytomas	Pheochromoblastomas
		BMT[a]	MT[b]			
I	F	53.7	7.4	1.8	7.4	1.8
II	F	40.0	3.3	3.3	18.3	/
I	M	11.3	/	16.1	30.6	/
	F	45.9	4.9	6.5	4.9	/
	M+F	28.4	2.4	11.4	17.9	/
II	M	5.0	/	13.3	28.3	1.7
	F	53.3	10.0	3.3	11.7	/
	M+F	29.2	5.0	8.3	20.0	0.8
III	M	7.0	1.9	7.6	22.8	0.6
	F	56.4	5.4	0.7	18.8	0.7
	M+F	30.9	3.6	4.2	20.8	0.6

[a]Benign and malignant tumors.
[b]Malignant tumors

Carcinogenicity

An increased percentage of rats bearing malignant tumors and an increased number of malignant tumors per 100 animals were found in offspring of both sexes treated with vinylidene chloride for 104 weeks. There was only limited evidence of a borderline increase in total malignant tumors in the other exposed groups, i.e., offspring treated for 15 weeks and breeders treated for 104 weeks. An increased incidence of leukemias was found in the vinylidene chloride exposed offspring; this increase was related to the length of treatment. No relevant differences were observed in the incidence of other tumors frequently occurring in untreated rats of the experimental unit colony. No unexpected tumors related to treatment were found.

1.5.2.10 [NTP (2015)]

Study reference:

National Toxicology Program 2015: Technical Report NTP TR 582; Toxicology and carcinogenesis of vinylidene chloride (CAS No. 75-35-4) in F344/N rats and B6C3F1/N mice (inhalation studies); National Institutes of Health, Public Health Service, U.S. Department on Health and Human Services.

Detailed study summary and results:

Nota*:* Information below related to study in rats

Test type

The protocol of the study was close to OECD guideline 451 (Carcinogenicity studies). The test described was designed and conducted to specifically characterize and evaluate the carcinogenic potential of vinylidene chloride as part of the US National Toxicological Program (NTP).

Test substance

Vinylidene chloride, manufactured by Dow Chemical Company (Freeport, TX), was obtained in one lot from Sigma-Aldrich. The material was identified as lot SB20019301. Analysis by GC/FID indicated that the test article was stabilized with approximately 300 ppm monomethyl ether hydroquinone (MEHQ) and that the overall purity was greater than 99.9%.

Test animals

The test was conducted on groups of 50 male and 50 female F344/N rats supplied by Taconic Farms, Inc. (Germatown, NY). Animals were 5 to 6 weeks old at the start of the study, and 109 to 111 weeks old at necropsy. They were housed individually. Diet and water were available ad libidum. The study started after a quarantine period of 12 days.

Administration/exposure

Rats were exposed to vinylidene chloride by whole body inhalation at 0, 25, 50 or 100 ppm in air for 6 hours/day, 5 days/week, and for 2 years. They were housed individually in exposure chambers.

The vinylidene chloride vapour was generated by pumping into a heated glass flask. Nitrogen entered the flask and assisted in vaporizing the chemical. Constant flows through the manifold and into all chambers was ensured. The inhalation exposure chambers (Harford Systems Division of Lab Products, Inc., Aberdeen, MD) were designed so that uniform vapor concentrations could be maintained throughout the chamber. A detector was used in the exposure chambers to ensure that vapour and not aerosol was produced. Chamber concentrations of vinylidene chloride was monitored by on-line gas chromatography. The average measured concentrations were in acceptable range: 25.0 ± 0.6 ppm, 50.1 ± 1.1 ppm and 100.0 ± 2.3 for the target concentrations of 25, 50 and 100 ppm respectively. No degradation of VDC was noted, nor impurity peaks with area greater than 0.1% of the total peak area were observed.

Clinical evaluation and pathology

Animals were observed twice daily. Body weights were recorded on day 1, weekly for the first 13 weeks, every 4 weeks through week 93, every 2 weeks thereafter, and at terminal kill. Clinical findings were recorded every 4 weeks through week 93, then every 2 weeks, and at terminal kill. No blood samples were collected either for haematology or clinical chemistry.

Complete gross examination and histopathology were performed on all rats. At necropsy, all organs and tissues were examined for grossly visible lesions. All major tissues (adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung, lymph nodes (mandibular, mesenteric, bronchial, and mediastinal), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland,

prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus) were prepared for microscopic examination.

A specific evaluation of renal proliferative lesions was conducted. Kidneys were step sectioned at 1 mm intervals from the residual cross sectional half of the right kidney and the longitudinal half of the left kidney of male rats, and four additional sections were obtained from each kidney.

The slides for microscopic evaluations, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The NTP PWG coordinator (consisting of the laboratory pathologist and other pathologists experienced in rodent toxicologic pathology) reviewed the selected tissues. This group examined the tissues without any knowledge of dose groups. Final diagnoses for reviewed lesions represented a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG.

Statistical methods

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958). Animals found dead of other than natural causes were censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

The Poly-k test was used to assess neoplasm and nonneoplastic lesion prevalence. This test modifies the Cochran-Armitage linear trend test to take survival differences into account. Unless otherwise specified, a value of k=3 was used. Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Reported P values are one sided.

Results and discussion

Survival in treated males groups are similar to survival in control group. For females, survival at 100 ppm was significantly lower than the control group.

Table 65: Survival rats (2-Year Inhalation Study of VDC)

	Control group	25 ppm	50 ppm	100 pm
MALE				
Animal initially in study	50	50	50	50
Early deaths:				
Moribond	21	15	23	27
Natural deaths	4	8	5	4
Animal surviving to study termination	25	27	22	19
Percent probability at end of study [a]	50	54	44	38
Mean survival (days) [b]	680	662	650	646
Survival analysis [c]	P=0.121	P=1.000	P=0.372	P=0.207
FEMALE				

Animal initially in study	50	50	50	50
Early deaths:				
Moribond	19	22	18	28
Natural deaths	1	2	2	3
Animal surviving to study termination	30	26	30 [d]	19
Percent probability at end of study [a]	60	52	58	38
Mean survival (days) [b]	705	681	678	675
Survival				
Survival analysis [c]	P=0.046	P=0.337	P=0.709	**P=0.029**

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal kill)
[c] Control group: result of the life table trend test (Tarone, 1975) / Treated groups: results of the life table pairwise comparisons (Cox, 1972)
[d] Includes one animal that died during the last week of the study

No clinical findings related to VDC exposure were observed in male rats; thinness was observed in approximately half of the 100 ppm females.
Mean body weights of treated groups of male and female rats showed similar mean body weight to those of the control groups throughout the study.

Table 66: Body weight in rats in grams (2-Year Inhalation Study of VDC)

	Average body weight (g)							
Weeks	**MALE**				**FEMALE**			
	Control group	**25 ppm (% of control)**	**50 ppm (% of control)**	**100 ppm (% of control)**	**Control group**	**25 ppm (% of control)**	**50 ppm (% of control)**	**100 ppm (% of control)**
1-13	231	233 (101)	231 (100)	230 (100)	157	157 (100)	155 (99)	152 (97)
14-52	428	432 (101)	423 (99)	425 (99)	254	257 (101)	253 (100)	250 (98)
53-103	516	520 (101)	500 (97)	505 (98)	354	361 (102)	355 (100)	344 (97)

At necropsy, fluid in the abdomen and multiple nodules on the peritoneum, particularly on the testicular tunics and epididymides, were grossly observed. According to the authors, these findings were associated with exposure to vinylidene chloride and resulted from the occurrence of mesothelioma.

Regarding histopathology, the statistically significant or biologically noteworthy findings were mainly changes in the incidences of malignant mesothelioma and mononuclear cell leukemia, and of neoplasms and/or non-neoplastic lesions of the thyroid gland, kidney, urinary bladder, nose, lung, liver, ovary, clitoral gland, and mesentery.

The incidences of malignant mesothelioma occurred with a positive trend and were significantly increased in all exposed groups of males. The peritoneal mesothelium covering the testis and epididymis was most often affected. One male exposed to 25 ppm also had mesotheliomas on the pleura and pericardium in addition to

the testicular and epididymal sites. In males, these neoplasms originated in the epididymis and testes, and disseminated throughout the peritoneum to multiple organs (intestines, mesentery, pancreas, prostate gland, spleen, and liver). Malignant mesothelioma occurred in one 25 ppm female (pleura, pericardium) and one exposed to 50 ppm (peritoneum); these incidences were greater than those in the control group and no malignant mesotheliomas have occurred in 700 females in the historical control database.

Global gene expression profiling of mesotheliomas arising in male rats exposed to VDC, compared to spontaneous mesotheliomas and to cultured rat mesothelial cells (Fred-PE cells), showed that mesotheliomas from VDC-exposed animals and control animals could be differentiated based on their genomic profiles, despite indistinguishable morphology. Mesotheliomas from VDC-exposed animals were distinguished from spontaneous mesotheliomas based on over-representation of genes associated with a proinflammatory response and immune dysregulation (alterations in pathways associated with proinflammatory cytokines and chemokines, and other various mediators or factors).

Table 67: Incidences of Malignant Mesothelioma in rats (2-Year Inhalation Study of VDC)

	Control group	25 ppm	50 ppm	100 ppm
MALE				
Malignant Mesothelioma (all organs) [a]				
Overall rate [b]	1/50 (2%)	12/50 (24%)	28/50 (56%)	23/50 (46%)
Adjuted rate [c]	2.4%	27.9%	63.4%	52.7%
Terminal rate [d]	0/25 (0%)	5/27 (19%)	10/22 (46%)	7/19 (37%)
First incidence (days)	562	535	500	449
Poly-3 test [e]	P<0.001	**P<0.001**	**P<0.001**	**P<0.001**
FEMALE				
Malignant Mesothelioma (all organs) [f]				
Overall rate [b]	0/50 (0%)	1/50 (2%)	1/50 (2%)	0/50 (0%)
Adjuted rate [c]	0.0%	2.4%	2.4%	0.0%
Terminal rate [d]	0/30 (0%)	1/26 (4%)	0/29 (0%)	0/19 (0%)
First incidence (days)	[g]	731 (T)	634	[g]
Poly-3 test [e]	[h]	[h]	[h]	[h]

(T) Terminal kill

[a] Male rats: historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 1/200 (0.5% ± 1.0%), range 0%-2%; all routes: 26/699 (3.7% ± 3.1%), range 0%-8%

[b] Number of animals with malignant mesothelioma per number of animals necropsied

[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[d] Observed incidence at terminal kill

[e] The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
Control group: P value is associated with the trend test
Exposed groups: P values correspond to pairwise comparisons between the control group and that exposed group.

[f] Female rats: historical incidence for inhalation studies: 0/200; all routes: 0/700

[g] Not applicable; no neoplasms in animal group

[h] Value of statistic not computed because all exposure groups have fewer than two neoplasms.

The incidence of C-cell adenoma was significantly increased in 100 ppm females, exceeded the historical control range for inhalation studies, and was at the upper end of the historical control range for all routes. Incidences of carcinoma were increased in all exposed groups of females, and this increase was statistically significant at 25 ppm. The incidences of carcinoma in all exposed groups of females exceeded the historical control range for inhalation studies, and the incidence in the 25 ppm group exceeded the historical control

range for all routes. The incidences of adenoma or carcinoma (combined) were significantly increased in 25 and 100 ppm females.

Table 68: Neoplasms of the Thyroid Gland (C-Cell) in female rats (2-Year Inhalation Study of 1,1-DCE)

FEMALE	Control group	25 ppm	50 ppm	100 ppm
Adenoma [a]				
Overall rate [b]	3/50 (6%)	4/50 (8%)	6/48 (13%)	11/50 (22%)
Adjusted rate [c]	6.6%	9.5%	14.6%	26.2%
Terminal rate [d]	3/30 (10%)	2/26 (8%)	4/28 (14%)	6/19 (32%)
First incidence (days)	731 (T)	625	579	669
Poly-3 test [e]	P=0.004	P=0.461	P=0.195	**P=0.012**
Carcinoma [f]				
Overall rate [b]	0/50 (0%)	6/50 (12%)	2/48 (4%)	2/50 (4%)
Adjuted rate [c]	0.0%	14.4%	4.9%	4.8%
Terminal rate [d]	0/30 (0%)	6/26 (23%)	1/28 (4%)	1/19 (5%)
First incidence (days)	[g]	731 (T)	670	670
Poly-3 test [e]	P=0.474	**P=0.011**	P=0.213	P=0.218
Adenoma or Carcinoma [h]				
Overall rate [b]	3/50 (6%)	10/50 (20%)	8/48 (17%)	13/50 (26%)
Adjuted rate [c]	6.6%	23.7%	19.3%	30.8%
Terminal rate [d]	3/30 (10%)	8/26 (31%)	5/28 (18%)	7/19 (37%)
First incidence (days)	731 (T)	625	579	669
Poly-3 test [e]	P=0.006	**P=0.023**	P=0.071	**P=0.003**

(T) Terminal kill

[a] Historical incidence for 2-year inhalation studies in control groups (mean ± standard deviation): 13/200 (6.5% ± 1.0%), range 6%-8%; all routes: 81/690 (11.7% ± 5.5%), range 6%-22%

[b] Number of animals with neoplasm per number of animals with thyroid gland examined microscopically

[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[d] Observed incidence at terminal kill

[e] The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
Control group: P value is associated with the trend test
Exposed groups: P values correspond to pairwise comparisons between the control group and that exposed group.

[f] Historical incidence for inhalation studies: 1/200 (0.5% ± 1.0%), range 0%-2%; all routes: 6/690 (0.9% ± 2.0%), range 0%-7%

[g] Not applicable; no neoplasms in animal group

[h] Historical incidence for inhalation studies: 14/200 (7.0% ± 1.2%), range 6%-8%; all routes: 87/690 (12.7% ± 5.8%), range 6%-22%

The incidence of mono-nuclear cell leukemia was significantly increased in 100 ppm females and exceeded the historical control ranges for inhalation studies and all routes of administration.

Table 69: Mononuclear Cell Leukemia in female rats (2-Year Inhalation Study of 1,1-DCE)

FEMALE	Control group	25 ppm	50 ppm	100 ppm
Mononuclear Cell Leukemia (all organs) [a]				
Overall rate [b]	10/50 (20%)	11/50 (8%)	13/50 (26%)	25/50 (50%)
Adjuted rate [c]	21.4%	24.6%	28.3%	54.6%
Terminal rate [d]	3/30 (10%)	4/26 (15%)	3/29 (10%)	8/19 (42%)
First incidence (days)	631	451	421	395
Poly-3 test [e]	P<0.001	P=0.457	P=0.300	**P<0.001**

[a] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 58/200 (29.0% ± 6.2%), range 20%-34%; all routes: 165/700 (23.6% ± 8.2%), range 10%-36%

[b] Number of animals with mononuclear cell leukemia per number necropsied

[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

[d] Observed incidence at terminal kill

[e] The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
Control group: P value is associated with the trend test
Exposed groups: P values correspond to pairwise comparisons between the control group and that exposed group.

In the standard evaluation of the kidney, two 25 ppm males, one 50 ppm male, and one 100 ppm male had renal tubule carcinomas. Although not statistically significant, the incidence in the 25 ppm group exceeded the historical control ranges for inhalation studies and all routes of administration.

There was a single incidence of renal tubule adenoma in 50 ppm females; no renal tubule adenomas have occurred in 692 females in the historical control database. Single incidences of renal tubule hyperplasia occurred in each exposed group of males, and slight increases in the incidences of this lesion occurred in 25 and 100 ppm females. This lesion did not occur in the concurrent control male group and was increased in severity in 100 ppm males. Hyperplasia of the transitional epithelium of the kidney occurred in one 50 ppm and two 100 ppm males.

Since there was evidence of a treatment-related effect in male rats, kidney step sections were performed for additional evaluation of proliferative lesions. The findings in male rats indicated increased incidences of renal tubule hyperplasia in all exposed groups. Several newly diagnosed incidences of renal tubule adenoma were observed in male rats, although there was not a significant difference between exposed animals and control groups. No additional carcinomas were observed as the result of step section review. The final combined incidences of renal tubule adenoma or carcinoma resulting from the kidney step section review indicated slightly increased incidences in 25 and 50 ppm male rats compared with control groups, but not in the 100 ppm group; one 25 ppm male had both an adenoma and a carcinoma.

Table 70: Incidences of neoplasms in kidneys in rats (2-Year Inhalation Study of VDC)

	Control group	25 ppm	50 ppm	100 ppm
MALE				
Standard Evaluation (single section)	50	50	49	50
Number of animals examined	0	1 (2.0)	1 (1.0)	1 (4.0)
Renal Tubule, Hyperplasia [a]	0	0	1 (3.0)	2 (1.5)
Transitional Epithelium, Hyperplasia [a]	0	2	1	1
Renal Tubule, Carcinoma [b]				
Extended evaluation (step sections)	50	50	49	50
Number of animals examined	3 (1.0)	5 (1.2)	5 (2.2)	7 (2.0)
Renal Tubule, Hyperplasia [a]	3	3	5	1
Renal Tubule, Adenoma [a]				
Combined evaluation (single and step sections)				
Number of animals examined	50	50	49	50
Renal Tubule, Hyperplasia [a]	3	5	6	8
Renal Tubule, Adenoma [a]	3	3	5	1
Renal tubule, Carcinoma [a]	0	2	1	1
Renal tubule Adenoma or Carcinoma				
Overall rate [c]	3/50 (6%)	4/50 (8%)	6/49 (12%)	2/50 (4%)
Adjuted rate [d]	7.2%	9.8%	15.7%	5.3%
Terminal rate [e]	3/25 (12%)	1/27 (4%)	4/22 (18%)	1/19 (5%)
First incidence (days)	729 (**T**)	631	502	718
Poly-3 test [f]	P=0.485N	P=0.484	P=0.194	P=0.546N

FEMALE				
Number of animals examined	50	50	49	50
Renal Tubule, Hyperplasia	1 (2.0)	2 (2.5)	0	2 (3.0)
Renal Tubule, Adenoma [g]	0	0	1	0

(T) Terminal kill
[a] Number of animals with lesion. The average severity grade of lesions is given in parentheses (1=minimal, 2=mild, 3=moderate, 4=marked)
[b] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 0/200; all routes: 1/697 (0.1% ± 0.5%), range 0%-2%
[c] Number of animals with neoplasm per number of animals with kidney examined microscopically
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in an exposure group is indicated by **N**.
Control group: P value is associated with the trend test
Exposed groups: P values correspond to pairwise comparisons between the control group and that exposed group.
[g] Historical incidence for inhalation studies: 0/199; all routes: 0/692

Carcinoma of the transitional epithelium occurred in two 25 ppm males; this incidence exceeded the historical control ranges for inhalation studies (0%; 0/199) and all routes of administration (0% to 2%; 1/698). Hyperplasia of the transitional epithelium of the urinary bladder occurred in one 50 ppm and two 100 ppm males. The biological significance of this neoplasm was judged uncertain by the authors, and the incidence of this neoplasm was not believed to be related to VDC exposure.

Table 71: Incidences of neoplasms in urinary system in rats (2-Year Inhalation Study of VDC)

	Control group	25 ppm	50 ppm	100 ppm
MALE				
Kidney				
Number of animals examined	50	50	49	50
Mesenchymal tumor malignant [a]	1 (2%)			1 (2%)
Renal tubule, carcinoma [a]		2 (4%)	1 (2%)	1 (2%)
Urinary system				
Number of animals examined	49	50	49	50
Transitional epithelium, carcinoma [a]		2 (4%)		
FEMALE				
Kidney				
Number of animals examined				
Mesenchymal tumor malignant [a]	50	50	50	50
Renal tubule, carcinoma [a]		1 (2%)		
		1 (2%)	1 (2%)	
Urinary system				
Number of animals examined	49	50	49	50
Transitional epithelium, carcinoma [a]		1 (2%)		

[a] Number of animals with lesion.

Adenoma of the respiratory epithelium was found in one 50 ppm and four 100 ppm male rats and one 100 ppm female rat. No respiratory epithelium adenomas have been seen in male historical controls. The incidence in 100 ppm females exceeded the historical control range for inhalation studies.

A variety of non-neoplastic lesions were observed in the nose of male and female rats.

- Turbinate atrophy, accompanied in most cases with turbinate hyperostosis, was found in every exposed male and female rats. The severity of both lesions increased with increasing exposure concentration, and were not observed in the control groups.
- Olfactory epithelium respiratory metaplasia occurred in most exposed rats with exposure concentration-related increases in severity. The incidences in 50 and 100 ppm males and in all exposed groups of females were significantly greater than those in the control groups.
- Olfactory epithelium squamous metaplasia was less commonly observed, and the incidence in the 100 ppm males was significantly increased.
- Respiratory epithelium hyperplasia occurred in male and female rats, and the incidences in 50 and 100 ppm males and in all exposed groups of females were significantly greater than those in the chamber controls.

Incidences of chronic active inflammation were significantly increased in all exposed groups of male and female rats, and the severities of the lesion increased with increasing exposure concentration. Thrombosis were sporadically observed in nasal vessels of male and female rats, and were significantly increased in 50 ppm males and 100 ppm females. Inflammatory polyp occurred in three 100 ppm females.

Table 72: Neoplasms and non-neoplastic lesions in nose and lung in rats (2-Year Inhalation Study of VDC)

	Control group	25 ppm	50 ppm	100 ppm
MALE				
Nose				
Number of animals examined	49	50	50	50
Turbinate, Atrophy [a]	0	50** (2.2)	50** (3.2)	50** (3.8)
Turbinate, Hyperostosis [a]	0	49** (2.1)	50** (2.6)	50** (2.9)
Olfactory Epithelium, Metaplasia, Respiratory [a]	3 (1.0)	49** (2.5)	49** (3.2)	48** (3.5)
Olfactory Epithelium, Metaplasia, Squamous [a]	0	0	1 (2.0)	5* (1.2)
Respiratory Epithelium, Hyperplasia [a]	5 (1.6)	8 (1.5)	22** (2.5)	31** (2.3)
Inflammation, Chronic Active [a]	9 (1.2)	36** (2.0)	45** (2.7)	48** (3.2)
Thrombosis [a]	4 (2.3)	4 (3.0)	11* (3.3)	7 (2.7)
Respiratory Epithelium, Adenoma [b]				
Overall rate [c]	0/49 (0%)	0/50 (0%)	1/50 (2%)	4/50 (8%)
Adjuted rate [d]	0.0%	0.0%	2.7%	10.5%
Terminal rate [e]	0/25 (0%)	0/27 (0%)	1/22 (5%)	3/19 (16%)
First incidence (days)	[g]	[g]	729 (**T**)	635
Poly-3 test [f]	p=0.004	[h]	p=0.483	p=0.051
Lung				
Number of animals examined	50	50	50	50
Alveolar Epithelium Hyperplasia [a]	7 (1.1)	18** (1.5)	14* (1.6)	14* (2.3)
FEMALE				
Nose				
Number of animals examined	50	50	50	50
Turbinate, Atrophy [a]	0	50** (2.8)	50** (3.3)	50** (4.0)
Turbinate, Hyperostosis [a]	0	50** (1.9)	50** (2.6)	50** (2.8)
Olfactory Epithelium, Metaplasia, Respiratory [a]	1 (1.0)	50** (2.8)	50** (3.1)	50** (3.6)
Respiratory Epithelium, Hyperplasia [a]	4 (1.3)	12* (1.6)	14** (1.7)	27** (2.1)
Inflammation, Chronic Active [a]	7 (1.4)	45** (1.8)	46** (2.0)	46** (2.9)
Thrombosis [a]	0	3 (2.3)	2 (2.0)	7 (2.3)
Polyp, Inflammatory [a]	0	0	0	3 (3.0)

Respiratory Epithelium, Adenoma [i]	0	0	0	1

***** Significantly different (P≤0.05) from the control group by the Poly-3 test
****** P≤0.01
(**T**) Terminal kill
[a] Number of animals with lesion. The average severity grade of lesions is given in parentheses (1=minimal, 2=mild, 3=moderate, 4=marked)
[b] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 0/198; all routes: 0/697
[c] Number of animals with neoplasm per number of animals with tissue examined microscopically
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
Control group: P value is associated with the trend test
Exposed groups: P values correspond to pairwise comparisons between the control group and that exposed group.
[g] Not applicable; no neoplasms in animal group
[h] Value of statistic cannot be computed.
i Historical incidence for inhalation studies: 0/200; all routes: 1/697 (0.1% ± 0.5%), range 0%-2%

In lung, the incidences of alveolar epithelium hyperplasia were significantly increased in all exposed groups of males, associated with an exposure concentration-related increase in severities.

In the liver, significantly increased incidences of chronic inflammation and of diffuse fatty change were observed in all exposed groups of rats, and its severity increased with increasing exposure concentration. Significantly increased incidences of necrosis occurred in the 50 ppm male and 50 and 100 ppm female rats. Incidences of cystic degeneration were significantly increased in the 100 ppm male and 50 and 100 ppm female rats.

Table 73: Non-neoplastics lesions of the liver in rats (2-Year Inhalation Study of VDC)

	Control group	25 ppm	50 ppm	100 ppm
MALE				
Number of animals examined	50	50	50	50
Inflammation Chronic [a]	28 (1.0)	46** (1.2)	46** (1.3)	44** (1.9)
Fatty Change, Diffuse [a]	4 (2.0)	19** (1.7)	18** (1.7)	26** (2.0)
Necrosis [a]	2 (2.5)	6 (2.8)	8* (2..6)	6 (2.3)
Degeneration, Cystic [a]	2 (2.0)	5 (2.8)	7 (1.9)	12** (2.1)
FEMALE				
Number of animals examined	50	50	50	50
Inflammation Chronic [a]	42 (1.0)	48* (1.4)	49** (1.4)	48** (2.1)
Fatty Change, Diffuse [a]	19 (1.2)	30* (1.7)	26 (1.7)	30** (2.0)
Necrosis [a]	0	3 (1.7)	5* (2.2)	11** (1.8)
Degeneration, Cystic [a]	0	2 (3.0)	4* (2.3)	7** (2.7)

* Significantly different (P≤0.05) from the chamber control group by the Poly-3 test
** P≤0.01
[a] Number of animals with lesion. The average severity grade of lesions is given in parentheses (1=minimal, 2=mild, 3=moderate, 4=marked).

In the genital system, incidences of bursa dilatation increased in an exposure concentration-related manner. Severities of bursa dilatation were increased in all exposed female groups. The biological relevance of this lesion in exposed rats is uncertain. The incidence of clitoral gland adenoma in 25 ppm females was greater than that in the control group and exceeded the historical control range for inhalation studies, but was within the historical control range for all routes of administration. In addition, the incidence of this neoplasm in the

concurrent control groups is the highest incidence in inhalation studies in the historical database. The incidence of clitoral gland carcinoma was increased in 100 ppm females. The biological significance of this neoplasm is uncertain; the incidence of this neoplasm was not believed to be related to test item exposure.

Table 74: Neoplastic and non-neoplastic lesions of the genital system in rats (2-Year Inhalation Study of VDC)

FEMALE	Control group	25 ppm	50 ppm	100 ppm
Ovary				
Number of animals examined	50	50	50	50
Cyst [a]			1 (2%)	1 (2%)
Bursa, dilatation [a]	5 (10%)	11 (22%)	17 (34%)	24 (48%)
Follicle, cyst [a]			1 (2%)	
Interstitial cell, hyperplasia [a]	1 (2%)			
Periovarian tissue, cyst			1 (2%)	
Clitoral gland				
Number of animals examined	47	48	45	48
Hyperplasia [a]		4 (8%)	1 (2%)	1 (2%)
Inflammation, chronic active [a]		1 (2%)		
Adenoma [a]	4 (9%)	8 (17%)	3 (7%)	4 (8%)
Carcinoma [a]	1 (2%)			5 (10%)

[a] Number of animals with lesion

In the mesentery, incidences of fat necrosis were prevalent in all exposed groups of female rats and severities of the lesion were unaffected by exposure concentration. Histologic evaluation of this lesion was only performed when gross lesions in the mesenteric fat were observed. The pathogenesis of this lesion and its biological significance are uncertain. However there is also a treatment- and exposure concentration-related increase in fat necrosis in the companion mouse study. Localized fat necrosis may be related to the inflammatory lesions in the liver.

Table 75: Neoplastic and non-neoplastic lesions of the mesentery in rats (2-Year Inhalation Study of VDC)

FEMALE	Control group	25 ppm	50 ppm	100 ppm
Mesentery				
Number of animals examined	13	20	23	24
Inflammation, chronic active [a]		1 (5%)		
Fat, hemorrhage [a]			1 (4%)	
Fat, necrosis [a]	13 (100%)	19 (95%)	22 (96%)	23 (96%)

[a] Number of animals with lesion

1.5.2.11 [NTP (2015)]

Study reference:

National Toxicology Program 2015: Technical Report NTP TR 582; Toxicology and carcinogenesis of vinylidene chloride (CAS No. 75-35-4) in F344/N rats and B6C3F1/N mice (inhalation studies); National Institutes of Health, Public Health Service, U.S. Department on Health and Human Services.

Detailed study summary and results:

Nota*:* Information below related to study in mice

Test type

The protocol of the study was close to OECD guideline 451 (Carcinogenicity studies). The test described was designed and conducted to specifically characterize and evaluate the carcinogenic potential of vinylidene chloride as part of the US National Toxicological Program (NTP).

Test substance

Vinylidene chloride, manufactured by Dow Chemical Company (Freeport, TX), was obtained in one lot from Sigma-Aldrich. The material was identified as lot SB20019301. Analysis by GC/FID indicated that the test article was stabilized with approximately 300 ppm monomethyl ether hydroquinone (MEHQ) and that the overall purity was greater than 99.9%.

Test animals

The test was conducted on groups of 50 male and 50 female B6C3F1/N mice from the NTP colony maintained at Taconic Farms, Inc. Animals were 5 to 6 weeks old at the start of the study, and 109 to 111 weeks old at necropsy. They were housed individually. Diet and water were available ad libidum. The study started after a quarantine period of 12 days.

Administration/exposure

Mice were exposed to VDC by whole body inhalation at 0, 6.25, 12.5 or 25 ppm in air for 6 hours/day, 5 days/week, and for 2 years. Animals were housed individually in exposure chambers.

The VDC vapour was generated by pumping into a heated glass flask. Nitrogen entered the flask and assisted in vaporizing the chemical. Constant flows through the manifold and into all chambers was ensured. The inhalation exposure chambers (Harford Systems Division of Lab Products, Inc., Aberdeen, MD) were designed so that uniform vapor concentrations could be maintained throughout the chamber. A detector was used in the exposure chambers to ensure that vapour and not aerosol was produced. Chamber concentrations of vinylidene chloride was monitored by on-line gas chromatography. The average measured concentrations were in acceptable range: 6.22 ± 0.16 ppm, 12.5 ± 0.3 ppm and 25.0 ± 0.4 for the target concentrations of 6.25, 12.5 and 25 ppm respectively. No degradation of VDC was noted, nor impurity peaks with area greater than 0.1% of the total peak area were observed.

Clinical evaluation and pathology

Animals were observed twice daily. Body weights were recorded regularly. Clinical findings were recorded every 4 weeks through week 93, then every 2 weeks, and at terminal kill. No blood samples were collected either for haematology or clinical chemistry. Complete gross examination and histopathology were performed on all mice. All major tissues (adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung, lymph nodes (mandibular, mesenteric, bronchial, and mediastinal), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus) were prepared for microscopic examination.

The slides for microscopic evaluations, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The NTP PWG coordinator (consisting of the laboratory pathologist and other pathologists experienced in rodent toxicologic pathology) reviewed the selected tissues. This group examined the tissues without any knowledge of dose groups. Final diagnoses for reviewed lesions represented a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG.

Statistical methods

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958). Animals found dead of other than natural causes were censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

The Poly-k test was used to assess neoplasm and nonneoplastic lesion prevalence. This test modifies the Cochran-Armitage linear trend test to take survival differences into account. Unless otherwise specified, a value of k=3 was used. Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Reported P values are one sided.

Results and discussion

Survival of 6.25 ppm males was significantly greater than that of the control group. Survival of 25 ppm males and 6.25 and 25 ppm females was significantly less than that of the control groups.

Table 76: Survival mice (2-Year Inhalation Study of 1,1-dichloroethylene)

	Control group	6.25 ppm	12.5 ppm	25 pm
MALE				
Animal initially in study	50	50	50	50
Early deaths:				
Moribond	12	5	14	19
Natural deaths	9	5	4	12

Animal surviving to study termination	29	40 [d]	32	19 [d]
Percent probability at end of study [a]	58	80	64	38
Mean survival (days) [b]	680	713	674	645
Survival analysis [c]	P=0.001	P=0.022N	P=0.791N	P=**0.038**
FEMALE				
Animal initially in study	50	50	50	50
Early deaths:				
Moribond	11	20	14	17
Natural deaths	3	5	6	9
Animal surviving to study termination	36	25	30	24 [d]
Percent probability at end of study [a]	72	50	60	48
Mean survival (days) [b]	687	667	688	653
Survival analysis [c]	P=0.064	**P=0.046**	P=0.399	**P=0.027**

[a] Kaplan-Meier determinations / [b] Mean of all deaths (uncensored, censored, and terminal kill)
[c] Control group: result of the life table trend test (Tarone, 1975) / Treated groups: results of the life table pairwise comparisons (Cox, 1972)
A lower mortality in an exposure group is indicated by **N**
[d] Includes one animal that died during the last week of the study

Exposure-related clinical findings were observed in 25 ppm males and included thinness and abnormal breathing. Exposure-related clinical findings observed in all exposed groups of females included abnormal breathing, thinness, and torso ventral mass.

Mean body weights of 12.5 and 25 ppm males were at least 10% less than those of the chamber control group after weeks 17 and 13, respectively. Mean body weights of 25 ppm females were at least 10% less after week 21, and 20% less for weeks 48 to 93 of the study.

Table 77: Body weight in mice in grams (2-Year Inhalation Study of VDC)

	Average body weight (g)							
	MALE				**FEMALE**			
Weeks	**Control group**	**6.25 ppm (% of control)**	**12.5 ppm (% of control)**	**25 ppm (% of control)**	**Control group**	**6.25 ppm (% of control)**	**12.5 ppm (% of control)**	**25 ppm (% of control)**
1-13	30.2	30 (99)	28.9 (96)	28.7 (95)	25.1	25.7 (102)	25.5 (101)	25.0 (99)
14-52	48.0	45.9 (96)	42.6 (89)	40.0 (83)	45.8	47.5 (104)	46.2 (101)	39.3 (86)
53-103	52.5	51.1 (97)	46.1 (88)	42.6 (81)	63.7	61.7 (97)	60.7 (95)	50.7 (80)

Gross lesions potentially related to VDC exposure were observed in the kidney of male mice. The lesions ranged from pale (1 mm cortical foci) to large (occasionally bilateral masses that often replaced normal renal parenchyma).

Regarding histopathology, the statistically significant or biologically noteworthy findings are mainly changes in the incidences of hemangioma and hemangiosarcoma and neoplasms and/or nonneoplastic lesions of the

kidney, liver, lung, small intestine, nose, mesentery, and uterus.

In the kidney, microscopic chemical-related lesions in males consisted of renal tubule hyperplasia, renal tubule adenoma, and renal tubule carcinoma. The incidences of adenoma, carcinoma, and adenoma or carcinoma (combined) were significantly increased in all exposed groups of males compared to those in the concurrent control group. These incidences exceeded the historical control ranges for inhalation studies, where none have occurred, and for all routes of administration. A renal tubule adenoma occurred in one 25 ppm female mouse, no such adenoma had been observed in 947 historical control male mice by all routes of exposure. No renal tubule neoplasms were observed in any other treated female mice, and no adenomas or carcinomas were observed in male or female control mice.

Similar to adenomas, most carcinomas occurred as solitary masses, but the incidences of bilateral carcinoma increased with increasing exposure concentration. In addition, three 12.5 ppm males had multiple carcinomas in one kidney, and four 12.5 ppm males had multiple carcinomas bilaterally.

The incidences of renal tubule hyperplasia were significantly increased in all exposed groups of males compared to that in the control groups.

The incidence of kidney cyst was significantly increased in 25 ppm males; this lesion also occurred in two females and one female in the 6.25 and 12.5 ppm groups, respectively.

The incidence of kidney nephropathy was significantly decreased in 12.5 ppm males (with a mild increase of severity).

Table 78: Neoplasms and Nonneoplastic Lesions of the Kidney in Mice (2-Year Inhalation Study of VDC)

	Control group	6.25 ppm	12.5 ppm	25 pm
MALE				
Number of animals examined	50	50	50	50
Renal Tubule, Hyperplasia [a]	0	8** (1.8)	22** (1.7)	16** (1.7)
Cyst [a]	1 (2.0)	1 (2.0)	5 (2.0)	7* (2.6)
Nephropathy [a]	44 (1.9)	46 (1.6)	37* (2.2)	44 (2.2)
Renal Tubule, Adenoma, Single [j]	0	5	15**	10**
Renal Tubule, Adenoma, Bilateral [j]	0	0	1	0
Renal Tubule, Adenoma, Multiple [j]	0	0	3	0
Renal Tubule, Adenoma (includes single, bilateral, and multiple) [b]				
Overall rate [c]	0/50 (0%)	5/50 (10%)	19/50 (38%)	10/50 (20%)
Adjuted rate [d]	0.0%	10.6%	44.2%	26.7%
Terminal rate [e]	0/29 (0%)	5/40 (13%)	15/32 (47%)	8/19 (42%)
First incidence (days)	[g]	729 (**T**)	600	525
Poly-3 test [f]	p<0.001	p=0.041	P<0.001	P<0.001
Renal Tubule, Carcinoma, Single [j]	0	6	17**	12**
Renal Tubule, Carcinoma, Bilateral [j]	0	1	7*	6*
Renal Tubule, Carcinoma, Multiple [j]	0	0	3	0
Renal Tubule, Carcinoma, Multiple Bilateral [j]	0	0	4	0
Renal Tubule, Carcinoma (includes single, bilateral, and multiple) [h]				

Overall rate [c]	0/50 (0%)	7/50 (14%)	31/50 (62%)	18/50 (36%)
Adjuted rate [d]	0.0%	14.7%	70.5%)	45.8%
Terminal rate [e]	0/29 (0%)	5/40 (13%)	24/32 (75%)	10/19 (53%)
First incidence (days)	[g]	619	429	537
Poly-3 test [f]	P<0.001	P=0.012	P<0.001	P<0.001
Renal Tubule, Adenoma or Carcinoma [i]				
Overall rate [c]	0/50 (0%)	11/50 (22%)	37/50 (74%)	27/50 (54%)
Adjuted rate [d]	0.0%	23.1%	81.9%	67.0%
Terminal rate [e]	0/29 (0%)	9/40 (23%)	27/32 (84%)	17/19 (90%)
First incidence (days)	[g]	619	429	525
Poly-3 test [f]	P<0.001	P<0.001	P<0.001	P<0.001
FEMALE				
Number of animals examined	50	50	50	50
Renal tubule, Adenoma [j]	0	0	0	1 (2%)
Cyst [j]	0	2 (4%)	1 (2%)	0
Nephropathy [j]	26 (52%)	28 (56%)	38 (76%)	35 (70%)

* Significantly different (P≤0.05) from the control group by the Poly-3 test / ** P≤0.01
(T) Terminal kill
a Number of animals with lesion.
The average severity grade of lesions is given in parentheses (1=minimal, 2=mild, 3=moderate, 4=marked)
b Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 0/298; all routes: 8/944 (0.9% ± 1.4%), range 0%-4%
c Number of animals with neoplasm per number of animals with kidney examined microscopically
d Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
e Observed incidence at terminal kill
f The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
Control group: P value is associated with the trend test
Exposed groups: P values correspond to pairwise comparisons between the control group and that exposed group.
g Not applicable; no neoplasms in animal group
h Historical incidence for inhalation studies: 0/298; all routes: 3/944 (0.3% ± 1.0%), range 0%-4%
i Historical incidence for inhalation studies: 0/298; all routes: 11/944 (1.2% ± 1.8%), range 0%-6%
j Number of animals with lesion.

In the liver, the incidence of hepatocellular adenoma (including multiple) was significantly increased in 12.5 ppm female mice compared to control group. The incidence of hepatocellular carcinoma (including multiple) was significantly increased in 25 ppm females. When combined, the incidences of hepatocellular adenoma or carcinoma were significantly increased in 12.5 and 25 ppm females. The incidences of hepatocellular adenoma in 12.5 and 25 ppm females and hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined) in all exposed female groups exceeded the historical control ranges for inhalation studies, but were within the historical control ranges for all routes of administration.

The incidences of hepatocholangiocarcinoma in exposed groups of males were higher than in the concurrent control groups, and exceeded the historical control range for inhalation studies but not that for all routes of administration. In females, hepatocholangiocarcinoma occurred in all exposed groups; this neoplasm has not been seen in 300 inhalation controls or 948 controls from all routes of study.

The incidences of hepatocellular adenoma (including multiple) were decreased in males in an exposure concentration-dependent fashion, and the incidences of hepatocellular carcinoma (including multiple) were decreased in 6.25 and 12.5 ppm males. The incidence of basophilic focus of the liver was significantly increased in 25 ppm males.

Table 79: Neoplasms and Nonneoplastic Lesions of the Liver in Mice (2-Year Inhalation Study of VDC)

	Control group	6.25 ppm	12.5 ppm	25 pm
MALE				
Number of animals examined	50	50	50	50
Basphilic focus [a]	2	4	2	7*
Hepatocellular adenoma multiple [a]	20	19	17	13
Hepatocellular adenoma (including multiple)				
Overall rate [b]	37/50 (74%)	35/50 (70%)	33/50 (66%)	25/50 (50%)
Adjuted rate [c]	77.6%	72.5%	73.8%	60.0%
Terminal rate [d]	21/29 (72%)	31/40 (78%)	25/32 (78%)	12/19 (63%)
First incidence (days)	443	619	429	471
Poly-3 test [e]	P=0.040**N**	P=0.361**N**	P=0.422**N**	P=0.046**N**
Hepatocellular carcinoma multiple [a]	8	1	4	8
Hepatocellular carcinoma (including multiple)				
Overall rate [b]	26/50 (52%)	19/50 (74%)	15/50 (30%)	29/50 (58%)
Adjuted rate [c]	55.0%	38.1%	33.2%	64.4%
Terminal rate [d]	11/29 (38%)	11/40 (28%)	7/32 (22%)	10/19 (53%)
First incidence (days)	443	521	508	425
Poly-3 test [e]	P=0.118	P=0.070**N**	P=0.026**N**	P=0.234
hepatocholangiocarcinoma [a, f]	1	2	2	3
FEMALE				
Number of animals examined	50	50	50	50
Hepatocellular adenoma multiple [a]	12	9	26*	17
Hepatocellular adenoma (including multiple) [g]				
Overall rate [b]	25/50 (50%)	21/50 (42%)	36/50 (72%)	29/50 (58%)
Adjuted rate [c]	55.3%	49.0%	77.6%	69.0%
Terminal rate [d]	20/36 (56%)	13/25 (52%)	25/30 (83%)	19/24 (79%)
First incidence (days)	509	471	524	443
Poly-3 test [e]	P=0.026	P=0.347**N**	P=0.015	P=0.126
Hepatocellular carcinoma multiple [a]	1	2	2	3
Hepatocellular carcinoma (including multiple) [h]				
Overall rate [b]	8/50 (16%)	14/50 (28%)	12/50 (24%)	17/50 (34%)
Adjuted rate [c]	18.2%	32.4%	27.2%	41.3%
Terminal rate [d]	6/36 (17%)	4/25 (16%)	8/30 (27%)	9/24 (38%)
First incidence (days)	611	478	611	415
Poly-3 test [e]	P=0.022	P=0.097	P=0.223	P=0.015
Hepatocellular adenoma or carcinoma [i]				
Overall rate [b]	28/50 (50%)	30/50 (60%)	37/50 (74%)	38/50 (76%)
Adjuted rate [c]	61.5%	65.4%	79.3%	84.4%
Terminal rate [d]	22/36 (61%)	14/25 (56%)	25/30 (83%)	21/24 (88%)
First incidence (days)	509	471	524	415
Poly-3 test [e]	P=0.003	P=0.434	P=0.041	P=0.009
hepatocholangiocarcinoma [a, j]	0	1	1	2

***** Significantly different (P≤0.05) from the chamber control group by the Poly-3 test / [a] Number of animals with lesion

[b] Number of animals with neoplasm per number of animals with liver examined microscopically

[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality / [d] Observed incidence at terminal kill

[e] Control grops: P value associated with the trend test / Exposed groups: P values corresponding to pairwise comparisons between the control group and exposed group.
The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
A negative trend or a lower incidence in an exposure group is indicated by **N**.

[f] Historical incidence for 2-year inhalation studies with control groups (mean ± standard deviation): 2/299 (0.7% ± 1.0%), range 0%-2%; all routes: 10/949 (1.1% ± 2.2%), range 0%-8%
[g] Historical incidence for inhalation studies: 105/300 (35.0% ± 8.8%), range 28%-50%; all routes: 378/948 (39.9% ± 18.7%), range 14%-78%
[h] Historical incidence for inhalation studies: 44/300 (14.7% ± 5.0%), range 8%-20%; all routes: 152/948 (16.0% ± 10.6%), range 4%-46%
[i] Historical incidence for inhalation studies: 133/300 (44.3% ± 8.6%), range 32%-56%; all routes: 448/948 (47.3% ± 19.3%), range 20%-82%
[j] Historical incidence for inhalation studies: 0/300; all routes: 0/948

The incidences of hemangioma in all exposed groups of females were increased compared to the concurrent control groups, and these incidences exceeded the historical control ranges for inhalation studies and all routes of administration. This neoplasm occurred in the liver, ovary, and uterus of exposed females and in the liver, bone marrow, and testes of exposed males. When all organs were combined, the incidence of hemangiosarcoma in 25 ppm females was greater than that in the concurrent control group and exceeded the historical control ranges for inhalation studies and all routes of administration. Incidences of hemangiosarcoma in female mice were primarily driven by the incidences of this neoplasm in the liver, in which the incidence of hemangiosarcoma in the 25 ppm group was significantly greater than that in the concurrent control group.

Hemangiosarcomas occurred in the liver, spleen, mediastinal lymph node, and skeletal muscle of males and females, in the bone marrow, lung, kidney, and thymus of males, and mesentery, ovary, uterus, urinary bladder, and in subcutaneous skin tissues of females. When all organs were combined, the incidence of hemangioma or hemangiosarcoma (combined) in 25 ppm females was significantly greater than that in the concurrent control group.

Table 80: Hemangioma and Hemangiosarcoma in female mice (2-Year Inhalation Study of VDC)

FEMALES	Control group	6.25 ppm	12.5 ppm	25 pm
LIVER [a]	50	50	50	50
Hemangioma [b]	0	1	0	2
Hemangiosarcoma				
Overall rate [c]	1/50 (2%)	1/50 (2%)	1/50 (2%)	6/50 (12%)
Adjuted rate [d]	2.3%	2.5%	2.3%	15.2%
Terminal rate [e]	1/36 (3%)	1/25 (4%)	1/30 (3%)	3/24 (13%)
First incidence (days)	731 (T)	731 (T)	731 (T)	508
Poly-3 test [f]	P=0.007	P=0.740	P=0.758	P=0.041
ALL ORGANS [a]	50	50	50	50
Hemangioma [g]	0	2	2	2
Hemangiosarcoma [h]	4	4	4	9
Hemangiosarcoma				
Overall rate [j]	4/50 (8%)	4/50 (8%)	4/50 (8%)	9/50 (18%)
Adjuted rate [d]	9.2%	9.9%	9.2%	22.5%
Terminal rate [e]	4/36 (11%)	3/25 (12%)	2/30 (7%)	5/24 (21%)
First incidence (days)	731 (T)	471	620	508
Poly-3 test [f]	P=0.044	P=0.603	P=0.643	P=0.084
Hemangioma or hemangiosarcoma [i]	4/50 (8%)	6/50 (12%)	6/50 (12%)	11/50 (22%)

Overall rate [j]	9.2%	14.9%	13.9%	27.5%
Adjuted rate [d]	4/36 (11%)	4/25 (16%)	4/30 (13%)	7/24 (29%)
Terminal rate [e]	731 (T)	471	620	508
First incidence (days)	P=0.018	P=0.324	P=0.368	P=0.027
Poly-3 test [f]				

(T) Terminal kill
[a] Number of animals examined microscopically or number necropsied (all organs)
[b] Number of animals with neoplasm
[c] Number of animals with neoplasm per number of animals examined microscopically
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] Control group: P value associated with the trend test / Exposed groups: P values corresponding to pairwise comparisons between the control grops and that exposed group.
The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[g] Historical incidence for 2-year inhalation studies with control groups (mean ± standard deviation): 0/300; all routes: 5/950 (0.5% ± 0.9%), range 0%-2%
[h] Historical incidence for inhalation studies: 21/300 (7.0% ± 2.1%), range 4%-10%; all routes: 50/950 (5.3% ± 3.9%), range 0%-12%
[i] Historical incidence for inhalation studies: 21/300 (7.0% ± 2.1%), range 4%-10%; all routes: 55/950 (5.8% ± 3.7%), range 2%-14%
[j] Number of animals with neoplasm per number of animals necropsied

In the lung, the incidence of alveolar/bronchiolar carcinoma (including multiple) in 12.5 ppm females was significantly increased and exceeded the historical control range for inhalation studies.

There were slight increases in the incidences of alveolar epithelium hyperplasia in the lung of exposed groups of males. However, there were no increased incidences of lung neoplasms in any exposed groups of males, despite the significantly increased incidence of alveolar/bronchiolar carcinoma in 12.5 ppm females.

Table 81: Neoplasms and Nonneoplastic Lesions of the Lung in Mice (2-Year Inhalation Study of VDC)

	Control group	6.25 ppm	12.5 ppm	25 pm
MALE				
Number of animals examined	50	50	50	50
Alveolar Epithelium Hyperplasia [a]	3 (1.3)	7 (1.3)	4 (1.8)	6 (2.3)
FEMALE				
Number of animals examined	50	50	50	49
Alveolar/bronchiolar Adenoma	2	4	2	2
Alveolar/bronchiolar Adenoma, Multiple	1	0	0	0
Alveolar/bronchiolar Adenoma (includes multiple)	3	4	2	2
Alveolar/bronchiolar Carcinoma	1	2	6	5
Alveolar/bronchiolar Carcinoma, Multiple	0	0	1	0
Alveolar/bronchiolar Carcinoma (includes multiple) [b]				
Overall rate [c]	1/50 (2%)	2/50 (4%)	7/50 (14%)	5/49 (10%)
Adjuted rate [d]	2.3%	4.9%	16.1%	12.7%
Terminal rate [e]	1/36 (3%)	0/25 (0%)	6/30 (20%)	1/24 (4%)
First incidence (days)	731 (T)	558	392	502
Poly-3 test [f]	P=0.038	P=0.477	P=0.030	P=0.080
Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate [c]	4/50 (8%)	5/50 (10%)	9/50 (18%)	7/49 (14%)
Adjuted rate [d]	9.2%	12.1%	20.6%	17.5%
Terminal rate [e]	4/36 (11%)	1/25 (4%)	8/30 (27%)	2/24 (8%)
First incidence (days)	731 (T)	478	392	502
Poly-3 test [f]	P=0.141	P=0.472	P=0.115	P=0.216

[a] Number of animals with lesion / The average severity grade of lesions is given in parentheses (1=minimal, 2=mild, 3=moderate, 4=marked)
[b] Historical incidence for 2-year inhalation studies with chamber control groups (mean ± standard deviation): 13/299 (4.4% ± 4.3%), range 0%-10%; all routes: 38/949 (4.0% ± 3.6%), range 0%-14%

[c] Number of animals with neoplasm per number of animals with lung examined microscopically
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] Control groups: P value associated with the trend test / Exposed groups: P values corresponding to pairwise comparisons between the control groups and that exposed group.
The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.

In the small intestine, carcinoma of the duodenum occurred in two 25 ppm males. One carcinoma occurred in the ileum of a 6.25 ppm male. Carcinomas of the duodenum or ileum have not been reported in the six inhalation studies included in the 2013 historical control database. Carcinoma also occurred in the jejunum of two 6.25 ppm males. Adenoma occurred in the ileum of a control male and in the duodenum of one 12.5 ppm male. The overall combined incidences of small intestine (duodenum, jejunum, or ileum) carcinoma in males were within the historical control ranges for inhalation studies and all routes of administration.
One adenoma occurred in the duodenum of a 12.5 ppm female, one adenoma and three carcinomas occurred in the ileum of 25 ppm females, and one ileum carcinoma occurred in a 6.25 ppm and a 12.5 ppm female. In 25 ppm females, the incidence of carcinomas of the ileum exceeded the historical control ranges for inhalation studies and all routes of administration as did the incidence of adenoma or carcinoma in all small intestine sites.

Table 82: Neoplasms of the Small Intestine in Mice (2-Year Inhalation Study of VDC)

	Control group	6.25 ppm	12.5 ppm	25 pm
MALE				
Small Intestine (Duodenum, Jejunum, or Ileum), Adenoma or carcinoma [a]				
Overall rate [b]	1/50 (2%)	3/50 (6%)	1/50 (2%)	2/50 (4%)
Adjuted rate [c]	2.4%	6.4%	2.4%	5.4%
Terminal rate [d]	1/29 (3%)	3/40 (8%)	1/32 (3%)	1/19 (5%)
First incidence (days)	729 (T)	729 (T)	729 (T)	563
Poly-3 test [e]	P=0.463	P=0.348	P=0.758	P=0.455
FEMALE				
Small Intestine (Duodenum, Jejunum, or Ileum), Adenoma or carcinoma [f]				
Overall rate [b]				
Adjuted rate [c]	2/50 (4%)	1/50 (2%)	2/50 (4%)	4/50 (8%)
Terminal rate [d]	4.6%	2.5%	4.6%	10.4%
First incidence (days)	1/36 (3%)	0/25 (0%)	1/30 (3%)	3/24 (13%)
Poly-3 test e	599	584	536	640
	P=0.141	P=0.531N	P=0.691	P=0.279

(T) Terminal kill
a Historical incidence for inhalation studies: 10/300 (3.3% ± 2.7%), range 0%-8%; all routes: 31/950 (3.3% ± 2.3%), range 0%-8%
b Number of animals with neoplasm per number of animals necropsied
c Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
d Observed incidence at terminal kill
e Control group: P value associated with the trend test / Exposed groups: P values corresponding to pairwise comparisons between the control groups and that exposed group.
The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
A lower incidence in an exposure group is indicated by **N**.
f Historical incidence for inhalation studies (includes jejunum): 4/300 (1.3% ± 1.6%), range 0%-4%; all routes: 10/950 (1.1% ± 1.4%), range 0%-4%

In the nose, exposure-related nonneoplastic lesions occurring included turbinate atrophy, hyperostosis, olfactory epithelium respiratory metaplasia, and olfactory epithelium hyaline droplet accumulation. Turbinate atrophy occurred in the vast majority of male and female mice exposed to VDC and the severity of the lesion increased with increasing exposure concentration. Incidences of hyperostosis increased in an exposure concentration-related fashion, and occurred in most male and female mice in the 12.5 and 25 ppm groups. Incidences of respiratory metaplasia in the olfactory epithelium were increased in all exposed groups of males and females, with exposure concentration-related increases in severities. The incidences of olfactory epithelium hyaline droplet accumulation were increased in all exposed groups of males and in 25 ppm females; the increases were significant in 12.5 ppm males and 25 ppm males and females. The incidence of respiratory epithelium hyperplasia was significantly increased in 25 ppm females.

Table 83: Non-Neoplastic lesions of the nose in Mice (2-Year Inhalation Study of VDC)

	Control group	6.25 ppm	12.5 ppm	25 pm
MALE				
Number Examined Microscopically	50	50	49	49
Turbinate, Atrophy [a]	0	46** (1.1)	46** (2.1)	47** (2.8)
Hyperostosis [a]	1 (2.0)	27** (1.3)	45** (2.1)	48** (2.2)
Olfactory Epithelium, Metaplasia, Respiratory [a]	17 (1.2)	39 ** (1.2	47 ** (1.6)	48** (1.8)
Olfactory Epithelium, Accumulation, Hyaline Droplet [a]	2 (1.0)	5 (1.0)	13** (1.3)	11** (1.3)
FEMALE				
Number Examined Microscopically	50	50	50	50
Turbinate, Atrophy [a]	0	46** (1.1)	50** (2.3)	49** (2.8)
Hyperostosis [a]	0	13** (1.2)	45** (2.0)	48** (2.2)
Olfactory Epithelium, Metaplasia, Respiratory [a]	3 (1.0)	29** (1.1)	49** (1.6)	50** (1.9)
Olfactory Epithelium, Accumulation, Hyaline Droplet [a]	18 (1.6)	18 (1.5)	13 (1.4)	32** (1.8)
Respiratory Epithelium, Hyperplasia [a]	33 (1.1)	41 (1.2)	39 (1.5)	43** (1.8)

****** Significantly different (P≤0.01) from the chamber control group by the Poly-3 test

[a] Number of animals with lesion; the average severity grade of lesions is given in parenthese (1=minimal, 2=mild, 3=moderate, 4=marked)

In the mesentery, increased incidences of fat necrosis of the mesentery occurred in exposed groups of females. Because this lesion was microscopically examined only in cases in which a gross lesion was observed in the mesenteric fat at the time of necropsy, the true incidence of this lesion is uncertain. There were also treatment- and exposure concentration-related increases in the incidences of fat necrosis in the companion rat study.

Table 84: Non-Neoplastics lesions of Mesentery in Mice (2-Year Inhalation Study of VDC)

	Control group	6.25 ppm	12.5 ppm	25 pm
MALE				
MESENTERY				
Number of animals examined [a]	6	9	6	3
Fat necrosis [b]	6 (100%)	8 (89%)	6 (100%)	1 (33%)
FEMALE				
MESENTERY				
Number of animals examined [a]	10	16	19	37

Fat necrosis [b]	8 (80%)	14 (88%)	15 (79%)	33 (89%)

[a] Number of animals examined microscopically at the site
[b] Number of animals with lesion

In the uterus, significantly increased incidences of uterus endometrium cystic hyperplasia occurred in all exposed groups of females. This is a common background lesion in aged mice, and its biologic relevance in this study is uncertain. The incidences of this lesion were not considered to be related to vinylidene chloride exposure.

Table 85: Non-Neoplastics lesions of Uterus in female Mice (2-Year Inhalation Study of VDC)

	Control group	6.25 ppm	12.5 ppm	25 pm
FEMALE				
MESENTERY				
Number of animals examined [a]	50	49	50	50
Endometrium, hyperplasia, cystic [b]	36 (72%)	41 (84%)	46 (92%)	46 (92%)

[a] Number of animals examined microscopically at the site
[b] Number of animals with lesion

1.5.3 Animal data by dermal exposure

1.5.3.1 [Van Duuren (1979)]

Study reference:

Van Duuren B.L., Goldschmidt B.M., Loewengart G., Smith A.C., Melchionne S., Seidman I. and Roth D. 1979: Carcinogenicity of Halogenated Olefinic and Aliphatic Hydrocarbons in Mice (publication), Journal of National Cancer Institute, 63(6).

Detailed study summary and results:

Test type

A carcinogenicity study on VDC was conducted on mice by dermal exposure. Three types of dermal exposure were tested: dermal initiation-promotion assay (tumor promoter phorbol myristate acetate applied on dorsal skin 14 days after the substance), repeated dermal applications (3 times/week) and weekly subcutaneous injections assay.

Test substance

The test item as described in the publication was 1,1-dichloroethylene obtained from Aldrich Chemical Co., Milwaukee, Wis. Purity checked by nuclear magnetic resonance 5.46(s)

Test animals

The study was conducted on male and female Ha:ICR Swiss mice (ARS PragueDawley, Madison, Wis.), 6-8 weeks old at the initiation of the study. Animals were housed 6 to a cage on sterile hardwood chips (Betta-Chip, Fisher & Son, Sound Brook, N.J.) in stainless-steel 11X7X8-inch cages. They fed diet (Purina Laboratory Chow) and water ad libitum.

Administration/exposure

Mice were initially shaved and whenever necessary. The substance was applied on dorsal skin using micropipette. Treatment was administered in ventilated hoods and mice housed there at least 2h.

Experimental groups consisted of 30 animals except for the control groups (untreated groups or those which received only phorbol myristate acetate (PMA)) which consisted of 90 or 100 animals.

Three types of dermal exposure were tested :

(1): In the first experiment, "initiation-promotion" experiment, VDC was administered at the dose levels of 121 mg/application/mouse, once only in 0.2 mL acetone, followed 14 days later by 5µg of PMA (used as promoter) in 0.2 mL acetone three times a week. Two concurrent PMA control groups were treated at the dose levels of 0.0025 mg/mouse (120 mice) and 0.0050 mg/mouse (90 mice). Another group of mice received treatment with 20 µg 7,12-dimethylbenz[a]anthracene (DMBA) plus TPA (30 mice) and served as positive control group. The duration of the test ranged from 428 to 576 days.

(2): In the second experiment, the test item was applied at the dose levels of 40 or 121 mg/application three times a week to the dorsal skin in acetone by micropipette. Two concurrent control groups were added: treated with only the vehicle acetone (30 mice) or without any treatment (100 mice). The duration of the test ranged from 440 to 594 days.

(3): In the third experiment, the mice were given weekly the dose level of 2.0 mg/injection/mouse, by subcutaneous injection in the left flank with a stainless-steel needle. The test compound was injected as 0.05 mL solution in trioctanoin as vehicle. Two concurrent control groups was treated with only the vehicle trioctanoin (30 mice) or received no treatment (100 mice). The duration of the test was 548 days.

Clinical evaluation and pathology

All animals were examined daily and weighed monthly. Skin lesions were diagnosed as papillomas when they reached approximately 1 mm and persisted for 30 days or more. Animals in poor health or with large tumor masses were killed. Except for the cranial region, animals were completely autopsied at the termination of the experiment or at death. Specimens of all abnormal-appearing tissues and organs were excised for histopathologic diagnosis.

For repeated skin applications, routine sections were taken of skin, liver, stomach, and kidney. For subcutaneous injections, tissues at the injection site and from the liver were taken. All tissue sections were fixed processed for histopathologic diagnosis.

The significant values P for tumor occurrence was calculated using the chi-square analysis and only P-value less than 0.05 were recorded in tables.

Results and discussion

The survival of all animals was normal, except for the control groups (PMA-treated groups), for which median survival times were shorter than in the test groups due to higher tumours incidence (median survival times ranged from 428 to 576 days for the different groups and was only 376 days for the PMA-treated groups).

In the initiation-promotion assay, a significant increase ($P < 0.005$) was observed in skin papillomas (nine in eight mice). In the repeated dermal application assay, no sarcomas were observed at the treatment site. Although 19 mice in the high-dose group and 12 in the low-dose group had lung tumours and 2 mice in the high-dose group had stomach tumours, the tumour incidence at both sites was not significantly different from controls (30 lung tumours and 5 stomach tumours). In the subcutaneous injection assay, after 548 days on test, none of the animals injected with VDC developed sarcomas at the injection site.

Table 86: Tumours in Mice exposed by dermal route to VDC (as initiating agent or after repeated application)

Compound	Initiation promotion test [a]			Repeated application [b]			
	Dose (mg/application/ mouse)	Days to first tumour	Mice with papillomas [c] /total papillomas	Dose (mg/application/ mouse)	Days to first tumour	Mice with papillomas /total papillomas	Number of mice with distant tumors [d]
VDC	121	271	8/9 (1) **P<0.005**	121	-	0	19 lung 2 stomach
				40	-	0	12 lung 0 stomach
PMA controls 120 mice 90 mice	0.0025 0.0050	141 449	9/10 (1) 6/7 (2)	-	-	-	-
Acetone 0.1 mL	-	-	-	0.1 mL	-	0	11 lung 2 stomach
No treatment 100 mice	-	-	-	-	-	0	30 lung 5 stomach

[a] Duration and median survival times were 428 to 576 days. The median survival time for positive controls (PMA) was 376 days.
[b] Duration was 440 – 594 days and median survival times were 317 to more than 589 days.
[c] Number of mice with squamous cell carcinoma are given in parentheses.
[d] All lung tumors are begnin papillomas; stomach tumours are papillomas of the forestomach and squamous cell carcinomas of the forestomach.
P values are given only when significant

Table 87: Incidence of tumours in Mice exposed by subcutaneous injections to VDC

Compound	Dose [a]	Days on test	Number of mice with local sarcomas [b]	P [c]
VDC	2.0 mg/injection.mouse	548	0	-
Trioctanoin	0.05 mL/injection/mouse	631	0	-

Untreated group (10 mice)	-	649	0	-

[a] Compound injected in left flank, once weekly, with 0.05 mL trioctanoin as vehicle.

[b] Sections of liver and injection sites were taken from autopsy for histopathologic examination.

No significant difference between the incidence of distant tumors in treated animals compared with no-treated and vehicle control groups was noted. When tested as initiating agent with PMA as promoter, VDC caused significant incidences of skin tumors. When tested by repeated applications, either by dermal application or subcutaneous injections, VDC did not induce skin papillomas or carcinomas. It can be concluded that VDC showed skin tumor-initiating activity with PMA as promoter but was inactive for mouse skin when applied repeatedly and by subcutaneous injection.

1.6 Specific target organ toxicity – repeated exposure

1.6.1 Animal data by oral route

1.6.1.1 [Quast (1983)]

Study reference:

Quast J.F., Humiston C.G., Wade C.E., Ballard J., Beyer J.E., Schwetz R.W. and Norris J.M 1983: A chronic toxicity and oncogenicity study in rats and subchronic toxicity study in dogs on ingested vinylidene chloride (publication), Fundamental and applied toxicology 1983, 3:55-62.

Rampy L. W., Quast J. F., Humiston C. G., Balmer M. F. and Schwetz B. A. 1977: Interim Results of Two-Year Toxicological Studies in Rats of Vinylidene Chloride Incorporated in the Drinking Water or Administered by Repeated Inhalation (publication), Environmental Health Perspectives, 21:33-43.

Detailed study summary and results:

Test type

A carcinogenicity study was conducted on rats by oral route for 2 years with a design was similar to OECD guideline 451. The animals were exposed to the target concentrations of 50, 100 or 200 ppm of VDC in drinking water (equivalent to 7, 10 and 20 mg/kg bw/day for the males and 9, 14 and 30 mg/kg bw/day).

For more details about the description and results of the study, see section Carcinogenicity, 1.5.1.3.

Conclusion

There were no significant differences between the groups of rats ingesting VDC and the control groups. The only adverse effect observed in the rats, evident only upon microscopic examination, was in the liver. An increase of hepatocellular fatty change in both sexes, in the females at all dose levels and in the males only at the 200 ppm level, was considered to be related to VDC. No exposure-related neoplastic changes occurred in the rats in any of the test groups.

1.6.1.2 [NTP (1982)]

Study reference:

National Toxicology Program 1982: Technical Report Series No. 228, Carcinogenesis Bioassay of Vinylidene Chloride (CAS No. 75-35-4) in F344 Rats and B6C3F1 Mice (Gavage Study); U.S. Department of health and human services. Public Health Service. National Institute of Health.

Detailed study summary and results:

Nota: Information below related to study in rats

Test type

A carcinogenicity study was conducted on vinylidene chloride. Rats were exposed to the substance by oral route (gavage) at dose levels of 1 or 5 mg/kg and for 2 years.

At the time of study period (1977), no international guidelines for carcinogenicity study were available. The study described was designed and conducted to specifically characterize and evaluate the carcinogenic potential of vinylidene chloride as part of the US National Toxicological Program (NTP). The design is close to the OECD Guideline 451 (Carcinogenicity studies).

For more details about the description and results of the study, see section Carcinogenicity, 1.5.1.4.

Conclusion

The only finding observed in this assay was the incidence increase of the chronic inflammation of the kidney in both male and female rats. This lesion is common in aging rats. A high mortality occurred in the control and low dose groups due to management problems.

1.6.1.3 [NTP (1982)]

Study reference:

National Toxicology Program 1982: Technical Report Series No. 228, Carcinogenesis Bioassay of Vinylidene Chloride (CAS No. 75-35-4) in F344 Rats and B6C3F1 Mice (Gavage Study); U.S. Department of health and human services. Public Health Service. National Institute of Health.

Detailed study summary and results:

Test type

A 90-day subchronic study was conducted to determine the doses of vinylidene chloride to be used in a 2-year chronic study. Five groups of 10 rats and 10 mice of either sex were administered VDC in corn oil by gavage for 13 weeks. The study was equivalent to OECD guideline 408 (Repeated Dose 90-Day Oral Toxicity in Rodents), however no international guideline was available at the time of the study (1976). It was part of the US National Toxicological Program (NTP).

The study is well described, but some deviations are noted. No data on ophtalmological and haematological examination are available.

Test substance

The test substance as cited in the report was vinylidene chloride, manufactured by Dow Chemical company (Freeport, TX). The lot identification used in this study was UTLX83844 with a minimum purity of 99%.

The results of purity and identity analyses were performed at Midwest Research Institute (Appendix E) and at Gulf South Research Institute. Three minor impurities were identified in the lot No. UTLX83844 by vapor-phase chromatography and mass spectrometry: trans-dichloroethylene (0.1%); cis-dichloroethylene (less than 0.1%); and a stabilizer, the monomethylether of hydroquinone (MEHQ), present at a concentration of 0.05%. It should be noted that 1,1-Dichloroethane, 1,2-dichloroethane, trichloroethylene, and vinyl chloride were not detected; if present, the concentrations were less than 0.1%, 0.04%, 0.1%, and 0.01%, respectively. The stability of vinylidene chloride in corn oil was determined at Midwest Research Institute by analyzing methanol extracts of vinylidene chloride - corn oil mixtures by vapor-phase chromatography.

Test animals

The test was conducted on four-week-old male and female F344/N rats and B6C3F1/1 mice obtained from NCI Fredrick Cancer Research Center (Frederick, MD). Animals were were observed for 5 weeks because of the presence of parasites or other diseases. They were housed five per cage (polybocarbonate cages), and all in the same room, without any other chemical tested in that room. Diet and water were available ad libidum.

Administration/exposure

Groups of 10 rats and 10 mice of either sex were exposed to vinylidene chloride in corn oil by gavage 5 times per week at 0, 5, 15, 40, 100, or 250 mg/kg body weight for 13 weeks. Vehicle control groups consisting of 10 males and 10 females of each species received corn oil alone. Rats received a dose volume of 5 ml/kg and mice received 10 ml/kg at the desired concentration to reach the tested dose level in rats.

After 90 days, the rats and mice were killed and necropsied. Mortality and morbidity were checked twice daily and animals were weighed every 2 weeks. Animals that were moribund and those that survived to the

end of the study were killed and necropsied. Gross and microscopic examinations were performed on major tissues, major organs, and all gross lesions from killed animals and from animals found dead unless precluded in whole or in part by autolysis or cannibalization. Representative tissues (skin, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, heart, salivary gland, liver, pancreas, stomach, small intestine, large intestine, kidneys, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate and seminal vesicles or uterus, testis or ovary, brain, thymus, larynx, and esophagus) from rats and mice receiving 250 mg/kg, from mice receiving 100 mg/kg, and from control animals were examined microscopically. Livers from all other dosed groups were also examined.

Results and discussion

Three female rats receiving 250 mg/kg died during the first week. Weight gain was depressed 20% for male rats receiving 250 mg/kg compared with controls.

All male mice receiving 250 mg/kg died within 24 hours; 9/10 females receiving 250 mg/kg died within 48 hours. Deaths occurred in 1/10 females receiving 5 mg/kg; 1/10 females receiving 15 mg/kg; 1/10 males receiving 40 mg/kg; and 2/10 males and 3/10 females receiving 100 mg/kg. A dose-related decrease in mean body weight gain was observed for male mice.

Table 88: Survival and mean body weights of Rats and Mice exposed to VDC by gavage for 13 weeks

		RAT		MICE	
Sex	**Dose (mg/kg)**	**Survival** [(a)]	**Weight change relative to controls (%)** [(b)]	**Survival** [(a)]	**Weight change relative to controls (%)** [(b)]
Male	**0**	10/10	/	10/10	/
	5	10/10	- 3	10/10	0
	15	10/10	- 4	10/10	**- 7**
	40	10/10	- 8	9/10	**- 14**
	100	10/10	- 8	8/10	**- 36**
	250	10/10	**-20**	0/10	/
Female	**0**	10/10	/	10/10	/
	5	10/10	- 5	9/10	- 33
	15	10/10	0	9/10	- 17
	40	10/10	- 8	10/10	- 17
	100	10/10	- 6	7/10	- 17
	250	7/10	- 11	1/10	0

(a): Number surviving / number per group

(a): Weight Change Relative to Controls =
{[Weight Change (Dosed Group) - Weight Change (Control Group)] / Weight Change (Control Group)} x 100

Table 89: Incidence of liver lesions in Rats and Mice exposed to VDC by gavage for 13 weeks

Species	**Dose (mg/kg)**	**Controls**		**5**		**15**		**40**		**100**		**250**	
	Sex	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
	Nb. Examined	9	10	10	10	10	10	10	10	10	10	10	10

RAT	**Centrilobular necrosis**	0	0	0	0	0	0	0	0	0	0	0	**3**
	Fibrosis, pigmentation, bile duct hyperplasia, hepatocellular atrophy	0	0	0	0	0	0	0	0	**3**	**4**	**10**	**7**
	Fatty metaporphosis	**4**	**0**	**7**	**5**	**7**	**7**	**8**	**3**	**2**	**2**	**0**	**1**
	Hepatocytomegaly	0	0	0	0	0	0	**1**	0	**6**	**3**	**10**	**7**
	Foci of cytoplasmic change (cellular alteration)	0	0	0	0	0	0	0	0	0	0	**3**	**3**
MICE	**Nb. Examined**	10	10	10	10	10	10	10	10	10	10	10	10
	Cellular necrosis	0	0	0	0	0	0	0	0	**2**	**2**	**5**	**5**
	Congestion	0	0	0	0	0	0	**2**	0	0	0	**8**	**1**
	Necrosis, NOS	0	0	0	0	0	0	0	0	0	0	2	0
	Cellular atypia	0	0	0	0	0	0	**2**	0	**7**	**6**	0	0
	Fatty metamorphosis	0	0	0	**1**	**2**	0	**2**	**2**	0	0	0	0
	Focal area of cellular alteration	0	0	**1**	0	0	0	0	0	0	0	0	0

Only the liver showed effects attributed to VDC.

In rats, severe centrilobular necrosis of the liver was observed in the 3 females that died. Hepatocytomegaly was seen in the rest of the rats that received 250 mg/kg, and in some male and female rats at 100 mg/kg. Various combinations of portal and subcapsular fibrosis, bile duct hyperplasia, pigmented macrophages, and hepatocellular atrophy were seen in all male rats and in female rats receiving 250 mg/kg. The rats receiving 100 mg/kg were affected to a much lesser degree (both in numbers and in severity). Foci of cytoplasmic change were seen in rats receiving 250 mg/kg. Fatty metamorphosis and/or cytoplasmic vacuolization occurred in the animals of most groups but had no distinct dose relationship.

Original examination of the lesions in the rat livers placed more emphasis on the changes seen in the rats at the lower doses whereas a review interpreted these changes as being much less important. Thus, the doses of 1 and 5 mg/kg bw, which were selected for the chronic study and were originally based on hepatotoxic effects, were probably too low.

In mice, centrilobular necrosis and congestion of the liver were observed in the males and females that died in the 250 mg/kg dose groups. Cellular atypia of the liver was observed in 7/10 males and 6/10 females receiving 100 mg/kg but not in animals receiving 250 mg/kg. At 40 mg/kg and below, severity of hepatic lesions was dose related. The incidence of hepatic lesions in males was higher than that in females. The most frequently encountered change in mice exposed to 40 mg/kg or less was slight or moderate fatty metamorphosis.

Hepatic effects were considered to be minimal at 5 mg/kg, and doses selected for the mice for the chronic study were 2 and 10 mg/kg body weight.

1.6.1.4 [NTP (1982)]

Study reference:

National Toxicology Program 1982: Technical Report Series No. 228, Carcinogenesis Bioassay of Vinylidene Chloride (CAS No. 75-35-4) in F344 Rats and B6C3F1 Mice (Gavage Study); U.S. Department of health and human services. Public Health Service. National Institute of Health.

Detailed study summary and results:

Nota: Information below related to study in mice

Test type

A carcinogenicity study was conducted on vinylidene chloride. Mice were exposed to the substance by oral route (gavage) at dose levels of 2 or 10 mg/kg and for 2 years (104 weeks).

The design is close to the OECD Guideline 451 (Carcinogenicity studies), with deviations: haematological examination, urinalysis, clinical chemistry were not performed, 2 doses were tested instead of 3 used, and animals were older than 6 weeks old at initiation.

For more details about the description and results of the study, see section Carcinogenicity, 1.5.1.5.

Conclusion

The only finding observed in this assay was the incidence increase of the necrosis of the liver in male mice but without clear evidence it was related to VDC administration.

1.6.1.5 [Quast (1983)]

Study reference:

Quast J.F., Humiston C.G., Wade C.E.., Ballard J., Beyer J.E., Schwetz R.W., Norris J.M. 1983: A Chronic Toxicity and Oncogenicity Study in RAts and Subchronic Toxicity Study in Dogs on Ingested Vinylidene Chloride (publication), Fundamental and applied toxicology, 3:55-62.

Detailed study summary and results:

Test type

A sub-chronic toxicity study was conducted in dogs exposed to VDC by gavage at the dose levels of 6.25, 12.5, or 25 mg/kg bw/day for 97 days. The study was similar to OECD guideline 409 (Repeated Dose 90-Day Oral Toxicity in Non-Rodents).

The study is well described study, however few deviations were noted: several clinical and haematological

parameters and organ weights were not examined and/or recorded; detailed results of body weight and histopathology are not available.

Test substance

The test item as described in the publication was VDC or vinylidene chloride or 1,1-dichloroethylene with a mimimum purity of 99.5%. It was obtained from Dow Chemical company U.S.A. The samples used in the studies were distilled to bring the residual MEHQ (mono methyl ether of hydroquinone) into the range of 1 to 5 ppm. Samples were analysed by gas chromatography, and the following impurities concentrations were obtained: vinyl bromide 4 ppm; vinyl chloride 3-50 ppm; trans-1,2-dichloroethene 138-1700 ppm; cis-1,2-dichchloroethene 24-680 ppm; 1,1,1-trichloroethane 2-60 ppm; 1,1,2-trichloroethane 48 ppm; Hydroquinone monomethyl ether (inhibitor) 2 ppm.

The test item formulations were prepared in peanut oil and and incorporated in gelatin capsules. Gelatin capsules for administration were filled weekly using the required amount of a 50% solution of VDC in peanut oil per capsule to provide the designated dosage. The stability of VDC in peanut oil was verified before the initiation of the studies.

Test animals

The study was conducted on male and female purebred beagle dogs, 8 months of age. Food and water were made available to the animals *ad libitum*.

Administration/exposure

Groups of 4 dogs/sex were administered VDC in peanut oil incorporated in a gelatin capsule at concentrations which provided 6.25, 12.5 or 25 mg VDC/kg bw/day for 97 days. A group of 4 control dogs/sex were daily administered a capsule containing an amount of peanut oil equivalent to that given to the dogs at the 25 mg/kg bw dose level. The dosages of 6.25, 12.5 and 25.0 were chosen to approximate 6250, 12 500 and 25 000 times, respectively, the amount of VDC ingested by a 75 kg person on a 1500 gram daily diet, assuming the food was in packages containing the compound and migration was 50 ppb in the f'ood.

The dogs were observed daily for signs of toxicity. Food consumption/pen was recorded twice each week and the average amount consumed/dog/day was calculated. Hematologic and clinical chemistry determinations, and urine analysis were conducted. The weights of the brain, heart, liver, kidneys and testes were recorded. Microscopic examination was conducted on a complete set of tissues.

Hematology and clinical chemistry determinations were made using an analysis of variance and Dunnett's test (Steel and Torrie, 1960). Since the dogs were housed together regression analysis was used to detect trends in food consumption.

Results and discussion

No exposure related changes in appearance and behavior, body weights or food consumption were observed at any tested dose levels. No significant exposure-related changes were observed in urinalysis, clinical chemistry or hematology determinations, as well as in organ weights and organ-to-body-weight ratios or in gross and microscopic examination at any dose level. There was no depletion of the non-protein sulfhydryl levels in the liver or kidneys.

The only pathologic changes observed were interpreted to be spontaneous. A single male dog receiving 12.5 mg/kg bw/day had kidneys which grossly appeared increased in size. However no significant histopathologic alterations were found. In addition, evaluation of hematologic, clinical clemistry, and urinalysis values of this dog did not reveal abnormalities. In view of these negative findings and the lack of other effects at the 25 mg/kg level, this finding in this one dog were considered not to be related to ingestion of VDC.

In conclusion, the results of the 97-day toxicity study in dogs demonstrated that 25 mg/kg bw/day, a level which did not alter the non-protein sulfhydryl levels in the liver or kidneys, did not elicit any evidence of toxicity.

1.6.2 Animal data by inhalation

1.6.2.1 [NTP (2015)]

Study reference:

National Toxicology Program 2015: Technical Report NTP TR 582; Toxicology and carcinogenesis of vinylidene chloride (CAS No. 75-35-4) in F344/N rats and B6C3F1/N mice (inhalation studies); National Institutes of Health, Public Health Service, U.S. Department on Health and Human Services.

Detailed study summary and results:

Test type

This study was designed and conducted to evaluate the toxic effects of repeated exposure to VDC and was used as range-finding study to provide preliminary informations to perform a main 3-month study. This study was part of the US National Toxicological Program (NTP). Groups of 5 male and 5 female rats or mice per dose were exposed to 1,1-dichloroethylene vapour by whole body inhalation at 0, 25, 50, 100, 200 or 400 ppm for 16 days (rats) or 17 days (mice).

The study was similar to OECD Guideline 412 (Subacute Inhalation Toxicity: 28-Day Study) with some acceptable deviations: ophtalmoscopic examination, hematology, clinical chemistry were not conducted; the treatment period was only for 16 days instead of 28 days.

Test substance

Vinylidene chloride, manufactured by Dow Chemical Company (Freeport, TX), was obtained in one lot from Sigma-Aldrich. The material was identified as lot SB20019301. Analysis by GC/FID indicated that the test article was stabilized with approximately 300 ppm monomethyl ether hydroquinone (MEHQ) and that the overall purity was greater than 99.9%.

Test animals

The test was conducted on groups of 5 male and 5 female F344/N rats or B6C3F1/N mice from the NTP colony maintained at Taconic Farms, Inc. (Germantown, NY). Animals were 4 to 5 weeks old at receipt, 5 to 7 days old at the start of the study, and 8 to 9 weeks at the necropsy date. They were housed individually. Diet and water were available ad libidum except during exposure period. The study started after a quarantine period of 11 days.

Administration/exposure

Rats and mice were exposed to VDC vapour by whole body inhalation at 0, 25, 50, 100, 200 or 400 ppm in air, for 16 days (rats) or 17 days (mice), 6 hours/day plus T90 (12 minutes) and 5 days/week. These wide-ranging exposure concentrations were selected based on reports in the literature that indicated significant differences in species- and strain-related sensitivity to the toxicity of vinylidene chloride. Animals were housed individually in exposure chambers.

The VDC vapour was generated by pumping into a heated glass flask. Nitrogen entered the flask and assisted in vaporizing the chemical. Constant flows through the manifold and into all chambers was ensured. The inhalation exposure chambers were designed so that uniform vapor concentrations could be maintained throughout the chamber. Chamber concentrations of vinylidene chloride was monitored by on-line gas chromatography. The average measured concentrations were in acceptable range: 25.1 ± 0.5 ppm, 50.1 ± 1.3 ppm, 99.7 ± 4.3 ppm (99.7 ± 4.1 ppm for mice), 200 ± 1.0 ppm and 398 ± 34 (396 ± 2.0 ppm for mice) for the target concentrations of 25, 50, 100, 200 and 400 ppm respectively.

Animals were observed twice daily. They were weighed initially, on days 6 and 13, and at the end of studies. Clinical findings were recorded daily before and after exposure and at the end of the studies. Clinical pathology was not assessed (no blood analysis for hematology or biochemistry). Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus. Histopathology was performed on 0, 100, 200, and 400 ppm rats and 0, 50, 100, 200, and 400 ppm mice. In addition to gross lesions and tissue masses, the eyes, kidney (except 50 ppm female mice), liver, lung, and nose were examined to a no-effect level.

Results and discussion

All male and nine of 10 female rats in the 200 and 400 ppm groups were found dead by day 2; one 400 ppm female was found dead on day 4. All other rats survived the entire study except one 25 ppm male removed

from the study due to chylothorax. All females and nine of 10 males rats exposed to 200 or 400 ppm became lethargic, while all females and four of five males exposed to 400 ppm developed ataxia. The final mean body weight gain of 100 ppm females rats was significantly less (6%) than that of the chamber controls.

All male mice exposed to 100 ppm or greater died within the first 4 days of exposure. All female mice exposed to 200 or 400 ppm were found dead following exposure on day 1. Two of five 50 ppm male mice and all 100 ppm males were lethargic. Abnormal breathing occurred in one of five 50 ppm males and four of five 100 ppm males. All 100 ppm female mice became thin, while one female exposed at this level also became lethargic, developed tremors, and was breathing abnormally. The mean body weight gains of 25 and 50 ppm male mice were significantly less (8% and 7% less, respectively) than that of the chamber controls.

Table 90: Survival and body weight of Mice and Rats in the 2-week inhalation study of VDC

Species	Sex	Concen-ration (ppm)	Survival [b]	Initial body weight [a] (g)	Final body weight [a] (g)	Change in body weight [a] (g)	Relative body weight [i] (%)
RATS	**Male**	0	5/5	93 ± 2	158 ± 2	66 ± 3	
		25	4/5 [c]	91 ± 1	150 ± 3	60 ± 4	95
		50	5/5	92 ± 3	159 ± 5	67 ± 3	100
		100	5/5	93 ± 1	154 ± 2	62 ± 2	97
		200	0/5 [d]	92 ± 2	-	-	
		400	0/5 [e]	92 ± 2	-	-	
	Female	0	5/5	84 ± 1	124 ± 2	40 ± 1	
		25	5/5	84 ± 1	125 ± 3	40 ± 3	101
		50	5/5	84 ± 2	122 ± 1	38 ± 2	98
		100	5/5	83 ± 2	117 ± 3	**34 ± 1*[2]**	94
		200	0/5 [d]	83 ± 1	-	-	
		400	0/5 [f]	84 ± 1	-	-	
MICE	**Male**	0	5/5	23.1 ± 0.6	26.6 ± 0.9	3.6 ± 0.6	
		25	5/5	23.6 ± 0.3	24.4 ± 0.9	**0.8 ± 0.6*[1]**	92
		50	4/5 [g]	23.6 ± 0.4	24.9 ± 0.2	**1.3 ± 0.6*[1]**	93
		100	0/5 [h]	23.7 ± 0.2	-	-	
		200	0/5 [d]	23.3 ± 0.4	-	-	
		400	0/5 [d]	23.3 ± 0.6	-	-	
	Female	0	5/5	19.7 ± 0.4	22.2 ± 0.4	2.5 ± 0.4	
		25	5/5	19.9 ± 0.2	21.8 ± 0.5	2.0 ± 0.5	98
		50	5/5	19.4 ± 0.4	21.4 ± 0.3	2.0 ± 0.4	96
		100	4/5 [g]	19.6 ± 0.4	22.2 ± 0.7	2.5 ± 0.2	100
		200	0/5 [d]	19.7 ± 0.4	-	-	
		400	0/5 [d]	19.1 ± 0.2	-	-	

*[1] Significantly different (P≤0.05) from the chamber control group by Dunnett's test
*[2] Significantly different (P≤0.01) from the chamber control group by Williams' test
[a] Weights and weight changes are given as mean ± standard error.
Subsequent calculations are based on animals surviving to the end of the study.
[b] Number of animals surviving at 16 (rats) or 17 (mice) days/number initially in group
[c] Day of the death: 10
[d] Day of deaths: 1 / [e] Day of deaths: 1, 2,2, 2, 2 / [f] Day of deaths: 2, 2, 2 ,2, 4 / [g] Day of the death: 5 / [h] Day of deaths: 3, 4, 4, 4, 4
[i] Final body weight relative to controls

In rats, absolute and relative kidney weights of all surviving groups of exposed males and females were significantly greater than those of the chamber controls. In males, relative lung weights were increased at 100 ppm compared to controls, and an increasing trend was observed in absolute and relative lung weights.

In mice, all surviving groups of exposed females, absolute and relative lung weights were significantly greater than those of the chamber controls. Absolute and relative liver weights of 50 and 100 ppm females and relative liver weights of 25 ppm females and 25 and 50 ppm males were significantly greater than those of the chamber controls.

Table 91: Organ weights for Rats in the 2-week inhalation study of VDC

RAT		Organ weights (g or mg) [a]					
		Controls	25 ppm	50 ppm	100 ppm	200 ppm	400 ppm
Sex	**Animals examined**	**5**	**5**	**5**	**5**	**0 [b]**	**0 [b]**
Male	**Necropsy body weight**	158 ± 2	150 ± 3	159 ± 5	154 ± 2	-	-
	HEART						
	Absolute	0.57 ± 0.01	0.55 ± 0.02	0.57 ± 0.02	0.56 ± 0.01	-	-
	Relative	3.590 ± 0.042	3.640 ± 0.079	3.559 ± 0.046	3.655 ± 0.043		
	KIDNEY						
	Absolute	0.61 ± 0.01	**0.70 ± 0.01****	**0.71 ± 0.02****	**0.71 ± 0.02****	-	-
	Relative	3.878 ± 0.045	**4.641 ± 0.054****	**4.460 ± 0.124****	**4.625 ± 0.074****		
	LIVER					-	-
	Absolute	7.06 ± 0.18	6.50 ± 0.16	6.84 ± 0.21	6.79 ± 0.13		
	Relative	44.561 ± 0.628	43.247 ± 0.147	43.045 ± 0.419	44.042 ± 0.578	-	-
	LUNG						
	Absolute	1.04 ± 0.02	1.14 ± 0.05	1.11 ± 0.07	1.20 ± 0.06	-	-
	Relative	6.557 ± 0.110	7.548 ± 0.248	6.991 ± 0.263	**7.776 ± 0.372***		
Female	**Animals examined**	**5**	**5**	**5**	**5**	**0 [b]**	**0 [b]**
	Necropsy body weight	124 ± 2	125 ± 3	122 ± 1	117 ± 3	-	-
	HEART						
	Absolute	0.45 ± 0.01	0.46 ± 0.01	0.47 ± 0.00	0.46 ± 0.01	-	-
	Relative	3.633 ± 0.044	3.680 ± 0.059	**3.874 ± 0.045****	**3.924 ± 0.068****		
	KIDNEY						
	Absolute	0.53 ± 0.01	**0.61 ± 0.01****	**0.59 ± 0.01***	**0.59 ± 0.02****	-	-
	Relative	4.299 ± 0.068	**4.904 ± 0.083****	**4.826 ± 0.079****	**5.084 ± 0.079****		
	LIVER					-	-
	Absolute	5.07 ± 0.13	4.96 ± 0.12	4.86 ± 0.08	4.87 ± 0.14		
	Relative	40.971 ± 0.433	39.867 ± 0.386	39.860 ± 0.846	41.676 ± 0.349	-	-
	LUNG						
	Absolute	0.88 ± 0.04	0.95 ± 0.05	0.95 ± 0.09	0.87 ± 0.04	-	-
	Relative	7.066 ± 0.214	7.643 ± 0.350	7.844 ± 0.747	7.414 ± 0.277		

***** Significantly different (P≤0.05) from the chamber control group by Williams' or Dunnett's test
****** P≤0.01
[a] Organ weights (absolute) and body weights are given in grams; organ-weight-to-body-weight ratios (relative) are given as mg
[b] No data because of 100% mortality before the end of the study

Table 92: Organ weights for Mice in the 2-week inhalation study of VDC

MOUSE		Organ weights [a]					
		Controls	25 ppm	50 ppm	100 ppm	200 ppm	400 ppm
Sex	**Animals examined**	**5**	**5**	**5**	**0 [b]**	**0 [b]**	**0 [b]**
	Necropsy	26.6 ± 0.9	24.4 ± 0.9	24.9 ± 0.2	-	-	-

Male	**body weight**						
	HEART						
	Absolute	0.13 ± 0.01	0.12 ± 0.01	0.12 ± 0.00	-	-	-
	Relative	4.952 ± 0.129	4.826 ± 0.059	4.728 ± 0.087			
	KIDNEY						
	Absolute	0.22 ± 0.02	0.23 ± 0.01	0.22 ± 0.01	-	-	-
	Relative	8.309 ± 0.292	**9.495 ± 0.137****	8.851 ± 0.301			
	LIVER				-	-	-
	Absolute	1.42 ± 0.07	1.48 ± 0.07	1.56 ± 0.03			
	Relative	53.190 ± 0.796	**60.483 ± 1.044****	**62.658 ± 0.713****	-	-	-
	LUNG						
	Absolute	0.18 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	-	-	-
	Relative	6.663 ± 0.215	**7.682 ± 0.279***	**7.751 ± 0.237***			
Female	**Animals examined**	**5**	**5**	**5**	**5**	**0** [b]	**0** [b]
	Necropsy body weight	22.2 ± 0.4	21.8 ± 0.5	21.4 ± 0.3	22.2 ± 0.7	-	-
	HEART						
	Absolute	0.12 ± 0.00	0.12 ± 0.01	0.11 ± 0.00	**0.10 ± 0.00***	-	-
	Relative	5.321 ± 0.125	5.580 ± 0.138	5.133 ± 0.111	**4.633 ± 0.071****		
	KIDNEY						
	Absolute	0.16 ± 0.01	**0.18 ± 0.01***	0.17 ± 0.00	0.16 ± 0.01	-	-
	Relative	7.026 ± 0.241	**8.340 ± 0.212****	**8.029 ± 0.134****	7.224 ± 0.204		
	LIVER					-	-
	Absolute	1.14 ± 0.03	1.23 ± 0.04	**1.30 ± 0.01***	**1.43 ± 0.07****		
	Relative	51.248 ± 0.827	**56.253 ± 0.396****	**60.528 ± 0.792****	**64.395 ± 1.497****	-	-
	LUNG						
	Absolute	0.15 ± 0.01	**0.21 ± 0.01****	**0.19 ± 0.01***	**0.21 ± 0.02****	-	-
	Relative	6.932 ± 0.514	**9.460 ± 0.297****	**9.055 ± 0.315****	**9.567 ± 0.456****		

***** Significantly different (P≤0.05) from the chamber control group by Williams' or Dunnett's test
****** P≤0.01
[a] Organ weights (absolute) and body weights are given in grams; organ-weight-to-body-weight ratios (relative) are given as mg
[b] No data because of 100% mortality before the end of the study

The liver was identified as a target organ for male and female rats and mice.

In rats, liver lesions consisted of centrilobular hepatocellular cytoplasmic alteration in 25, 50, and 100 ppm males and females and centrilobular hepatocellular necrosis in the 200 and 400 ppm groups. Necrosis was associated with early deaths observed in in the 200 and 400 ppm groups. The cytoplasmic alteration is suggestive of a degenerative lesion in the lower dose groups, which culminates in hepatocellular necrosis at higher doses.

In mice, liver lesions consisted of hepatocellular necrosis in males and females exposed to 100, 200, or 400 ppm vinylidene chloride, and there was evidence of hepatocellular regeneration in the 100 ppm female group.

The kidney was also a target organ for male and female rats and male mice in the 2-week studies.

In rats, increased kidney weights were observed in both sexes. Kidney lesions consisted of tubule cast formation in the renal papillae in the 200 and 400 ppm males and females.

In male mice, lesions were more severe, characterized not only by granular cast formation, but also proximal renal tubule necrosis in all dosed males. Incidences of marked renal tubule necrosis coincided with early deaths in all male mice exposed to 100 ppm or greater. Incidences of minimal to moderate renal tubule necrosis and granular casts occurred in the 25 and 50 ppm male groups. Mild to moderate renal tubule regeneration occurred in 25 and 50 ppm males that survived until terminal sacrifice. In female mice, kidneys were not affected.

The nose was also a target organ for male and female mice in the 2-week study. Lesions in the nose included respiratory epithelial necrosis in all 100, 200, and 400 ppm males and 200 and 400 ppm females

Table 93: Incidence of selected non-neoplastic lesions in Mice and Rats in the 2-week inhalation study of VDC

Species	Sex	Lesions	Number of animals with lesion [b]					
			Control	25 ppm	50 ppm	100 ppm	200 ppm	400 ppm
RATS	Male	**LIVER[a]**	5	5	5	5	5	5
		Centrilobular, necrosis	0	1 (2.0)	0	0	**5** (4.0)**	**5** (4.0)**
		Centrilobular, cytoplasmic alteration	0	**4* (2.8)**	**5** (3.0)**	**5** (3.0)**	0	0
		KIDNEY[a]						
		Papilla, renal tubule, casts	5	0	0	5	5	5
			0			0	**5** (3.2)**	**4* (2.5)**
	Female	**LIVER[a]**	5	5	5	5	5	5
		Centrilobular, necrosis	0	0	0	0	**5** (4.0)**	**5** (4.0)**
		Centrilobular, cytoplasmic alteration	0	**5** (2.4)**	**5** (3.0)**	**5** (2.6)**	0	0
		KIDNEY[a]						
		Papilla, renal tubule, casts	5	0	0	5	5	5
			0			0	**5** (3.0)**	**5** (3.2)**
MICE	Male	**NOSE[a]**	5	5	5	5	5	5
		Respiratory Epithelium, necrosis	0	0	1 (1.0)	**5** (1.0)**	**5** (1.0)**	**5** (1.0)**
		LIVER[a]	5	5	5	5	5	5
		Necrosis	0	0	1 (1.0)	**5** (3.0)**	**5** (4.0)**	**5** (4.0)**
		KIDNEY[a]	5	5	5	5	5	5
		Renal tubule, necrosis	0	**5** (1.2)**	**5** (1.6)**	**5** (4.0)**	**5** (4.0)**	**5** (4.0)**
		Cast granular	0	**5** (1.8)**	**5** (2.2)**	**5** (2.2)**	**5** (4.0)**	**5** (4.0)**
		Renal tubule regeneration	0	**5** (2.8)**	**4* (3.0)**	0	0	0
	Female	**NOSE[a]**	5	0	5	5	5	5
		Respiratory epithelium, necrosis	0		0	1 (1.0)	**5** (1.0)**	**5** (1.0)**
		LIVER[a]	5	0	5	5	5	5
		Necrosis	0		0	**5** (1.6)**	**5** (4.0)**	**5** (4.0)**
		Regeneration	0		0	**4* (2.0)**	0	0

* Significantly different (P≤0.05) from the chamber control group by the Fisher exact test
** P≤0.01
[a] Number of animals with tissue examined microscopically
[b] In parenthesis, average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

1.6.2.2 [NTP (2015)]

Study reference:

National Toxicology Program 2015: Technical Report NTP TR 582; Toxicology and carcinogenesis of vinylidene chloride (CAS No. 75-35-4) in F344/N rats and B6C3F1/N mice (inhalation studies); National Institutes of Health, Public Health Service, U.S. Department on Health and Human Services.

Detailed study summary and results:

Test type

This study was designed and conducted to evaluate the toxic effects of repeated exposure to VDC and was used as range-finding study for the carcinogenesis study. It was part of the US National Toxicological Program (NTP). Groups of 10 male and 10 female rats per dose were exposed to VDC by whole body inhalation at 0, 6.25, 12.5, 25, 50 or 100 ppm in air for 90 days.

The study was similar to OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day Study) with some acceptable deviations: ophtalmoscopic examination was not conducted; not all the haematology parameters listed in the guideline were analysed; not all the clinical chemistry parameters were analysed.

Test substance

Vinylidene chloride, manufactured by Dow Chemical Company (Freeport, TX), was obtained in one lot from Sigma-Aldrich. The material was identified as lot SB20019301. Analysis by GC/FID indicated that the test article was stabilized with approximately 300 ppm monomethyl ether hydroquinone (MEHQ) and that the overall purity was greater than 99.9%.

Test animals

The test was conducted on male and female F344/N rats from the commercial colony at Taconic Farms, Inc. (Germantown, NY). Animals were 4 weeks old at receipt, 5 to 7 days old at the start of the study, and 19 to 20 weeks at the necropsy date. They were housed individually. Diet and water were available ad libidum except during exposure period. Groups of 10 male and 10 female animals were exposed per group. The study started after a quarantine period of 12 days.

Administration/exposure

Rats were exposed to VDC vapour by whole body inhalation at 0, 6.25, 12.5, 25, 50 or 100 ppm in air for 6 hours/day, 5 days/week, and for 90 days. Groups of 10 male and 10 female clinical pathology rats were exposed to the same concentrations for 23 days. Animals were housed individually in exposure chambers.

The VDC vapour was generated by pumping into a heated glass flask. Nitrogen entered the flask and assisted in vaporizing the chemical. Constant flows through the manifold and into all chambers was ensured. The

inhalation exposure chambers were designed so that uniform vapor concentrations could be maintained throughout the chamber. Chamber concentrations of vinylidene chloride was monitored by on-line gas chromatography. The average measured concentrations were inacceptable range: 6.28 ± 0.12, 12.6 ± 0.2, 25.1 ± 0.5 ppm, 50.4 ± 1.0 ppm and 100.0 ± 2.2 for the target concentrations of 6.25, 12.5, 25, 50 and 100 ppm respectively.

Animals were weighted initially, then approximately weekly and at the end of the study. Blood was collected for hematology and clinical chemistry analyses. Necropsies were performed on all core study animals. The heart, right kidney, liver, lungs, right testis, and thymus were weighed. Complete histopathologic examinations were performed on 0 and 100 ppm groups. In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung, lymph nodes (mandibular, mesenteric, bronchial, and mediastinal), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.

Results and discussion

All rats survived until the end of the study. No exposure-related clinical findings were observed. Final mean body weights and body weight gains of exposed groups were similar to those of the chamber control groups. No exposure-related clinical findings or gross lesions were observed.

Slight increases (≤ 6%) in hemoglobin concentrations and erythrocyte counts were observed in 100 ppm male and female rats on day 3. In addition, on day 3 the hematocrit was also slightly increased in 100 ppm males. These changes ameliorated by day 23 and were consistent with a transient hemoconcentration associated with mild dehydration as the rats acclimated to exposure.

Exposure concentration-related minimal to mild (≤ 10%) increases were observed in total protein and globulin concentrations on days 3 and 23 in both male and female rats in various exposed groups, but most consistently at 100 ppm. In addition, albumin was minimally increased (≤ 5%) in 100 ppm males and 25 ppm or greater female rats on day 23. Urea nitrogen concentrations were minimally increased in 50 and 100 ppm male and female rats on day 3, and 50 and 100 ppm males and 6.25 ppm or greater females on day 23. The total protein, albumin, globulin, and urea nitrogen concentrations returned to chamber control levels by week 14 and were consistent with possible mild dehydration, not confirmed as water consumption was not recorded.

Increased alkaline phosphatase activities were observed in the 50 and 100 ppm groups on days 3 and 23 in male rats and on day 23 in female rats. While increased alkaline phosphatase activity is considered an indicator of cholestasis, the increases were of minimal severity and transient, and bile acid concentrations,

another marker for cholestasis, were unchanged or decreased, suggesting that these changes represent a transient alteration in hepatic metabolism rather than cholestasis. Sorbitol dehydrogenase (SDH) activities were increased in 100 ppm females on day 3 and in 100 ppm males and 50 and 100 ppm females on day 23. In addition, alanine aminotransferase (ALT) activities were increased on day 3 in 50 and 100 ppm male rats and day 23 in 100 ppm male rats. Both SDH and ALT are considered markers of hepatocellular injury. These increases were transient, not being observed at week 14, and minimal histopathologic changes were observed in the liver at study termination; therefore, these changes are consistent with mild transient hepatocellular injury.

Relative kidney weights of 6.25, 12.5, and 100 ppm in males, absolute and relative kidney weights of 12.5 ppm or greater females were significantly greater than those of the chamber controls.

Table 94: Organ weights and organ-weight-to-body-weight ratios for Rats in the 3-month inhalation study of VDC

		Organ weights (g or mg) [a]					
		Controls	**6.25 ppm**	**12.5 ppm**	**25 ppm**	**50 ppm**	**100 ppm**
Sex	**Animals examined**	**10**	**10**	**10**	**10**	**10**	**10**
MALE	**Necropsy body weight**	326 ± 7	332 ± 6	337 ± 5	319 ± 6	340 ± 6	322 ± 5
	HEART						
	Absolute	0.89 ± 0.02	0.93 ± 0.02	0.88 ± 0.02	0.86 ± 0.02	0.92 ± 0.02	0.89 ± 0.03
	Relative	2.713 ± 0.022	2.807 ± 0.068	2.628 ± 0.031	2.700 ± 0.023	2.694 ± 0.033	2.757 ± 0.055
	KIDNEY						
	Absolute	0.99 ± 0.02	1.07 ± 0.04	1.05 ± 0.03	1.01 ± 0.03	1.08 ± 0.02	1.05 ± 0.02
	Relative	3.026 ± 0.028	**3.208 ± 0.091***	**3.129 ± 0.035***	3.171 ± 0.039	3.167 ± 0.029	**3.245 ± 0.038****
	LIVER						
	Absolute	10.63 ± 0.32	10.61 ± 0.26	10.46 ± 0.27	9.77 ± 0.27	10.59 ± 0.32	10.23 ± 0.28
	Relative	32.562 ± 0.400	31.951 ± 0.510	31.034 ± 0.404	**30.596 ± 0.376****	31.062 ± 0.456	31.697 ± 0.412
	LUNG						
	Absolute	1.66 ± 0.06	1.59 ± 0.06	1.60 ± 0.04	1.59 ± 0.06	1.71 ± 0.09	1.50 ± 0.05
	Relative	5.075 ± 0.148	4.781 ± 0.132	4.766 ± 0.115	4.986 ± 0.128	5.003 ± 0.194	4.665 ± 0.129
	TESTIS						
	Absolute	1.343 ± 0.026	1.353 ± 0.019	1.346 ± 0.022	1.316 ± 0.020	1.346 ± 0.017	1.314 ± 0.021
	Relative	4.122 ± 0.056	4.082 ± 0.073	4.004 ± 0.061	4.135 ± 0.083	3.963 ± 0.068	4.085 ± 0.077
	THYMUS						
	Absolute	0.335 ± 0.014	0.358 ± 0.011	0.326 ± 0.012	0.314 ± 0.015	0.344 ± 0.13	0.328 ± 0.017
	Relative	1.025 ± 0.032	1.080 ± 0.033	0.969 ± 0.036	0.985 ± 0.047	1.014 ± 0.038	1.018 ± 0.052
F	**Animals examined**	**10**	**10**	**10**	**10**	**10**	**6**
	Necropsy body weight	203 ± 3	205 ± 6	206 ± 4	201 ± 4	205 ± 4	195 ± 2
	HEART						
	Absolute	0.61 ± 0.01	0.60 ± 0.01	0.61 ± 0.01	0.61 ± 0.02	0.63 ± 0.02	0.60 ± 0.01
	Relative	2.978 ± 0.055	2.926 ± 0.039	2.980 ± 0.038	3.017 ± 0.048	3.077 ± 0.044	3.046 ± 0.042
	KIDNEY						
	Absolute	0.64 ± 0.01	0.67 ± 0.02	**0.69 ± 0.02***	**0.68 ± 0.01***	**0.72 ± 0.01****	**0.71 ± 0.02****
	Relative	3.155 ± 0.041	3.280 ± 0.055	**3.356 ±**	**3.393 ±**	**3.512 ±**	**3.645 ±**

E				**0.043****	**0.040****	**0.025****	**0.058****
M	**LIVER**						
A	Absolute	5.84 ± 0.10	5.72 ± 0.24	5.87 ± 0.18	5.51 ± 0.14	5.97 ± 0.16	5.94 ± 0.16
L	Relative	28.741 ± 0.345	27.921 ± 0.470	28.538 ± 0.588	27.452 ± 0.505	29.175 ± 0.598	30.393 ± 0.495
E	**LUNG**						
	Absolute	1.08 ± 0.02	1.11 ± 0.03	1.10 ± 0.02	1.09 ± 0.03	1.12 ± 0.05	1.07 ± 0.02
	Relative	5.303 ± 0.080	5.415 ± 0.100	5.338 ± 0.128	5.429 ± 0.125	5.451 ± 0.248	5.454 ± 0.070
	THYMUS						
	Absolute	0.279 ± 0.010	0.265 ± 0.013	0.268 ± 0.011	0.255 ± 0.008	0.274 ± 0.009	0.266 ± 0.008
	Relative	1.372 ± 0.040	1.303 ± 0.069	1.301 ± 0.048	1.270 ± 0.032	1.342 ± 0.046	1.359 ± 0.037

***** Significantly different (P≤0.05) from the chamber control group by Williams' or Dunnett's test
****** P≤0.01
[a] Organ weights (absolute) and body weights are given in grams; organ-weight-to-body-weight ratios (relative) are given as mg organ weight/g body weight (mean ± standard error).

Microscopic lesions of the nose were noted in both sexes of rats. A combination of lesions in the nasal epithelium composed of olfactory epithelium atrophy, mineralization, and necrosis and turbinate atrophy was observed with generally increasing severity with increasing exposure. A no-effect level was not observed, although turbinate atrophy was not seen in rats exposed to 6.25 ppm, and most of the lesions were minimal in rats exposed to 12.5 ppm or less. Necrosis was not associated with inflammation.

Table 95: Incidences of Selected Non-neoplastic Lesions in Rats exposed to VDC by inhalation for 3 Months

Sex	Lesions	Number of animals with lesion [b]					
		Control	6.25 ppm	12.5 ppm	25 ppm	50 ppm	100 ppm
Male	**NOSE[a]**	10	10	10	10	10	10
	Olfactory Epithelium, Atrophy	0	**4* (1.0)**	**10** (1.0)**	**10** (1.7)**	**10** (2.2)**	**10** (2.7)**
	Olfactory Epithelium, Mineralization	0	**10** (1.3)**	**10** (2.0)**	**10** (2.9)**	**10** (3.0)**	**10** (2.6)**
	Olfactory Epithelium, Necrosis	0	2 (1.0)	**6* (1.0)**	**9** (1.0)**	**7** (1.7)**	**10** (1.6)**
	Turbinate, Atrophy	0	0	**10** (1.0)**	**10** (2.0)**	**10** (2.2)**	**10** (3.0)**
	LIVER[a]	10	10	10	10	10	10
	Centrilobular, Cytoplasmic Alteration	1 (1.0)	1 (1.0)	**6* (1.7)**	**10** (1.8)**	**10** (2.0)**	**10** (1.9)**
Female	**NOSE[a]**	10	10	10	10	10	10
	Olfactory Epithelium, Atrophy	0	2 (1.0)	**10** (1.0)**	**10** (1.3)**	**10** (1.7)**	**10** (2.4)**
	Olfactory Epithelium, Mineralization	0	**5* (1.0)**	**9** (1.3)**	**10** (1.9)**	**10** (2.1)**	**10** (2.3)**
	Olfactory Epithelium, Necrosis	0	1 (1.0)	**3 (1.3)**	**6** (1.5)**	**10** (2.2)**	**10** (1.6)**
	Turbinate, Atrophy	0	0	**10** (1.0)**	**10** (2.0)**	**10** (2.2)**	**10** (3.0)**
	LIVER[a]	10	10	10	10	10	10
	Vacuolization, Cytoplasmic	0	0	0	0	**10** (1.1)**	**10** (1.0)**

* Significantly different (P≤0.05) from the chamber control group by the Fisher exact test
** P≤0.01
[a] Number of animals with tissue examined microscopically
[b] In parenthesis, average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

In the liver of male rats, centrilobular cytoplasmic alteration was significantly increased at 12.5 ppm or greater. In females, cytoplasmic vacuolization was observed at 50 and 100 ppm.

Decreased incidences of nephropathy were observed in male rats exposed to vinylidene chloride. The higher exposure concentration groups had histologically normal kidneys. This lesion is a common background finding in the F344/N rat and was unrelated to vinylidene chloride exposure.

In conclusion, the target organs were identified to be nose and liver on both sexes. While increased kidney weights were observed in males and females, there were no corresponding histopathologic changes in the kidney.

1.6.2.3 [Rampy (1977)]

Study reference:

Rampy L. W., Quast J. F., Humiston C. G., Balmer M. F. and Schwetz B. A. 1977: Interim Results of Two-Year Toxicological Studies in Rats of Vinylidene Chloride Incorporated in the Drinking Water or Administered by Repeated Inhalation (publication), Environmental Health Perspectives, 21:33-43.

Quast J.F., McKenna M.J., Rampy L.W. and Norris J.M. 1986: Chronic toxicity and oncogenicity study on inhaled vinylidene chloride in rats (publication), Fundamental and applied toxicology, 6:105-144.

Detailed study summary and results:

Test type

A carcinogenicity study on VDC was conducted on rats.

Three groups (control group and two treated groups) of 86 male and 86 female Sprague-Dawley rats were exposed for 18 months to VDC vapour, 6 hours/day, 5 days/week and for 18 months.

Some deviations from the guideline OECD 451 were noted: exposure period was for only 18 months; doses were changed after 1 month of treatment; only 2 doses were tested; and infection by mycoplasma pulmonis near the end of the study unvalidated potential pulmonary findings and lethality incidences.

For more details about the description and results of the study, see section 1.5.2.8 in Carcinogenicity section.

Conclusion

The Authors concluded that the only adverse effect related to VDC exposure resulted in minimal fatty changes in liver at both 25 and 75 ppm (100 and 300 mg/m3, respectively). This finding was statistically significant in female rats at the highest tested dose level.

1.6.2.4 [Lee (1977)]

Study reference:

Lee C.C., Bhandari J.C., Winston J. M., House W. B., Peters P. J., Dixon R. L. and Woods J. S. 1977: Inhalation Toxicity of Vinyl Chloride and Vinylidene Chloride (publication), Environmental Health

Perspectives, 21:25-32.

Lee C.C., Bhandari J.C., Winston J. M., House W. B., Peters P. J., Dixon R. L. and Woods J. S. 1978: Carcinogenicity of Vinyl Chloride and Vinylidene Chloride, Journal of Toxicology and Environmental health, 4:15-30.

Detailed study summary and results:

Test type

A chronic toxicity study on VDC was conducted on rats and mice exposed by inhalation.

Animals were exposed to VDC vapour at 220 mg/m3 for 12 months, 6 hours daily and 5 days weekly. A concurrent negative control received untreated air in the same conditions.

For more details about the description and results of the study, see sections 1.5.2.6 and 1.5.2.7 in Carcinogenicity section.

Conclusion

In rats, fatty change was observed in livers of most of the rats treated with VDC.

In mice, changes in the liver were also found (liver enlarged and basophilic hepatocytes, enlarged nuclei, mitotic figures or polyploidy, microfoci of mononuclear cells, focal degeneration and necrosis). The incidence and severity of these lesions progressed with the lengths of exposure.

1.6.2.5 [Maltoni (1977)]

Study reference:

Maltoni C., Cotti G., Morisi L. and Chieco P. 1977: Carcinogenicity bioassays of vinylidene chloride Research plan and early results (publication), Medicina Del Lavoro, 68(4) 241-262.

Maltoni C., Cotti G., and Chieco P. 1984: Chronic toxicity and carcinogenicity bioassays of Vinykidene chloride, Acta oncologica 5 (2).

Roberts S.M., Jordan K.E., Warren D.A., Britt J.K. and James R.C. 2002: Evaluation of the carcinogenicity of 1,1-dichloroethylene (vinylidene chloride) (review article or handbook), Regulatory Toxicology and Pharmacology, 35:44-55.

United States Environmental Protectin Agency 1984: Health assessment document for Vinylidene chloride; Final report EPA/600/8-83/031F

Detailed study summary and results:

Nota*:* Information below related to study in rats

Test type

A carcinogenicity study on VDC was conducted in rats exposed by inhalation. Six groups (one control group and five treated groups) of male and female Sprague-Dawley rats were treated with VDC by vapour for 52 weeks, 4 hours daily and 4-5 days weekly.

Deviations from OECD guideline 451 were noted. The treatment period was for one year, and for 4-5 days per week. The number of animals were insufficient (30 per sex and per group instead of 50 required in the guideline). Following the 52-week exposure, animals were observed until spontaneous death. Only the early results are available. Except histology examinations for tumours analysis, no information either on other examinations (hematology, clinical chemistry, urine analysis, body weight, organ weight…) or statistical methods are available. Several shortcomings of the study design and reporting lead to difficulties in the interpretability of the study results.

For more details about the description and results of the study, see section Carcinogenicity, 1.5.2.1.

Conclusion

Non-neoplastic changes were found in the liver (hepatocyte vacuolization, cloudy swelling, fatty degeneration, necrobiosis and necrosis) both in the control and treated animals. The findings were observed more frequently in 150-200 ppm group (57.6%) than in control (20.5%).

1.6.2.6 [Prendergast (1967)]

Study reference:

Prendergast J.A., Jones R.A., Jenkins L.J. Jr. and Siegel J. 1967: Effects on experimental animals of long-term inhalation of trichloroethylene, carbon tetrachloride, 1,1,1-trichloroethane, dichlorodifluoromethane, and 1,1-dichloroethylene (publication), Toxicology and applied pharmacology, 10:270-289.

Detailed study summary and results:

Test type

Two types of studies were conducted, namely, “continuous” 90-day exposures to simulate submarine conditions, and “repeated” daily 8-hour exposures, 5 days/week, over a period of 6 weeks to simulate exposures of the type encountered in industry. Groups of different animal species were exposed: Long-Evans or Sprague-Dawley rats, Hartley guinea pigs, squirrel monkeys, New Zealand albino rabbits and beagle dogs.

No guideline was followed. The study was conducted according to "Guide for laboratory animal facilities and care" prepared by the Committee on the Guide for laboratory animal resources, National academy of Science-National Research Council, Washington, D.C.". This data is old (from 1967); the design and results are not sufficiently detailed. The age of the animals was not specified, no organ weights and very light information on hematology, biochemistry and histopathological examination results.

Test substance

The test item described in the publication was vinylidene chloride or 1,l –dichloroethylene, obtained from K & K Laboratories, Plainview, New York, with a point of 83.5°C and a minimum purity of 98%.

Test animals

The typical group of animals exposed consisted of 15 Long-Evans or Sprague-Dawley rats, 15 Hartley guinea pigs, 3 squirrel monkeys, 3 New Zealand albino rabbits, and 2 beagle dogs. Several exposures were conducted depending on the tested concentrations and on the species: a group of males and females of each species was exposed to a single dose level of 395 mg/m3 (or 100 ppm), 8 hour/day, 5 days/week, for 6 weeks (repeated exposure). Male and female groups of each species were exposed continuously for 90 days to 20, 61, 101 and 189 mg/m3 (or 5, 16, 26 and 48 ppm), except rabbits which were exposed only to the single concentration of 101 mg/m3 .

Table 96: Total number of animals per species exposed to VDC

		Total number of animals per group (per sex and per dose)				
Tested concentration (mg/m3) [a]	**Type of study [b]**	**Rat (Long-Evans or Sprague-Dawley)**	**Guinea pig (Hartley)**	**Rabbit (New Zealand albino)**	**Dog (Beagle)**	**Monkey (Squirrel)**
395 ± 32	Repeated	15	15	3	2	3
189 ± 6.2	Continous	15	15	-	2	9
101 ± 4.4	Continous	15	15	3	2	3
61 ± 5.7	Continous	15	15	-	2	9
20 ± 2.1	Continous	45	45	-	6	21
0 (Control group)	-	304	314	48	34	57

[a] Mean ± SD.
[b] Repeated = 30 exposures, 8 hr/day, 5 days/week; Continous = continuous 90-day exposure.

No information on age and weight at the start of the study is available.

Administration/exposure

Each test animal was weighed prior to the exposure, at monthly intervals, and at the termination of the run, Total and differential leukocyte counts, hemoglobin concentrations, and microhematocrits were obtained on all animals except the monkeys before and after the exposure. All animals were routinely checked for visible signs of toxicity, such as marked alterations in behavior, physical appearance, respiration pattern, locomotor activity, and prostration. At the termination of each study, animals were sacrificed, autopsied, and sections of heart, lung, liver, spleen, and kidney were taken for histopathologic evaluation. In several of these studies selected biochemical determinations were made in an effort to detect early alterations.

Results and discussion

No mortality was noted at any dose levels and in any species during the repeated treatment at the dose levels of 395 mg/m3.

During continuous treatment, no rabbits or dogs died at any dose levels. In guinea pigs, the mortality was 7/15, 3/15 and 3/15 and 2/45 at 189, 101, 61 and 20 mg/m3, respectively. In monkeys, 3/9 and 1/21 deaths were observed at 189 mg/m3 and 20 mg/m3, respectively. Only 2/45 rats died at the lowest dose level of 20 mg/m3. No visible toxic signs were noted in any survivors of any species.

Rabbits, dogs and monkeys lost weight. During the exposure to 395, 189, 61 and 20 mg/m3, rats gained less weight than the controls, but without any dose-related trend.

For all species, variations were noted in pre- and post-exposure values of the hematological components studied, none of these changes was considered significant. Biochemistry analysis was conducted only on rats and guinea pigs at 189 and 20 mg/m3 (continous exposure). For both species, elevation in liver alkaline phosphatase activity and in serum glutamic-pyruvic transaminase activity were observed during the 189 mg/m3 exposure. Variations observed in liver lipid values fell within control limits and were judged as not significant of an adverse effect.

At 395 mg/m3 (repeated treatment), gross examination revealed normal organs in all animals with the exception of one rat that had a gelatinous material on the kidney and bloody urine in the bladder. Gross examination revealed mottled livers in a majority of animals at 189 mg/m3 (continuous exposure), in several animals at 61 mg/m3 and in about 1/3 of animals at 20 mg/m3. White or bluish-gray spots and nodules were found on the lungs of number of rats treated at 101 mg/m3.

Histopathologic examination was performed on sections of heart, lung, liver, spleen, and kidney from all species as well as on sections of brain, spinal cord, and adrenal gland from dogs and monkeys, and thyroid gland from dogs. At the dose level of 189 mg/m3, sections of liver from dogs, monkeys, and rats showed morphologic changes which consisted of fatty metamorphosis, focal necrosis, hemosiderin deposition, lymphocytic infiltration, bile duct proliferation, fibrosis and pseudo-lobule formation. These changes were the most severe in dogs. At the same dose level, nuclear hypertrophy of the tubular epithelium was observed in the kidney of all rats. These hepatic changes in dogs, monkeys, and rats and the renal changes in rats are considered to be a direct result of the exposure.

One adrenal gland from a dog contained a cortical adenoma composed of cells of the zona glomerulosa type. Non-specific inflammatory changes were found in the lungs for all concentrations but were not attributed to the exposure. Non-specific inflammatory was also observed in the liver and kidneys of all species at the dose level of 101 mg/m3, but with lesser degree than in lungs.

Table 97: Mortality and body weights change for animals exposed to VDC

	Tested concentration (mg/m3)	Type of study[b]	Mortality	Body weight change	
				Starting weight (g for rats, guinea pigs and monkeys (kg for rabbits and dogs)	Change (%)
Rat	**395**	Repeated[a]	0/15	268 (203 -354)	+ 17.5
	189	Continous[a]	0/15	275 (223 -392)	+ 4.3
	101	Continous[a]	0/15	234 (175 -307)	+ 31.6
	61	Continous[a]	0/15	331 (246 -450)	+ 7.6
	20	Continous[a]	2/45	315 (202 -531)	+ 27.0
	Control	-	7/304	286 (192 -440)	+ 35.5
Guinea Pig	**395**	Repeated[a]	0/15	340 (234 -389)	+ 67.0
	189	Continous[a]	7/15	469 (405 -534)	+ 50.3
	101	Continous[a]	3/15	418 (360 -495)	+ 74.0
	61	Continous[a]	3/15	447 (363 -567)	+ 55.3
	20	Continous[a]	2/45	490 (342 -622)	+ 58.6
	Control	-	2/314	418 (274 -646)	+ 69.0
Rabbit	**395**	Repeated[a]	0/3	3.80 (3.55 -4.05)	- 3.6
	101	Continous[a]	0/3	3.35 (3.10 -3.65)	- 4.1
	Control	-	0/34	2.6 (2.1 -3.9)	+ 33.8
Dog	**395**	Repeated[a]	0/2	8.3 (8.0 -8.6)	+ 9.3
	189	Continous[a]	0/2	9.6 (9.5 -9.7)	- 8.3
	101	Continous[a]	0/2	9.6 (9.5 -9.7)	- 5.2
	61	Continous[a]	0/2	8.3 (8.0 -8.6)	+ 13.3
	20	Continous[a]	0/6	12.3 (9.0 -14.8)	-2.2
	Control	-	0/34	10.4 (7.6 -14.6)	+12.4
Monkey	**395**	Repeated	0/3	606 (572 -627)	- 5.9
	189	Continous	3/9	718 (508 -820)	- 28.1
	101	Continous	2/3	759 (759)	- 6.1
	61	Continous	0/9	667 (505 -1008)	- 10.3
	20	Continous	1/21	740 (602 -962)	+ 9.8
	Control	-	1/57	651 (382 -952)	+ 0.8

[a] Repeated = 80 exposures, 8 hr/day, 5 days/week; Continous = continuous 90-day exposure.

Table 98: Biochemical effects of continuous exposure to VDC

	Tested concentration (mg/m3)	Type of study[a]	Biochemical parameters parameters (mean +/- SD)			
			Serum urea nitrogen (mg/100 mL)	Liver lipids (%)	Liver alkaline phosphatase (µmole/min/g)	Serum glutamic pyruvic transaminase (µmole/min/mL)
	189	Continous	17 +/- 4	12.0 +/- 7.7	0.21 +/- 0.07	34 +/- 13

Rats	**20**	Continous	22 +/- 2	9.4 +/- 2.1	0.11 +/- 004	9 +/- 4
	Control	-	20 +/- 4	9.1 +/- 1.8	0.12 +/- 0.05	11 +/- 6
Guinea pigs	**189**	Continous	20 +/- 2	5.9 +/- 1.4	0.19 +/- 0.04	> 70
	20	Continous	24 +/- 2	9.1 +/- 2.4	0.08 +/- 0.03	11 +/- 3
	Control	-	24 +/- 5	11.0 +/- 3.6	0.08 +/- 0.03	10 +/- 5

[a] Continous = continous 90-day exposure / Repeated = Thirty exposures 8h/day and 5 days/week

In brief, repeated exposure to 395 mg/m3 (100 ppm) produced no deaths or visible toxic signs. Hematological and growth data did not reveal any evidence of a toxic response, and histopathological findings were negative in all species. No toxic effects were obtained in this exposure at 395 mg/m3.

Continuous exposure of animals to VDC at levels of 189, 101, 61, and 20 mg/m3 resulted in no significant mortality. Body weight data for the continuous studies contained several deviations from the normal, but only the 189 mg/m3 study resulted in definite growth depression in all species. Histopathological examination of tissues from the surviving experimental animals revealed that only morphologic alterations in liver and kidney observed at 189 mg/m3 were considered to be a direct result of the exposure.

1.6.2.7 [Anonymous (2004)]

Study reference:

See confidential Annex

Detailed study summary and results:

Test type

A sub-chronic toxicity study was conducted according to OECD Guidelines 413 (Subchronic Inhalation Toxicity: 90-Day Study) and 424 (Neurotoxicity study in rodents). Deviations were identified: ophtalmoscopic examination, haematology, clinical chemistry were not conducted; only one dose was tested. The objective of this study was to provide data on neurotoxicity of VDC in rats upon administration by inhalation for 6 hours/day, 5 days/week and for 90 days.

This study was conducted under GLP.

Test substance

The study was conducted on vinylidene chloride (CAS 75-35-4), batch number 150503-01, provided from Solvin France s.a., Tavaux. VDC was stabilised with 60-80 ppm butylated hydroxytoluene (BHT). Based on analyses, the chemical used in the study was 99.97% pure and contained as major impurities chlorinated ethylenes and acetylenes (< 300 ppm).

Test animals

The study was conducted on male and female albino Wistar outbred rats (Crl☹WI)WU BR) obtained from a

colony maintained under SPF-conditions at Charles river Deutschland, Sulzfeld, Germany. Age of animal at the time of start of the treatment period was 55-62 days.

Administration/exposure

Two groups of 10 male and 10 female rats were exposed: the control group was exposed to clean air and the test group was exposed to a test atmosphere at a VDC target concentration of 100 ppm (or 401 mg/m3), for 6 hours/day, 5 days/week over a period of 90 days.

Animals were exposed to the test atmosphere in a nose-only inhalation chamber. Control group was exposed to clean air in similar conditions. To generate atmosphere, the test substance was evaporated by a glass evaporator and was directed towards the animal nose. The VDC concentration in the test atmosphere was determined with a total carbon analyzer, from samples taken continuously from the exposure unit. The overall concentration of the test substance was 98.9 ± 1.7 ppm, close to the target concentration of 100 ppm.

Clinical and neurobehavioural observations, growth, food consumption and food conversion efficiency, organ weight (liver), gross examination at necropsy, microscopic examination of liver and muscle, and detailed microscopic examination of peripheral nervous system organs were evaluated. Feed and drinking water were provided ad libitum from the arrival of the rats until the end of the study except during exposure and neurobehavioral assessments.

Results and discussion

All animals survived until scheduled necropsy. Incidental findings observed among a few animals comprised sparsely haired skin (in 5 of 10 female rats), tail wound encrustation and eyes encrustations.

A slight but significant reduction ($p \leq 0.01$) in body weight was observed in female rats exposed to VDC. This reduction increased with time during exposure, and was considered related to the treatment. However, no decrease body weight gain was observed.

No significant difference in food consumption was observed between groups.

Regarding the neurobehaviour, most functional domains of the nervous system tested appeared unaffected. In the neuromuscular domain, small differences were observed in the parameters gripstrenght and landing footsplay between the 100 ppm group and the control group. In male rats, after 13 weeks of exposure, significantly lower gripstrength and footsplay were observed in the VDC exposed group. Both parameters appear to show a gradual significant decrease with time during the 91-day exposure period. In females rats, a gradual decrease observed over time during the 91-day exposure period for VDC group did not reach the level of significance.

No significant change in liver weights, either on absolute or relative weights, were noted in males or females. No macroscopic changes were observed.

Microscopic examination of the liver indicated very slight to slight mononuclear cell aggregates and necrotic hepatocytes, hepatocellular vacuolation and hepatocellular hypertrophy in both sexes in VDC-exposed

groups, as well as in control groups.

Hepatocellular hypertrophy was observed mainly in the male rats of the VDC group and occurred mainly centrilobularly, a statistically significant difference was observed between the male rats of the VDC group and the control group.

Regarding neuropathology, microscopic examination indicated significant incidence of axonal degeneration in the sural nerve in female rats and in the tibial nerve of male rats (within the range of the normal background pathology of rats in this strain and age).

Table 99: Incidence of significant microscopic findings in rats exposed to VDC by inhalation

Changes	Incidence (number of animals with lesions)			
	Males		Females	
	Control group	100 ppm	Control group	100 ppm
Nervus suralis				
Number of animals examined	5	5	5	5
Single axonal degeneration	3	3	1	**5***
Nervus tibialis				
Number of animals examined	5	5	5	5
Single axonal demyelination	0	1	0	0
Single axonal degeneration	4	**0***	2	2
Few axonal degeneration	0	**5****	3	3
Liver				
Number of animals examined	10	10	10	10
Diffuse hepatocellular vacuolation	1	1	0	2
Periportal and midzonal hepatocellular vacuolation	1	0	0	0
Midzonal hepatocellular vacuolation	0	3	5	2
Centrolobular and midzonal hepatocellular vacuolation	2	2	2	6
Centrolobular hepatocellular hypertrophy	1	**8****	0	1
Diffuse hepatocellular hypertrophy	0	0	0	1
Mononuclear cell aggregates/necrotic hepatocyes	7	4	3	3

Statistic: 2-side Fisher's exact test between the control and each of the treatment groups.

***** $p \leq 0.05$ / ****** $P \leq 0.01$ / ******* $p \leq 0.001$

In conclusion, in the conditions of the study, VDC did not affect the nervous system. The centrilobular hepatocellular hypertrophy, although slight, observed in the males rats was considered as an adverse effect of VDC on the liver.

1.6.2.8 [Anonymous (1979b)]

Study reference:

See confidential Annex

Detailed study summary and results:

Test type

Rats were exposed to VDC vapour at concentrations of 120 and 400 mg/m3 for 6 weeks, 5 days/week and 6h/day.

Test substance

The substance cited in the report is vinylidene chloride or 1,1-dichloroethene. No infomation about purity or batch is available.

Test animals

The study was conducted on male and female Sprague-Dawley rats, obtained from SPF-Zucht, Firma WIGA. At the initiation study, they were 25 days old, with an average weigth of 86 g for males and 82 g for females. At receipt, and after an acclimatation period of 3 days, an adaptation period of 5 days was applied (rat were exposed at fresh air (2000 L/Hr) during 6 hours per day). They were fed diet and water ad libidum.

Administration/exposure

Two groups of 20 male and 20 female rats were exposed whole body at the target concentrations of 120 and 400 mg/m3 (or 30 or 100 ppm, respectively) of VDC in air. An additional group was exposed in the same conditions to fresh air, and served as control. Five animals were treated per chamber.

The rate flow in the chamber was 2000L/h measured by a rotameter. A syringe with 5 mL of VDC is cooled by a water flow at 16 °C. A constant volume of VDC is evaporated with infusion material. ("UNITA I" from B. Braun, Melsungen). The concentrations were verified. The concentration average of the two groups were 118.8 mg/m3 in the first group (for a target concentration of 120 mg/m3) and 392.4 mg/m3 in the second group (for a target concentration of 400 mg/m3).

External appearance and behavior of the animals were monitored daily, and the mortality was checked daily. Haematology determination, biochemistry and enzymology analysis and urine analysis (Glutamat-Pyruvat-Transaminase and Alkaline Phosphatase) were conducted. Animals were sacrified 3 days after the last exposure. Macroscopic observation, organ weight (heart, liver, kidney, spleen, testis, thyroid, adrenal gland and lung), body weight (on 20 rats) and histology on major organs (eart, aorta, traohea, lung, esophagus, stomach, small intestine, colon, parotid glandular, liver, pancreas, spleen, thymus, lymph nodes, epididymis prostate, semen bubble, ovaries, uterus, skeletal muscle, teeth, skin, eye with optic nerve) were conducted. Statistical analysis on weights was conducted using variance analysis using t-test. Yates' correction and Chi-

squared tests were used for urinalysis.

Results and discussion

No mortality and no clinical signs were observed. No data are available on food consumption. No adverse effect was observed either in haematology or clinical chemistry analysis. No significant variation was observed in body weight or body weight gain.

Variations of absolute or relative organ weights were observed. An increase of absolute kidney weight at 400 mg/m3 and a decrease of adrenal gland at 120 mg/m3 were noted only in females. Increase of relative kidney weight was found in males and females at 400 mg/m3, and only in females at 120 mg/m3. Increase of the relative liver weight was observed in females at 400 mg/m3, and a decrease of the adrenal gland in females at 120 mg/m3. It should be noted that none of these findings were statistically significant.

No relevant histopathology findings was observed. Several pulmonary changes was noted related to the killing mechanism. However the spleen and the kidneys show minor variations in the normal morphological field. Others isolated cases showed insignificant changes (heart, trachea, gland submandibulaire, thyroid).

1.6.2.9 [Gage (1970)]

Study reference:

Gage J.C. 1970: The subacute inhalation toxicity of 109 industrial chemicals (publication), Brit. J. industr. Med., 27:1-18.

Detailed study summary and results:

Test type

In a subacute inhalation toxicity study, vinylidene chloride was administered to 4 Alderley Park rats/sex/concentration by whole body exposure at concentrations of 802 and 2005 mg/m3 for 6 hours/day, 5 days/week and for 4 weeks.

In this assay, 109 substances has been tested and only very few data about either methods or results are available.

Test substance

The substance, as specified in the publication, is l,l-dichloroethene or vinylidene chloride with a boiling point of 31.9°C. No more information is available.

Test animals

The study was conducted on Alderley Park specific-pathogen-free rats with an average weight of 200 g.

They were maintained in the exposure chamber for periods of up to 6 hours, and between repeated daily

exposures they were returned to their cages where food and water were freely available.

Administration/exposure

Groups of 4 males or females rats were treated for 4 weeks, 6-hour/day and 5 days/week, at the dose levels of 200 and 500 ppm (802 and 2005 mg/m3), i.e. a total of 20 dosing days.

Results and discussion

At 2000 mg/m3 (or 500 ppm), nose irritation, retarded weight gain and liver cell degeneration were observed. At 800 mg/m3 (or 200 ppm), only slight nose irritation and no significant findings were noted at the autopsy. No other information is available.

1.6.2.10 [NTP (2015)]

Study reference:

National Toxicology Program 2015: Technical Report NTP TR 582; Toxicology and carcinogenesis of vinylidene chloride (CAS No. 75-35-4) in F344/N rats and B6C3F1/N mice (inhalation studies); National Institutes of Health, Public Health Service, U.S. Department on Health and Human Services.

Detailed study summary and results:

Nota*:* Information below related to study in mice

Test type

This study was designed and conducted to evaluate the toxic effects of repeated exposure to VDC and was used as range-finding study for the carcinogenesis study. It was part of the US National Toxicological Program (NTP). Groups of 10 male and 10 female mice per dose were exposed to 1,1-dichloroethylene by whole body inhalation at 0, 6.25, 12.5, 25, 50 or 100 ppm (female only) in air for 90 days.
The study was similar to OECD Guideline 413 (Subchronic Inhalation Toxicity: 90-Day Study).

Test substance

Vinylidene chloride, manufactured by Dow Chemical Company (Freeport, TX), was obtained in one lot from Sigma-Aldrich. The material was identified as lot SB20019301. Analysis by GC/FID indicated that the test article was stabilized with approximately 300 ppm monomethyl ether hydroquinone (MEHQ) and that the overall purity was greater than 99.9%.

Test animals

The test was conducted on male and female B6C3F1/N mice from the NTP colony maintained at Taconic

Farms, Inc. Animals were 4 to 5 weeks old at receipt, 5 to 7 days old at the start of the study, and 19 to 20 weeks at the necropsy date. They were housed individually. Diet and water were available ad libidum except during exposure period. Groups of 10 male and 10 female animals were exposed per group. The study started after a quarantine period of 12 days.

Administration/exposure

Mice were exposed to VDC vapour by whole body inhalation at 0, 6.25, 12.5, 25, 50 or 100 ppm (female only) in air for 6 hours/day, 5 days/week, and for 90 days. Animals were housed individually in exposure chambers.

The VDC vapour was generated by pumping into a heated glass flask. Nitrogen entered the flask and assisted in vaporizing the chemical. Constant flows through the manifold and into all chambers was ensured. The inhalation exposure chambers were designed so that uniform vapor concentrations could be maintained throughout the chamber. Chamber concentrations of vinylidene chloride was monitored by on-line gas chromatography. The average measured concentrations were in acceptable range: 6.28 ± 0.12, 12.6 ± 0.2, 25.1 ± 0.5 ppm, 50.4 ± 1.0 ppm and 100.0 ± 2.2 for the target concentrations of 6.25, 12.5, 25, 50 and 100 ppm respectively.

Animals were weighted initially, then approximately weekly and at the end of the study. Blood was collected for hematology and clinical chemistry analyses. Necropsies were performed on all core study animals. The heart, right kidney, liver, lungs, right testis, and thymus were weighed. Complete histopathologic examinations were performed on 0, 50 (male), and 100 (female) ppm mice. In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx, liver, lung, lymph nodes (mandibular, mesenteric, bronchial, and mediastinal), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.

Results and discussion

Two 50 ppm males and four 100 ppm females died during the first week of the study; all other mice survived until terminal kill. There were no exposure-related clinical findings.

Table 100: Survival and body weight of Mice exposed to VDC by inhalation

Sex	Concentration (ppm)	Survival [b]	Initial body weight [a]	Final body weight [a]	Change in body weight [a] (g)	Final weight relative to Controls (%)

			(g)	(g)		
Male	0	10/10	23.2 ± 0.4	39.4 ± 1.2	16.2 ± 1.1	-
	6.25	10/10	23.4 ± 0.3	37.8 ± 0.5	14.3 ± 0.3	96
	12.5	10/10	23.2 ± 0.2	**35.5 ± 0.6****	**12.3 ± 0.6****	90
	25	10/10	23.4 ± 0.2	**35.5 ± 0.8****	**10.1 ± 0.8****	85
	50	8/10 [c]	23.9 ± 0.2	**33.0 ± 0.5****	**10.0 ± 0.4****	94
Female	0	10/10	19.6 ± 0.2	35.2 ±1.2	15.6 ± 1.2	-
	6.25	10/10	19.5 ± 0.4	**30.8 ± 0.6****	**11.4 ± 0.7****	88
	12.5	10/10	20.1 ± 0.3	**31.9 ± 0.9****	**11.8 ± 0.7****	91
	25	10/10	19.8 ± 0.3	**30.9 ± 0.8****	**11.1 ± 0.6****	88
	50	10/10	19.6 ± 0.4	**28.7 ± 0.6****	**9.2 ± 0.6****	82
	100	6/10 [c]	19.5 ± 0.4	**29.9 ± 0.8****	**10.0 ± 0.4****	85

** Significantly different (P≤0.01) from the chamber control group by Williams' test
[a] Weights and weight changes are given as mean ± standard error.
Subsequent calculations are based on animals surviving to the end of the study.
[b] Number of animals surviving at 14 weeks/number initially in group
[c] Week of deaths: 1

The final mean body weights and body weight gains of all exposed groups of females and of males exposed to 12.5 ppm or greater were significantly less than the those of the chamber control groups.

Exposure concentration-related decreases (≤ 8%) in erythrocyte counts, hemoglobin concentrations, and hematocrit values occurred at the end of the study in 12.5, 25, and 50 ppm male mice. Female mice had decreased erythrocyte counts in the 50 and 100 ppm groups, but the decreases (≤ 4%) were less than those in males. In addition, hemoglobin concentration and the hematocrit value were decreased in 50 ppm female mice, but not in the 100 ppm group.

In males, absolute kidney weights of all exposed groups were significantly less than that of the chamber control group. In females, absolute and relative liver weights of 12.5 ppm or greater females were significantly greater than those of the chamber controls. Relative liver weights were also increased in 6.25 ppm females. Absolute and relative kidney and lung weights of 100 ppm females were significantly greater than those of the chamber controls. Other organ weight differences were considered to be related to reduced body weight either for male or female mice.

Table 101: Organ weights and organ-weight-to-body-weight ratios for Mice in the 3-month inhalation study of VDC

		Organ weights (g) [a]					
		Controls	**6.25 ppm**	**12.5 ppm**	**25 ppm**	**50 ppm**	**100 ppm**
Sex	**Animals examined**	**10**	**10**	**10**	**10**	**8**	-
M	**Necropsy body weight**	39.4 ± 1.2	37.8 ± 0.5	**35.5 ± 0.6****	**33.5 ± 0.8****	**33.0 ± 0.5****	-
	HEART						
	Absolute	0.16 ± 0.01	0.15 ± 0.00	0.15 ± 0.01	0.15 ± 0.00	0.15 ± 0.01	-
	Relative	4.090 ± 0.083	3.950 ± 0.061	4.171 ± 0.104	**4.516 ± 0.084****	**4.548 ± 0.121****	
	KIDNEY						
	Absolute	0.32 ± 0.01	**0.28 ± 0.01****	**0.26 ± 0.01****	**0.25 ± 0.01****	**0.25 ± 0.01****	-
	Relative	8.073 ±	**7.390 ±**	**7.217 ±**	**7.607 ± 0.162**	**7.421 ± 0.234**	

A L E		0.180	**0.120***	**0.199****			
	LIVER						
	Absolute	1.60 ± 0.05	1.52 ± 0.03	1.62 ± 0.04	1.65 ± 0.05	1.72 ± 0.08	-
	Relative	40.528 ± 0.231	40.138 ± 0.502	**45.631 ± 0.986****	**49.264 ± 0.948****	**51.978 ± 1.596****	
	LUNG						
	Absolute	0.21 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	0.21 ± 0.01	-
	Relative	5.271 ± 0.135	5.248 ± 0.123	**5.878 ± 0.162***	**6.379 ± 0.194****	**6.390 ± 0.262****	
	TESTIS						
	Absolute	0.113 ± 0.002	0.115 ± 0.002	0.111 ± 0.003	0.116 ± 0.002	0.110 ± 0.003	-
	Relative	2.887 ± 0.072	3.033 ± 0.041	**3.138 ± 0.067***	**3.471 ± 0.090****	**3.335 ± 0.102****	
	THYMUS						
	Absolute	0.050 ± 0.003	0.053 ± 0.002	0.047 ± 0.002	0.055 ± 0.002	0.052 ± 0.002	-
	Relative	1.258 ± 0.073	1.395 ± 0.060	1.338 ± 0.045	**1.638 ± 0.052****	**1.575 ± 0.050****	
F E M A L E	**Animals examined**	**10**	**10**	**10**	**10**	**10**	**6**
	Necropsy body weight	35.2 ± 1.2	**30.8 ± 0.6****	**31.9 ± 0.9****	**30.9 ± 0.8****	**28.7 ± 0.6****	**29.9 ± 0.8****
	HEART						
	Absolute	0.15 ± 0.00	0.14 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.15 ± 0.01	0.16 ± 0.00
	Relative	4.176 ± 0.113	**4.589 ± 0.127****	**4.630 ± 0.087****	**4.985 ± 0.061****	**5.123 ± 0.147****	**5.297 ± 0.096****
	KIDNEY						
	Absolute	0.21 ± 0.01	0.21 ± 0.00	0.22 ± 0.01	0.23 ± 0.00	0.22 ± 0.00	**0.24 ± 0.01****
	Relative	6.119 ± 0.175	**6.791 ± 0.125****	**6.990 ± 0.149****	**7.308 ± 0.167****	**7.680 ± 0.161****	**8.114 ± 0.219****
	LIVER						
	Absolute	1.43 ± 0.03	1.41 ± 0.03	**1.55 ± 0.04***	**1.77 ± 0.04****	**1.63 ± 0.05****	**1.87 ± 0.08****
	Relative	40.788 ± 0.973	**45.680 ± 0.880****	**48.769 ± 0.632****	**57.129 ± 0.447****	**56.632 ± 0.571****	**62.493 ± 1.149****
	LUNG						
	Absolute	0.24 ± 0.01	0.22 ± 0.01	0.25 ± 0.01	0.23 ± 0.01	0.25 ± 0.02	**0.29 ± 0.01****
	Relative	6.755 ± 0.325	7.009 ± 0.165	**7.841 ± 0.333***	**7.602 ± 0.291***	**8.613 ± 0.493****	**9.631 ± 0.261****
	THYMUS						
	Absolute	0.058 ± 0.003	0.057 ± 0.003	0.057 ± 0.002	0.067 ± 0.005	0.063 ± 0.002	0.060 ± 0.005
	Relative	1.653 ± 0.091	1.835 ± 0.086	1.795 ± 0.080	**2.162 ± 0.146****	**2.191 ± 0.044****	**2.003 ± 0.144****

***** Significantly different (P≤0.05) from the chamber control group by Williams' or Dunnett's test
****** P≤0.01
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

Gross lesions potentially related to exposure were observed in the lung (5/10) and liver (1/10) of 100 ppm female mice and the liver (1/10) and kidney (2/10) of 50 ppm male mice. Lung lesions included pale to white, 1 to 7 mm diameter foci; affected livers were mottled and/or red, and affected kidneys were diffusely pale and/or granular. Kidney lesions, limited to males, consisted of renal tubule necrosis and protein casts formation in mice that experienced early death and nephropathy in those that survived to terminal kill. Marked necrosis of the renal tubules and protein cast formation occurred in two 50 ppm males. Minimal to moderate nephropathy occurred in the 12.5, 25, and 50 ppm male groups. Decreases in the erythrocyte counts, hemoglobin concentration and hematocrit values also occurred in the same exposed groups.

Non-neoplastic lesions of the liver included necrosis in male and female mice and centrilobular hepatocyte hypertrophy in female mice. Necrosis was marked in early death 100 ppm females and mild in early death 50 ppm males. Hepatic necrosis was not evident in the 50 ppm mice that survived to terminal kill. Mild to moderate centrilobular hepatocyte hypertrophy was observed in six 100 ppm female mice.

Laryngeal lesions consisted of necrosis and respiratory epithelium hyperplasia and squamous metaplasia. Necrosis was minimal and was only seen in early death 100 ppm females. Respiratory epithelium hyperplasia occurred in most 100 ppm females and respiratory epithelium squamous metaplasia occurred in a few males and many females exposed to 25 ppm or greater, with slight increases in severities and incidences in the female mice.

Exposure-related lung lesions were limited to 100 ppm female mice and consisted of bronchial epithelium necrosis and histiocytic inflammation. Bronchial epithelium necrosis occurred in one early death female and five females that survived to terminal kill, and histiocytic inflammation occurred in all of the females that survived to terminal kill. Small numbers of neutrophils were present within alveoli, the interstitium, and bordering vessels.

Several females exposed to 100 ppm vinylidene chloride had minimal to moderate necrosis of the nasal respiratory epithelium and minimal turbinate atrophy. Male mice did not develop exposure-related nasal lesions. Respiratory epithelium necrosis of the nose occurred in all early death female mice. Turbinate atrophy occurred in four 100 ppm females.

Table 102: Incidences of Selected Non-neoplastic Lesions in Mice exposed to VDC by inhalation for 3 Months

Sex	Lesions	Number of animals with lesion [b]					
		Control	6.25 pm	12.5 ppm	25 ppm	50 ppm	100 ppm
Male	**KIDNEY[a]**	10	10	10	10	10	-
	Renal Tubule Necrosis	0	0	0	0	2 (4.0)	-
	Renal Tubule, Casts Protein	0	0	0	0	2 (4.0)	-
	Nephropathy	0	0	**5* (1.2)**	**10** (1.9)**	**8** (2.5)**	-
	LARYNX[a]	10	10	10	10	10	-
	Respiratory Epithelium, Metaplasia, Squamous	0	0	0	1 (1.0)	**4* (1.0)**	-
	LIVER[a]	10	10	10	10	10	-
	Necrosis	0	0	0	0	2 (2.0)	-
Female	**LARYNX[a]**	10	10	10	10	9	10
	Respiratory Epithelium, Hyperplasia	0	0	0	0	0	**8** (1.4)**
	Respiratory Epithelium, Metaplasia, Squamous	1 (1.0)	0	1 (2.0)	3 (1.3)	**9** (1.8)**	**7** (2.4)**
	Necrosis	0	0	0	0	0	**4* (1.0)**
	LIVER[a]	10	10	10	10	10	10
	Necrosis	0	0	1 (1.0)	0	0	**4* (4.0)**
	Hepatocyte, Centrilobular, Hypertrophy	0	0	0	0	0	**6** (2.8)**
	LUNG[a]	10	10	10	10	10	10
	Bronchus, Epithelium, Necrosis	0	0	0	0	0	**6** (2.7)**
	Inflammation, Histiocytic	0	0	0	0	0	**6** (1.7)**
	NOSE[a]	10	10	10	10	10	10
	Respiratory Epithelium, Necrosis						

	Turbinate, Atrophy	0 0	0 0	0 0	0 0	0 0	**4* (2.5)** **4* (1.0)**

* Significantly different (P≤0.05) from the chamber control group by the Fisher exact test
** P≤0.01
[a] Number of animals with tissue examined microscopically
[b] In parenthesis, average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

In conclusion, the target organs were identified to be larynx and liver in both sexes, nose in female mice, and kidney only in male mice.

1.6.2.11 [Maltoni (1977)]

Study reference:

Maltoni C., Cotti G., Morisi L. and Chieco P. 1977: Carcinogenicity bioassays of vinylidene chloride Research plan and early results (publication), Medicina Del Lavoro, 68(4) 241-262.

Maltoni C., Cotti G., and Chieco P. 1984: Chronic toxicity and carcinogenicity bioassays of Vinykidene chloride, Acta oncologica 5 (2).

Roberts S.M., Jordan K.E., Warren D.A., Britt J.K. and James R.C. 2002: Evaluation of the carcinogenicity of 1,1-dichloroethylene (vinylidene chloride) (review article or handbook), Regulatory Toxicology and Pharmacology, 35:44-55.

United States Environmental Protectin Agency 1984: Health assessment document for Vinylidene chloride; Final report EPA/600/8-83/031F

Detailed study summary and results:

Nota*:* Information below related to study in mice

Test type

A carcinogenicity study on VDC was conducted on mice exposed by inhalation. Six groups (one control group and five treated groups) of male and female were treated with VDC by vapour for 52 weeks, 4 hours daily and 4-5 days weekly.

Numerous deviations from OECD guideline 451 were noted: the treatment period is only for one year, and only for 4-5 days per week; the number of animals is insufficient (30 per sex and per group instead of 50 required in the guidelines). Only the early results are available; only incidences about effects in liver and the kidneys were reported; no information either on other examinations (hematology, clinical chemistry, urine analysis, body weight, organ weight…) or statistical methods are available; several shortcomings of the study design and reporting lead to difficulties in the interpretability of the study results.

For more details about the description and results of the study, see section Carcinogenicity, 1.5.2.2.

Conclusion

Non-neoplastic changes, mainly typical necrosis, were found in the liver (hepatocyte vacuolization, swelling, fatty degeneration and necrosis) and in the kidney (swelling and necrosis of tubular cells, amyliodosis of glomureli and chronic nephritis), and both in the control and treated animals. The findings in the liver did not indicate a clear relationship with exposure to VDC. A higher incidence of more pronounced regressive changes were found in kidneys with renal adenocarcinoma.

1.6.2.12 [Maltoni (1977)]

Study reference:

Maltoni C., Cotti G., Morisi L. and Chieco P. 1977: Carcinogenicity bioassays of vinylidene chloride Research plan and early results (publication), Medicina Del Lavoro, 68(4) 241-262.

Maltoni C., Cotti G., and Chieco P. 1984: Chronic toxicity and carcinogenicity bioassays of Vinykidene chloride, Acta oncologica 5 (2).

Roberts S.M., Jordan K.E., Warren D.A., Britt J.K. and James R.C. 2002: Evaluation of the carcinogenicity of 1,1-dichloroethylene (vinylidene chloride) (review article or handbook), Regulatory Toxicology and Pharmacology, 35:44-55.

United States Environmental Protectin Agency 1984: Health assessment document for Vinylidene chloride; Final report EPA/600/8-83/031F

Detailed study summary and results:

Nota*:* Information below related to study in hamsters

Test type

A carcinogenicity study on VDC was conducted on hamsters exposed by inhalation.

Deviations from OECD guideline 451 were noted: the treatment period is for one year, for 4-5 days per week and only one dose was tested; the number of animals is insufficient (30 per sex in the treated group and 18 or 17 per sex in the control group, instead of 50 required in the guidelines); only the early results are available; except histology examinations for tumours analysis, no information either on other examinations (hematology, clinical chemistry, urine analysis, body weight, organ weight…) or statistical methods are

available; several shortcomings of the study design and reporting lead to difficulties in the interpretability of the study results.

For more details about the description and results of the study, see section Carcinogenicity, 1.5.2.23.

Conclusion

No particular changes were found, in either treated or control group.

2 ENVIRONMENTAL HAZARDS

2.1 Degradation

2.1.1 Ready biodegradability (screening studies)

2.1.1.1 [Anonymous (1989, 1992)]

Study reference:

See confidential Annex

Detailed study summary and results:

Test type:

The ready biodegradability of VDC under aerobic conditions was assessed in a closed bottle test performed according to OECD TG 301D.

Test substance:

The substance used in the study is reported as 1,1-dichloroethene (Lot No. FBY01) with a purity of ≥ 99%. MEHQ (Hydroquinone monomethyl ether) was reported as impurity at a concentration of 200 ppm. Supplier information for the test substance is not provided.

Materials and methods:

The inoculum used in the test was activated sludge obtained from a sewage treatment plant in Fukuoka Prefecture. The sludge was filtered through filter paper (No. 2), and the first 200 ml were discarded. The remaining filtrate was aerated and inoculated. The viable bacteria count was 1.0 x 10^5/mL. A suspension of one drop from a pipette with a sharp tip in 1 L of inorganic mineral nutrient solution was used as the test suspension. The test substance concentration was 9.7 mg/L. Aniline (2.0 mg/L) was used as reference

substance. In addition, a simultaneous inhibition test was carried out to check the degradation of aniline in the presence of 1,1-dichloroethylene.

The test solutions were incubated in a stirring incubator in the dark at 20 ± 1°C under airtight conditions. The total test duration was 28 days. Test vessels were removed from the incubator after 0, 5, 15 and 28 days and the amount of dissolved oxygen was measured by the Winkler sodium azide method.

At the end of the test, the amount of the test substance remaining in the test vessel was measured by gas chromatography (GC).

Results:

The biodegradation of VDC based on the oxygen consumption was 0% after 28 days and therefore VDC is considered as not readily biodegrable.

Degradation of reference material (aniline) calculated from oxygen consumption (BOD) was 73% at day 28 after the start of the test, thus confirming that the test conditions were valid. In the presence of VDC, the biodegradation of aniline was 69% after 28 days and therefore the test substance is not considered inhibitory at 9.7 mg/L. The measurement of VDC by GC at the end of the test showed that remaining amount was 76%.

2.1.1.2 [Tabak (1981)]

Study reference:

Tabak H. et al. 1981: Biodegradability studies with organic priority pollutant compounds. (publication), Journal of the Water Pollution Control Federation 53(10): 1503–1518

Detailed study summary and results:

Test type:

The aerobic biodegradation of VDC was assessed in a static-culture flask-screening procedure of Bunch and Chambers (Jour. Water Poll. Control Fed., 39, 181 (1967)) modified.

Test substance:

The substance used in the study is 1,1-dichloroethene. No information about the purity, impurities, batch number or supplier are provided.

Materials and methods:

The biodegradability of VDC was tested in a static-culture flask-screening procedure of Bunch and Chambers. Settled domestic wastewater was used as microbial inoculum. Biochemical oxygen demand (BOD) dilution water containing 5 mg of yeast extract per litre was used as the synthetic medium.

The test substance concentrations were 5 and 10 mg/L. Phenol was used as reference substance. Test solutions were incubated for 7 days under static conditions at 25°C in the dark followed by three weekly

subcultures (adaptation) totaling 28 days of incubation. Blank (inoculum) controls and abiotic (volatilization) controls were included. The microbial degradation of VDC was followed by GC analysis and TOC determination.

The study with VDC was conducted as part of a test battery in which the biodegradability of 114 industrial chemicals was determined and therefore no specific information is provided on the methods used for the test with VDC.

Results:

The loss of VDC determined by GC analysis and TOC determination was 78% and 45% after 7 days at initial concentrations of 5 and 10 mg/L, respectively. After a subsequent subculture of 7 days (gradual adaptation from the microorganisms), the loss of 1,1-dichloroethylene was 100%. The abiotic (volatilization) controls showed a loss of 15 to 24%, at 10 and 5 mg/L over 10 days, respectively. The results of this study showed that VDC is biodegradable under aerobic conditions, with a higher degradation rate following a first experimental period of 7 days.

2.1.1.3 [Fogel (1986)]

Study reference:

Fogel & al. 1986: Biodegradation of chlorinated ethenes by a methane-utilizing mixed culture. (publication), Appl Environ Microbial 51:720-724.

Detailed study summary and results:

Test type:

The biodegradability of VDC under anaerobic conditions was assessed in the presence of a mixed culture isolated from sediment and containing methane-utilizing bacteria.

Test substance:

The test substance, 1,1-dichloroethylene was supplied by Aldrich Chemical Co., with an analytical purity of 99%. No further information on the test substance (impurities and batch number) is provided.

Materials and methods:

The inoculum used in the test was a mixed culture of methane-utilizing bacteria obtained by methane enrichment of a sediment sample. Inoculum and mineral medium (a total of 50 mL) were added to 160 mL serum bottles which were then sealed with a Teflon-coated septum and crimped aluminium cap.

Air (20 mL) was withdrawn from each bottle by using a syringe and replaced with 20 mL of methane. An abiotic control, consisting of a duplicated culture which was killed by injecting 1 mL of 0.1 g/mL sodium azide solution was included. 1,1-dichloroethylene was injected through the Teflon septa of the test vessels.

Agitation during 15 to 30 minutes allowed the volatile test compounds to be equilibrated between the liquid en gas phases. At regular intervals samples were taken by a syringe and the amount of the test substance was measured by gas chromatography (GC).

Results:

VDC was degraded from 630 to 200 µg/L after 2 days of incubation. The corresponding amount of degradation in the abiotic control was from 520 to 350 µg/L (i.e. around 30% of loss of substance). Gas chromatograms indicated that no volatile chlorinated transformation products were produced. The results of this study showed that VDC is biodegradable under anaerobic, methanotrophic conditions.

2.1.2 Aquatic simulation tests

2.1.2.1 [Wilson (1986)]

Study reference*:*

Wilson B.H. et al. 1986: Biotransformation of Select Alkylbenzenes and Halogenated Aliphatic Hydrocabons in Methanogenic Aquifer Material: A Microcosm Study (publication), Environmental Science & Technology 20(10): 997-1002

Detailed study summary and results:

Test type:

The behaviour of commonly occurring contaminants (including VDC) under anaerobic conditions was examined in microcosms constructed with authentic aquifer material that receives municipal landfill leachate and is known to support methanogenesis.

Test substance:

The substance used in the study is 1,1-dichloroethene with a purity of at least 97% (minimum purity for all the tested contaminants). No information about the actual purity, impurities, batch number or supplier are provided.

Materials and methods:

The inoculum used in the test was aquifer material obtained from sites adjacent to the Norman (Oklahoma, US) landfill located on the north bank of the South Canadian River. Solid samples were taken by digging down to the region of methanogenesis and scooping up the aquifer material into sterilized canning jars. Aquifer water was collected from the same site. The redox potential (vs. Ag/AgCl) of the aquifer material was -50 mV.

All manipulations were performed in an anaerobic glovebox to ensure the maintenance of methanogenic conditions. Aquifer material was slurried by the addition of 15% (by weight) aquifer water and then poured into 160 mL serum bottles, resulting in approximately 100 g of aquifer material (wet weight) in each experimental unit. The microcosms were dosed by the addition of 2.0 mL of the dosing solution and hereafter each unit was sealed with a Teflon-coated silicone septum and a crimp cap seal immediately after dosing. The sterile (abiotic) controls were autoclaved overnight at 121°C prior to dosing. The initial average concentration of 1,1-dichloroethylene was 124 µg/L in the living microcosms and 136 µg/L in the autoclaved microcosms. The microcosms were stored upside down in the dark at 17°C (the average groundwater temperature of shallow aquifers in Central Oklahoma).
At regular intervals samples two to four replicate samples were taken by a syringe and the amount of the test substance was measured by gas chromatography (GC/FID). GC/MS was used to obtain information on the material used and to confirm the identity of metabolites.

Results:
The disappearance of VDC was not rapid and a lag period of 16 weeks was required before significant degradation occurred compared to autoclaved controls. After 40 weeks, there was a decrease of 60 to 100 % of the concentration of VDC compared with autoclaved control. After 40 weeks there was a decrease of 80 to 100 % of the concentration of VDC compared with initial concentration. Vinyl chloride was identified as a metabolite of VDC.

2.2 Bioaccumulation

2.2.1.1 [Anonymous (1991, 1992)]

Study reference:
See confidential Annex
Detailed study summary and results:
Test type:
The bioconcentration of VDC was studied in common carp (Cyprinus carpio) under flow-through conditions according to OECD TG 305C and GLP.

Test substance:
The substance used in the study is reported as 1,1-dichloroethene (Lot No. FAX01) with a purity of ≥ 99%. As impurity MEHQ (Hydroquinone monomethyl ether) was reported at a concentration of 200 ppm. Supplier information for the test substance is not provided.

Materials and methods:

In the study, adult common carp (*Cyprinus carpio*), were acclimatized under flow-through conditions at 25 ± 2°C for 37 days in the acclimation tank and 19 days in the test tanks prior to testing. The fish were held in ground water with continuous monitoring of temperature, pH and dissolved oxygen and regular monitoring of other chemical and physical charcteristics. The average weight and length per fish was 22.2 g and 9.5 cm, respectively. The lipid content at test initiation was 3.9%. The fish were fed twice daily with mixed feed pellets for carp at a total rate of approx. 2% of their body weight per day. Feeding was stopped on the day before sampling.

Two dose groups exposed to nominal test substance concentrations of 500 and 50 µg/L (12 fish/group) and a vehicle control group (5 fish) were followed for 6 weeks. The fish were exposed to the test substance in 100 litre glass test tanks suitable for volatile substances under flow-through conditions. The test substance was dissolved into N,N-dimethylformamide to prepare a 10 g/L test substance solution which was further diluted with deionized water to obtain the stock solutions for the 2 concentration levels. The stock solutions (2 mL/min) and test water (800 mL/min) were supplied to the test tanks at a rate of 1155 L/day. During the test the dissolved oxygen concentration ranged between 5.6 and 7.0 mg/L (control group) and 4.2 – 6.2 mg/L (exposure groups).

Analysis of the test solutions was carried out twice weekly during the exposure period, a total of 12 times in the two exposure groups. Analysis of the test fish was carried out at 2, 3, 4 and 6 weeks after the start of the exposure in the two exposure groups. Analysis of the control group fish was carried out before exposure and at the end of the exposure. The VDC concentration was measured by using a validated GC-MS method.

Results:

External visual inspection of the test fish showed no abnormalities. The measured VDC concentrations in the test solutions were maintained on a constant level throughout the test (486 – 493 µg/L and 46.8 – 47.8 µg/L, respectively for the two exposure groups). The bioconcentration factors were 2.5–6.4 (500 µg/L) and <13 (50 µg/L) during the 6 weeks exposure period and it is therefore concluded that VDC has a low potential for bioaccumulation.

2.3 Acute toxicity

2.3.1 Short-term toxicity to fish

2.3.1.1 [Anonymous (1977), Dill (1980)]

Study reference:

Dill A.C., McCarty, W.M, Bartlett, E.A. 1977: Vinylidene chloride toxicity to fathead minnows in a flow-through system (study report), Report date: Oct 13, 1977

See also confidential Annex

Detailed study summary and results:

Test type:

The acute toxicity of VDC was assessed in *Pimephales promelas* using a flow-through system. This study was conducted in accordance with the EPA guideline 'Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians' EPA-660/3-75-009 (1975).

Test substance:

The test substance, VDC with a purity of 99.5% was obtained from C. C. Kazmierski, Inorganic Chemicals TS&D. The impurities were identified as 1,2-dichloroethylene (cis and trans isomers).

Materials and methods:

In the study, adult fathead minnows (*Pimephales promelas*), acclimatized at 12°C for 10 days prior to testing were used. The fish were held in raw dechlorinated Lake Huron water that is well characterized on a regular basis. The average weight and length per fish was 2.0 g and 41 mm, respectively.

The fish were exposed to the test substance under flow-through conditions (1 liter of fresh solution was supplied every seven minutes to the exposure aquaria with a volume of 6.6 liters). Nine dose groups with nominal concentrations of 40, 50, 70, 95, 125, 170, 225, 300 and 400 mg/L, a water control group and a vehicle control (methanol) group (all 10 fish/group) were followed during 13 days and mortality and sublethal effect (loss of equilibrium) was recorded on a daily basis. The VDC concentration was measured 8 times per concentration during the study using a GC-FID method. The exposure concentrations used in the LC50 calculations were the average of the 8 measurements.

Results:

Loss of body equilibrium (swimming disorientation) was the major sublethal effect noted in the study and the affected fish died by day 7. The test temperature was 12°C and the pH ranged from 7.8 – 8.0. The LC50 of 1,1-dichloroethene was 108 (95 % CI: 84.6 – 117.4) mg/l after 96 hours, then stabilized at 29 (95 % CI: 25.0 – 34.1) mg/l from days seven until the test ended.

2.3.1.2 [Buccafusco (1981)]

Study reference:

Buccafusco R.J., Ells S.J. and LeBlanc G.A. 1981: Acute Toxicity of Priority Pollutants to Bluegill (Lepomis macrochirus) (publication), Bull. Environm. Contam. Toxicol. 26:446-452.

Detailed study summary and results:

Test type:

The toxicity of VDC on *Lepomis macrochirus* was tested as part of a study testing 64 chemical compounds for acute toxicity under static conditions according to the guideline "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (U.S. EPA 1975, EPA-660/375-009)".

Test substance:

The substance used in the study is 1,1-dichloroethylene with a minimum purity of 80% (minimum purity for all the tested contaminants). No information about the actual purity, impurities, batch number or supplier are provided.

Materials and methods:

In the study, young bluegill sunfish (*Lepomis macrochirus*), obtained from a commercial fish supplier were used. The fish were held in well-water with weekly monitoring of chemical and physical charcteristics. The average weight of the fish at study inititation ranged between 0.32 – 1.2 g. Feeding was discontinued 48 hours prior to testing. Since the test substance is volatile, capped test jars with a volume of 15 liters were used to control volatilization. Ten fish were randomly selected and added within 30 minutes prior to the addition of the test chemical; the jars were capped immediately following addition of the test chemical. The study was conducted as part of a test battery with several (64) other industrial chemicals and no information is provided on the methods and specific conditions for the test with VDC.

Results:

Measurements of water quality characteristics for all the tested chemicals revealed that dissolved oxygen concentrations were within a range of 9.7 mg/L at the beginning of an exposure to 0.3 mg/L after 96 h exposure. The pH of the test solution ranged from 7.9 – 6.5, and temperature ranged from 21 to 23 °C. The LC50 (96 h) on *Lepomis macrochirus* was determined to be 74 mg/L (95 % CI: 57 – 91 mg/L, nominal concentration).

2.3.1.3 [Dawson (1977)]

Study reference:

Dawson G.W. 1977: The acute toxicity of 47 industrials chemicals to fresh and saltwater fishes (publication), Journal of Hazardous Materials, 1 (1975-1977):303-318.

Detailed study summary and results:

Test type:

The acute toxicity of VDC to fish was tested on the freshwater species *Lepomis macrochirus* and the saltwater species *Menidia beryllina* under static conditions. No guideline was followed. The study was

performed in a static test system with aeration of the water and results expressed in terms of nominal concentrations.

Test substance:

In the publication, the substance is described as vinylidene chloride (1,1-dichloroethylene). No information about the purity, impurities, batch number or supplier are provided.

Materials and methods:

In the study, adult bluegill sunfish (*Lepomis macrochirus*) and tidewater silversides (*Menidia beryllina)*, were acclimatized for 14 days prior to testing. The bluegill sunfish were obtained from a commercial hatchery while the tidewater silversides were collected from "Horseshoe Bay" at Sandy Hook, New Jersey. Potable well water, obtained from an underground source in Passaic County, was used as the holding water and dilution water for the freshwater species. The pH of the water was 7.6 – 7.9 with a hardness of 55 mg/L (as $CaCO_3$). The same potable well water was mixed with “Instant Ocean” synthetic sea water salt mix until a specific gravity of 1.018 was achieved, and used for the saltwater species.

The test temperature was 23°C (*Lepomis macrochirus*) and 20°C (*Menidia beryllina*). The average length at study initiation was 33 to 75 mm (*L. macrochirus*) and 40 to 100 mm (*M. beryllina*). The fish were not fed during the test. For *L. macrochirus*, five dose groups with nominal concentrations of 132, 180, 320, 560 and 750 mg/L were used and for *M. beryllina*, four dose groups with nominal concentrations of 180, 250 and 320 mg/L. The freshwater solutions were aerated if dissolved oxygen was being depleted, but not during the first 24 hours. The salt water solutions were continuously aerated during the test. The test duration was 96 hours and mortality counts were taken daily.

The study with VDC was conducted as part of a test battery with 47 industrial chemicals and no information is provided on the methods used for the test with VDC.

Results:

No data on pH, dissolved oxygen concentration and test substance concentration during the test were reported. The LC50 (96 h) on *Lepomis macrochirus* was determined to be 220 mg/L. The LC50 (96 h) on *Menidia beryllina* was determined to be 250 mg/L (no CI mentionned).

2.3.1.4 [Heitmuller (1981)]

Study reference:

Heitmuller P.T., Hollister T.A. and Parrish P.R. 1981: Acute toxicity of 54 Industrial Chemicals to Sheepshead Minnows (*Cyprinodon variegatus*) (publication), Bull. Environm. Contam. Toxicol., 27:596-604.

Detailed study summary and results:

Test type:

The toxicity of VDC was tested on the marine species *Cyprinodon variegatus* according to the guideline "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (U.S. EPA 1975, EPA-660/375-009)". The study was conducted as part of a test battery with 54 industrial chemicals. The study was conducted under static conditions and results were expressed in terms of nominal concentrations.

Test substance:

The substance used in the study is 1,1-dichloroethylene with a minimum purity of 80% (minimum purity for all the tested contaminants). No information about the actual purity, impurities, batch number or supplier are provided.

Materials and methods:

In the study, 14 – 28 days old juvenile sheepshead minnows (*Cyprinodon variegatus*) obtained from he EPA's Environmental Research Laboratory, with a length of 8 – 15 mm at study initiation were used. Fish were maintained in flowing, filtered seawater of ambient salinity from 10-31 ‰, and a temperature from 25 – 31°C.

Ten fish were used per concentration, the fish were not fed during test test. Dissolved oxygen was measured at test initiation and daily thereafter; pH was measured in the control, low and high test concentrations at test initation and after 96h of testing. The test solutions were not aerated during the test. Mortality was recorded on a daily basis. The study with VDC was conducted as part of a test battery with 54 industrial chemicals and therefore no information is provided on the methods and specific conditions used for the test with VDC.

Results:

The 96h LC50 of VDC on *Cyprinodon variegatus* was determined to be 250 (95 % CI: 200 – 340) mg/L.

2.3.2 Short-term toxicity to aquatic invertebrates

2.3.2.1 [Anonymous (2010)]

Study reference:

See confidential Annex

Detailed study summary and results:

Test type:

The acute toxicity of VDC to *Daphnia magna* was assessed in a GLP compliant study according to the OECD Guideline 202.

Test substance:

The test substance, as cited in the report "1,1-dichloroethene", was supplied by Solvay, Solvin France S.A., batch 9M135, with an analytical purity of 99.95%.

Materials and methods:

In the study, *Daphnia magna* Straus from a stock breeding in the laboratory were used. Based on the results of a preliminary test, the study was conducted at nominal concentrations of 0, 25, 32.8, 43.2, 57.4, 76 and 100 mg/L. The study was performed using 30 mL glass flasks stoppered with PTFE bungs and sealed with aluminium caps in order to avoid the loss of the test item. Approximately 90% of the final volume of the test solutions was added into the test flasks. Five *Daphnia magna* aged from 6 to 24 hours were added into each test flask, which were then totally filled with the test solution. Four replicates were prepared for each concentration and control group (dilution water without test substance). Test flasks were incubated in the dark at a temperature of 20.5 ± 1°C. Immobility was recorded after 24 and 48 hours. Dissolved oxygen and pH were measured at the highest concentration and in the control at the beginning and at all concentrations and in the control at the end of the test.

The VDC concentration was measured at test initiation and at the end of the test using a validated GC-MS method. The EC50 values were determined based on geometric average values of initial and final measure concentrations being 0 (< LOD), 16.0, 20.5, 29.4, 36.8, 49.3 and 70.9 mg/L.

Results:

No immobility was observed in the control glass beakers. The dissolved oxygen concentration in the test solutions was higher than 60% of the air saturation value. The 48h EC50 based on immobility was calculated with Probit analysis and was equal to 37 mg/L. No 95% confidence intervals could be determined.

2.3.2.2 [Dill (1980)]

Study reference:

Dill D.C., McCarty, W.M, Alexander, H.C., Bartlett, E.A. 1980: Toxicity of 1,1-dichloroethylene (Vinylidene chloride) to aquatic organisms. Environmental Research Laboratory, U.S. Environmental Protection Agency, Duluth, Minnesota. Report date: July 1980

Detailed study summary and results:

Test type:

The toxicity of VDC was tested on *Daphnia magna* under static conditions. This study was conducted in accordance with the EPA guideline 'Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians' EPA-660/3-75-009 (1975). Results are expressed in terms of nominal concentrations.

Test substance:

The test substance, 1,1-dichloroethylene with a purity of >99.5% was obtained from C. C. Kazmierski, Inorganic Chemicals TS&D. The impurities were identified as 1,2-dichloroethylene (cis and trans isomers).

Materials and methods:

Daphnia magna, less than 24 hours old, were used in the 48 - hour static toxicity test. The daphnids were held in carbon filtered raw dechlorinated Lake Huron water that is well characterized on a regular basis. Stock cultures were maintained in 18L glass aquaria at 17°C ±1°C, with 16-hour light/8-hour dark cycle. Stock solutions of vinylidene chloride (VDC) were prepared in methanol both to dilute the test material and to facilitate rapid mixing with water. The final volume of the test solution was 200 mL in a 250 mL glass test beaker. It is not mentioned that the test beakers were closed during the experimentation. A water and a solvent control with methanol were also set. Ten water fleas were added to each beaker and the beakers set in a constant temperature incubator (17°C) having a 16-hour light / 8-hour dark cycle. There were three beakers for each exposure concentration and each control. Mortality data were recorded at 24 and 48 hours. Dead organisms were not removed from any test beaker during the test. The static water flea test EC50 values were calculated using nominal VDC water concentrations. A computer program of Finney's methods of probit analysis (Finney, 1952) was utilized to calculate the EC values, the confidence interval, and the slope of the regression curve.

Results:

The 48-hour static EC50 for daphnids was 11.6 (9.0 to 14.0) mg/L.

2.3.2.3 [LeBlanc (1980)]

Study reference:

LeBlanc GA 1980: Acute Toxicity of Priority Pollutants to Water Flea (*Daphnia magna*) (publication), Environm. Contam. Toxicol., 24:684-691.

Detailed study summary and results:

Test type:

The toxicity of VDC was tested on *Daphnia magna* under static conditions according to the guideline "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (U.S. EPA 1975, EPA-660/375-009)". Static test system was used and results are expressed in terms of nominal concentrations. The study with VDC was conducted as part of a test battery with several other industrial chemicals

Test substance:

The substance used in the study is 1,1-dichloroethylene with a minimum purity of 80% (minimum purity for all the tested contaminants). No information about the actual purity, impurities, batch number or supplier are provided.

Materials and methods:

In the study, *Daphnia magna* (≤ 24 hours old) originating from laboratory stocks were used. Two-litre test vessels were filled with 500 mL of test solution and 15 daphnids were added. Since the test substance is volatile, the test vessels were covered with a plastic wrap secured with an elastic band. A minimum of 5 test concentrations, and one replicate per concentration was used. Observations for mortality were made at 24 and 48 hours of exposure. During the test, the dissolved oxygen concentration, pH and temperature of the test solutions were measured at test initiation and termination.

The study was conducted as part of a test battery with several other industrial chemicals and therefore no information is provided on the methods and specific conditions for the test with VDC.

Results:

Dissolved oxygen concentrations for all the chemicals tested ranged from 6.5 – 9.1 mg/L during the 48-hour exposure period and the temperature of test solutions was maintained at 22 ± 1°C. The EC50 (48h) of 1,1-dichloroethene on *Daphnia magna* was determined to be 79 mg/L (95 % CI: 62 – 110 mg/L).

2.3.3 Algal growth inhibition tests

2.3.3.1 [Geyer (1985)]

Study reference:

Geyer H., Scheunert I. and Korte F. 1985: The effects of organic environmental chemicals on the growth of the alga Scenedesmus subcapitatus: a contribution to environmental biology (publication), Chemosphere, 14(9):1355-1369.

Detailed study summary and results:

Test type:

The toxicity of VDC was tested on *Scenedesmus subspicatus* according to the test guideline of the Federal Environmental Agency (Umweltbundesamt). Results were expressed as nominal concentrations and closed test systems were used.

Test substance:

The substance used in the study is 1,1-dichloroethylene with a purity of 99%. No information about the impurities, batch number or supplier are provided.

Materials and methods:

300-mL Erlenmeyer containing 80 mL of test solutions and closed with Kapsenberg caps were used. The treatment groups and the controls consisted of 4 replicates each containing 10000 cells/mL at test initiation. The test solutions were incubated at a temperature of 22 ± 2°C and under constant illumination at an intensity of 120 $\mu E \cdot m^{-2} \cdot s^{-1}$. The flasks were shaken 2 to 3 times per day. After 0, 72 and 96 hours, the cell growth of cell suspensions was measured at 578 nm using a ZEISS spectrophotometer.

The study was conducted as part of a test battery with several other industrial chemicals and therefore no information is provided on the methods and specific conditions for the test with VDC.

Results:

The EC10 and EC50 at 96 h on *Scenedesmus subspicatus* was determined to be 240 mg/L and 410 mg/L, respectively. No further details on the results (CI, pH variation) are provided.