26-Week Carcinogenicity Study of 1,2-Dichloroethane by Dermal Application in CB6F1-Tg rasH2 Mice

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Background and Objective

A typical 26-week rasH2 mice carcinogenesis study has been approved as an alternative to the conventional bioassay indicated by regulatory authorities.

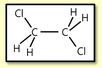
However, percutaneous absorption plaster has not been scope of application in the recognition of alternatives.

It has been reported that 1,2-dichloroethane (DCE) caused lung carcinogenicity by repeated topical application to back skin of CD-1(ICR) mice.

In the present study, we examined whether or not the carcinogenic potential of DCE could be detected by typical 26-week carcinogenicity study through topical application to rasH2 mice.

1,2-dichloroethane (DCE)

Molecular formula: $C_2H_4Cl_2$



Manufacture:
Wako Pure Chemicals Industries, Ltd.
Cas No.107-06-2

Appearance and property:
Colorless liquid with chloroform-like odor.

Mutagenicity tests:
Positive in *in vivo* and *in vitro* mutagenicity tests.
(Positive in in vivo DNA damage test and negative in micronucleus Test)

The common use:
The most common use of 1,2-dichloroethane is in the production of vinyl chloride which is used for making a variety of plastic and vinyl products.

Carcinogenicity:
DCE is categorized group 2B carcinogen by IARC (1999)
(Possibly carcinogenic to humans)

Animal experimental data:
Incidence of lung tumors, but not skin tumors, significantly increased in female ICR mice by topical application of DCE at a dose of 126 mg/day (440 to 594 day).
(Journal of the National cancer Institute, Vol. 63, No.6, 1433-1439, 1979)
Toxic effects on the kidney such as regeneration of tubular cell and karyomegaly were seen when DCE was administered by mixing with the drinking water for 13-week period. (NTP TOX 4, NIH Publication No.91-3123, 1991)

Material and Method

Animal: Both sexes of rasH2 mice 7-week old (10 mice in each sex per group)

Supplier: CLEA Japan Inc.

Test chemical:
Acetone (100 μL, 1 time/day)
80% ethanol (100 μL, 1 time/day)
1,2-dichloroethane (200 μL, 3 times/week)

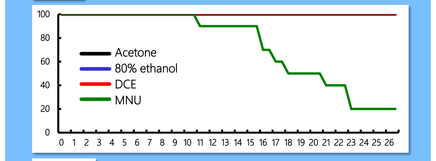
Administration route and period:
Topical application for 26 weeks

Positive control:
N-Nitroso-*N*-methylurea (MNU) was administered by single i.p. injection at a dose of 75 mg/kg

Observation and Examination Items:
Survival rate, body weight, gross pathological examination, organ weights and histopatholoical examination.

Statistical analyses:
The variances in body weight, and organ weight data were assessed using the F test. If homogeneous, the data analyzed with Student's *t* test and if not then with Welch's test. The incidences of gross pathology and histopathology variances were evaluated with the Fisher's exact probability test. The Wilcoxon test was employed for comparison of lesions with degrees. The levels of significance were set at $P<0.05$ and $P<0.01$.

<Male>



<Female>

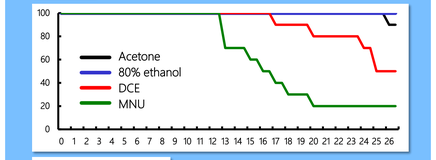


Fig.1 Survival rate

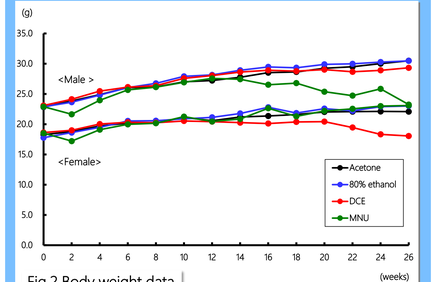


Fig.2 Body weight data

Table 1 Gross pathological findings (Lung)

Findings	Male				Findings	Female			
	Acetone	80% ethanol	DCE	MNU		Acetone	80% ethanol	DCE	MNU
Discolored	0	0	0	0	Discolored	0	0	2	0
Discolored spot/area, single	0	1	3	2	Adhesion to diaphragm	0	0	1	0
Discolored spot/area, several	0	0	0 **	0	Discolored spot/area, single	0	1	1	2
Discolored spot/area, multiple	0	0	4	1	Discolored spot/area, several	0	0	1 *	2 *
Discolored nodule, single	1	0	1	0	Discolored spot/area, multiple	0	0	3	0
Discolored nodule, several	0	0	0	0	Discolored nodule, single	0	0	0	0
Discolored nodule, multiple	0	0	3	0	Discolored nodule, several	0	0	2 **	0
					Discolored nodule, multiple	0	0	5	0

**; P<0.01 (vs. acetone)

*, **; P<0.05 or 0.01 (vs. acetone)

Table 2 Organ weight data (Lung)

Test Chemicals	Male		Test Chemicals	Female	
	Absolute weight (g)	Relative weight (%)		Absolute weight (g)	Relative weight (%)
Acetone	0.164±0.051	0.541±0.171	Acetone	0.147±0.011	0.649±0.034
80% ethanol	0.155±0.012	0.509±0.046	80% ethanol	0.145±0.006	0.621±0.041
DCE	0.194±0.035	0.657±0.120	DCE	0.567±0.055**	3.186±0.222**
MNU	0.141±0.001	0.646±0.125	MNU	0.154±0.011	0.720±0.004

**; P<0.01 (vs. acetone)

Table 3 Histopathological findings (Lung)

Findings	Male				Female			
	Acetone	80% ethanol	DCE	MNU	Acetone	80% ethanol	DCE	MNU
No. of animals / group	10	10	10	10	10	10	10	10
Hyperplasia, bronchiolo-alveolar	0	0	1	0	0	0	6 **	1
Adenoma, bronchiolo-alveolar	0	1	8 **	3	0	1	7 **	3
Adenocarcinoma, bronchiolo-alveolar	0	0	5 *	0	0	0	10 **	1
Hemangiosarcoma	1	0	0	0	0	0	0	0

*, **; P<0.05 or 0.01 (vs. acetone)

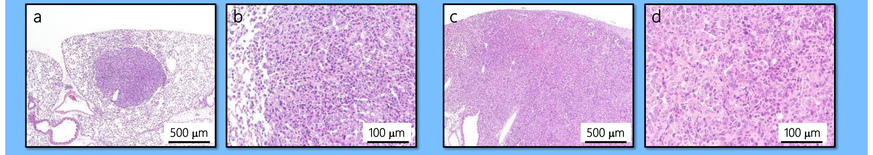


Fig. 3 H&E staining for typical adenoma (a and b) and adenocarcinoma (c and d)

Table 4 Histopathological findings (other than lung)

Organ & Findings	Male				Female			
	Acetone	80% ethanol	DCE	MNU	Acetone	80% ethanol	DCE	MNU
No. of animals / group	10	10	10	10	10	10	10	10
Spleen								
Hemangioma	0	0	0	2	0	0	0	1
Hemangiosarcoma	0	0	0	2	0	0	0	1
Bone marrow								
Hemangioma	0	0	0	1	0	0	0	0
Thymus								
Hemangioma	0	0	0	1	0	0	0	1
Tongue								
Papilloma, squamous cell	0	0	0	1	0	0	0	0
Stomach								
Papilloma, squamous cell	0	1	1	9 **	0	0	1	9 **
Carcinoma, squamous cell	0	0	0	1	0	0	0	1
Duodenum								
Adenoma	0	0	0	0	0	0	0	1
Jejunum								
Adenocarcinoma	0	0	0	2	0	0	0	0
Ileum								
Adenoma	0	0	0	0	0	0	0	1
Adenocarcinoma	0	0	0	1	0	0	0	1
Pancreas								
Degeneration, acinar	0	0	0	0	0	0	9 **	2
Liver								
Sarcoma, NOS	0	0	0	1	0	0	0	0
Kidney								
Degeneration, tubule	0	0	0	0	0	0	10 **	0
Karyomegaly	0	0	10 **	0	0	0	10 **	0
Adenoma, renal cell	0	0	0	1	0	0	0	1
Carcinoma, renal cell	1	0	0	0	0	0	0	0
Ovary								
Hemangioma	-	-	-	-	1	0	0	0
Uterus								
Adenoma	-	-	-	-	0	0	0	2
Hemangioma	-	-	-	-	0	0	0	1
Polyp, endometrial stromal	-	-	-	-	0	0	1	1
Adenocarcinoma	-	-	-	-	0	0	0	1
Hemangiosarcoma	-	-	-	-	1	0	0	0
Musculature								
Myopathy	10	7	8	7	9	10	8	5
Skin/subcutis								
Papilloma, squamous cell	0	0	0	3	0	0	0	4 *
Eye								
Atrophy of retina	0	0	0	9 **	0	0	0	4 *
Harderian gland								
Adenoma	0	0	0	0	0	0	0	3
Abdominal cavity				[1]a				[1]a
Hemangiosarcoma	-	-	-	1	-	-	-	1
All sites				[6]a	[1]a			[7]a
Malignant lymphoma	-	-	-	6	1	-	-	6
Sarcoma, histiocytic	-	-	-	-	-	-	-	1
Urethra						[1]a		[4]a
Papilloma, transitional cell	-	-	-	-	-	1	-	4

*, **; P<0.05 or <0.01 (vs. acetone) a : Numbers in square bracket are for animals examined microscopically.

Results

Survival rate

Dead or moribund animals were found after week 17 in DCE treated female mice and their numbers gradually increased and the survival rate reached 50% at the end of the study.
In the positive control group, both of male and female mice were dead or sacrificed moribundly during the experimental period, and survival rate reached 20% at the end of the study.

Body weight data

The final body weights were significantly decreased in female mice treated with DCE, but not in males as compared with control.

Gross pathological findings (Table 1)

Discolored area or nodule of lung were observed in 9 and 10 mice of male and female DCE group.

Organ weight data (Table 2)

Absolute and relative weight of lung in female DCE group were higher with statistical significance as compare to the acetone group.

Histopathological findings (Table 3 and 4)

The gross lesions were diagnosed as alveolar hyperplasia, adenoma and adenocarcinoma of lung. The incidence of adenoma and adenocarcinoma was 80% and 50% in male and 70% and 100% in female mice, respectively, and statistical significance was observed as compared to the acetone group (0%).
Various neoplastic lesions were seen in MNU treated group and sensitivity of rasH2 mice used in this study was confirmed.

Conclusions

The lung carcinogenicity of 1,2-dichloroethane could be detected by the repeated topical application in rasH2 mice for 26 weeks. Acetone and 80% ethanol did not show carcinogenicity in this study.

Thus, present study indicated that the carcinogenicity of the substance topically applied to skin could be elucidated by this 26-week carcinogenesis study using rasH2 mice.

COI
The authors have no financial conflicts of interest to disclose concerning the presentation