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DOW THE DOW CHEMICAL COMPANY

MIDLAND, MICHIGAN 48674

March 6, 1986

FYI-91-001135

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Dr. Louis Borghi
Dynamac Corporation
The Dynamac Building
11140 Rockville Pike
Rockville, MD 20852

Dear Dr. Borghi:

Enclosed is a copy of the recently completed inhalation teratology study for ethyl chloride (CAS #75-00-3) which I mentioned in our February 19, 1986, telephone conversation. The reviewer, Dr. Tim Landry, requests that you bring the information to the attention of Dr. Joseph Roycroft, the National Toxicology Program (NTP), who is a colleague of his.

Dr. Brink, Executive Secretary of the Interagency Testing Committee, has the exposure information which was submitted in response to your original request for information.

I hope these materials will be useful in the ITC discussions of this chemical.

Sincerely,

June Turley

June W. Turley, Ph.D.
Regulatory and Legislative Issues
Health and Environmental Issues
1803 Building
517/636-5443

P

Attachment

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ETHYL CHLORIDE: INHALATION TERATOLOGY STUDY IN CF-1 MICE

By:

B. H. Scortichini, K. A. Johnson, J. J. Momany-Pfruender, and T. R. Hanley, Jr.

Reviewed By:

T. D. Landry

January 30, 1986

Mammalian and Environmental Toxicology Research Laboratory
Health and Environmental Sciences, USA
Dow Chemical U.S.A.
Midland, Michigan 48674

DOW CONFIDENTIAL

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SUMMARY

The objective of this study was to evaluate the teratogenic potential of inhaled ethyl chloride (EtCl) in CF-1 mice. Groups of 30 bred mice were exposed to EtCl vapors at concentrations of 0, 500, 1500 or 5000 ppm (0, 1.3, 4.0 and 13.2 mg/ℓ air) for 6 hours per day on days 6 through 15 of gestation. No significant effects on maternal body weights, liver weights, reproductive parameters or fetal body weights were observed at any of the exposure levels tested. Examination of fetal mice for external, visceral and skeletal malformations failed to produce any indication of a teratogenic response. A small increase in the incidence of foramina of the skull bones (small centers of unossified bone) suggestive of, at most, very slight fetotoxicity was observed only at the high exposure concentration (5000 ppm). In conclusion, exposure to ethyl chloride at concentrations up to 5000 ppm during the period of major organogenesis did not produce a teratogenic response in fetal mice.

INTRODUCTION

Ethyl chloride (monochloroethane, EtCl, CAS No. 75-00-3) is a colorless, flammable gas that is reportedly sweet tasting and has an ethereal odor. Ethyl chloride has been used as a chemical intermediate, as an anesthetic, and, to a limited degree, as a refrigerant. Few toxicologic problems have been encountered in industry. The major industrial hazards appear to be due to fire and explosion (Torkelson and Rowe, 1981).

The acute toxicity of EtCl was first studied in consideration of its use as a general anesthetic and as a refrigerant. Sayers et al. (1929) reported acute toxicity in guinea pigs exposed to EtCl vapor. A 30-minute exposure to 9.1% EtCl caused some deaths; however, exposure to 1% for 810 minutes caused no deaths. A 2-hour LC50 of 152 mg/l (57,600 ppm) for rats has been reported (Troshina, 1964). In humans, 19,000 ppm caused weak analgesia within 12 minutes; exposure to 33,000 ppm rapidly resulted in incoordination, cyanosis (within 8.5 minutes), and nausea (Davidson, 1926).

A two-week inhalation toxicity study was conducted by Landry et al. (1981). Fischer 344 rats and Beagle dogs were exposed to 0, 1600, 4000, or 10,000 ppm EtCl for 6 hr/day for 2 weeks. The only reported effect was a slight increase in mean relative liver weights of male rats exposed to 4000 and 10,000 ppm. There were no histopathologic effects observed.

The National Toxicology Program (1981) has recently conducted a 13-week inhalation toxicity test of ethyl chloride. Fischer 344 rats and B6C3F1 mice were exposed to concentrations of 0, 2500, 5000, 10,000 and 19,000 ppm daily for 13 weeks. The "Prechronic Test Phase Review" identified no histopathological changes in rats or mice related to the ethyl chloride exposures. Hyperactivity was reported in mice exposed to

10,000 and 19,000 ppm during exposure, and for a short period after exposure. This hyperactivity was observed during weeks 7 to 13 of the 13-week study, and females were reportedly more affected than males. The mean relative liver weight of female mice and male rats exposed to 19,000 ppm appeared to be increased relative to controls. Based on these findings, the NTP chose 15,000 ppm as the highest exposure concentration for the chronic study.

A probe study was conducted to establish appropriate exposure levels for the definitive teratology study in mice. Groups of 8-10 bred female CF-1 mice were exposed to 0, 5000, 10,000 and 15,000 ppm of EtCl for 6 hrs/day on days 6 through 15 of gestation. Animals at all exposure levels exhibited increased activity during exposure as compared to controls. In addition, most of the treated mice exhibited stereotypic activity characterized by highly repetitive running patterns. Body weights on day 16 of gestation and body weight gains on days 10-16 of gestation were significantly decreased for all treatment groups as compared to controls. No treatment-related effects on reproductive parameters were observed at any exposure level and gross necropsy revealed no treatment-related lesions (Scortichini et al., 1985). Based on the significantly decreased body weight gains, exposure levels of 500, 1500, and 5000 ppm were selected for the definitive teratology study.

The objective of this study was to evaluate the teratogenic potential of inhaled ethyl chloride in mice. This study was conducted in accordance with the spirit of FDA Good Laboratory Practice Procedures for Non-Clinical Studies (Federal Register, December 22, 1978), the Environmental Protection Agency (EPA): Good Laboratory Practice Procedures (Federal Register, November 29, 1983) and the Standard Operating Procedures of the Mammalian and Environmental Toxicology Research Laboratory.

MATERIALS AND METHODS

Test Material:

Chemical Name: Ethyl Chloride

Structural Formula: CH_3CH_2Cl

Molecular Weight: 64.5 Physical State: Gas
Abbreviation: EtCl Purity: 99.7% (by specification)
Vapor Pressure: 1106 mmHg @ 23.5°C
Explosive Limits: 3.8 to 15.4%

The test material (Ref. #901-77858-1) was obtained from Air Products and Chemicals (Tamaqua, PA). Analysis of the test material indicated a purity of 99.9%.

Test Animals:

Stock supplies of male and female CF-1 mice were obtained from Charles River Breeding Laboratories, Inc. (Portage, MI). This strain was selected as the test species based upon its historical use for teratology studies within this laboratory. Upon receipt at the laboratory[1], the mice were examined for health status by the laboratory veterinarian and acclimated to the laboratory conditions for at least two weeks prior to breeding. Animals were housed in wire bottom cages in a room designed to control temperature at approximately 22°C, relative humidity at approximately 50%, and a 12 hour photoperiod. Adult virgin females weighing approximately 25-30 grams at breeding were bred to adult males (two females to one male) from the same strain. Day 0 of gestation was determined by the presence of a vaginal plug upon visual examination. Randomization of the animals into test groups was performed using computer-generated tables of random numbers based on their day 0 of gestation. All animals placed on study were uniquely identified by means of numbered metal ear tags. Food (Certified Laboratory Animal Chow No. 5002, Ralston Purina Company, St. Louis, MO) and municipal tap water were available ad libitum except during actual inhalation exposure periods. Analysis of Purina Certified Chow was provided by Ralston Purina Company to confirm that the diet provides adequate nutrition, and to quantify the levels of selected contaminants associated with the formulation process. Analysis of tap water was performed according to the Standard Operating Procedures of the Mammalian and Environmental Toxicology Research Laboratory.

[1]Fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

Experimental Design:

Groups of 30 bred female CF-1 mice were exposed by inhalation to levels of 500, 1500 and 5000 ppm of EtCl for 6 hr/day on days 6 through 15 of gestation. The high exposure concentration was selected on the basis of the results of a probe study conducted in this laboratory where increased activity during exposure and decreased maternal body weights were seen at 5000 ppm. Other concentrations were selected at regularly decreasing intervals. A control group of comparable size was exposed to room air in an inhalation chamber in a manner similar to the treated groups.

Exposure Conditions:

Mice were exposed in 157 liter stainless steel and glass Rochester-type inhalation chambers. Airflow was maintained at approximately 30 liters/min. Test atmospheres of EtCl were generated by pumping from Tedlar* gas sampling bags containing 100% EtCl into a chamber inlet duct. The EtCl was mixed with air in this duct, and the mixture was drawn into the chambers at approximately 30 liters/min. An FMI pump (Fluid Metering Inc., Oyster Bay, NY) was used to pump the EtCl gas into the inlet duct.

Analysis of chamber atmospheres was performed with a Wilks Miran I infrared analyzer set at 10.2 μ wavelength. The chambers were sampled at approximately 2 liters/min using an automated stream selector. The infrared spectrometer was utilized in conjunction with a microcomputer that records the chamber concentration. Each chamber was analyzed at least once/two hours.

The infrared spectrometer was calibrated with SARAN@ bags containing known concentrations of EtCl. Chamber EtCl concentrations were determined by interpolation (by the microcomputer) from a standard curve. Daily calibration checks were conducted while the exposures were in progress. Nominal chamber concentrations were estimated based on the chamber airflow (~30 liters/min) and the EtCl flow rate into the chamber. The EtCl flow rate was estimated based on calibration of the FMI pump with a soap bubble flowmeter.

*Trademark of DuPont, Inc.
@Trademark of The Dow Chemical Company.

Daily maximum and minimum chamber temperatures were recorded, and a single daily determination of relative humidity was made.

Maternal Observations:

Animals were observed daily throughout the experimental period for indications of toxicity from the test material. Body weights for mice were recorded on gestation days 6, 9, 12, 15 and 18; food and water consumptions were recorded for each mouse at 3-day intervals beginning on day 6 of gestation. Maternal liver weights and gravid uterine weights were recorded at the time of cesarean section; sections of liver were saved in neutral, phosphate-buffered 10% formalin, but were not examined histologically.

Fetal Observations:

Test animals were sacrificed by carbon dioxide inhalation on day 18 of gestation and the uterine horns exteriorized through a mid-line abdominal incision. The following data were recorded: 1) number and position of fetuses *in utero*, 2) number of live and dead fetuses, 3) number and position of resorption sites, 4) weight and sex of each fetus and 6) any gross external alteration. The uteri of apparently nonpregnant animals were stained with a 10% solution of sodium sulfide (Kopf *et al.*, 1964) and examined for evidence of implantation sites. This procedure was conducted solely to calculate the incidence of pregnancy; data obtained was not used to calculate the incidence of resorption. One-half of each litter, selected using a table of random numbers (Steel and Torrie, 1960), was examined immediately by dissection under a low power microscope for evidence of visceral alterations (Staples, 1974). For all fetuses, the heart was examined for cardiac anomalies.

The heads of mouse fetuses examined by dissection were removed, placed in Bouin's fixative and examined by the serial sectioning technique of Wilson (1965). All fetuses were then preserved in alcohol, eviscerated and subsequently cleared and stained with alizarin red-S (Dawson, 1926) and examined for skeletal alterations.

Statistical Evaluation:

Descriptive statistics (means and standard deviations) are reported for food and water consumption. Maternal body weights and body weight gains, fetal body weights (litter means) and absolute and relative organ weights were evaluated by Bartlett's test for equality of variance (Winer, 1971). Based on the outcome of Bartlett's test, a parametric (Steel and Torrie, 1960) or nonparametric (Hollander and Wolfe, 1973) analysis of variance (ANOVA) was performed. If the ANOVA was significant, analysis was performed by Dunnett's test or the Wilcoxon Rank Sum test with Bonferroni's correction (Miller, 1966). Statistical evaluation of resorptions among litters and the fetal population and frequency of fetal alterations was performed using a censored Wilcoxon test (Haseman and Hoel, 1974) with Bonferroni's correction. Number of implants and litter size were analyzed with a non-parametric ANOVA followed by the Wilcoxon Rank-Sum test (Hollander and Wolfe, 1973) with Bonferroni's correction. The pregnancy rate was analyzed by the Fisher exact probability test (Siegel, 1956). Statistical outliers were identified by a sequential outlier test (Grubbs, 1969), but only outlying food and water consumption values were excluded.

The nominal alpha levels used are as follows:

Bartlett's Test of Variance	α=0.01
Analysis of Variance	α=0.10
Dunnett's Test	α=0.05, two-sided
Wilcoxon Rank Sum Test	α=0.05, two-sided with Bonferroni correction
Fisher's Test	α=0.05, one-sided
Censored Wilcoxon Test	α=0.05, one-sided
Grubb's Test	α=0.02, two-sided

Because numerous measurements are statistically compared in the same group of animals, the overall false positive rate (Type I errors) is much greater than the cited alpha levels would suggest. Therefore, the final interpretation of the numerical data considered statistical analyses along with other factors such as dose-response relationship and whether the results were significant in light of other biologic and pathologic findings.

RESULTS

Table 1 summarizes the chamber exposure concentrations (analytical and nominal), temperature (maximum and minimum) and relative humidity for the entire exposure period. The mean time-weighted average (TWA) ethyl chloride concentrations were very close to the targeted values. Moreover, there was close agreement between the mean daily TWA analytical concentrations and the mean daily nominal concentrations, indicating that test material losses were minimized in the vapor generator and exposure system.

Exposure of pregnant mice to ethyl chloride via inhalation at concentrations up to 5000 ppm produced no signs of maternal toxicity in the present study. No significant effects were noted in demeanor or general appearance, body weights (Table 2), weight gains during pregnancy (Table 3) or liver weights (Table 4) at any of the exposure concentrations tested, nor were there any apparent effects on feed or water consumption observed in exposed mice when compared to controls (Tables 5 and 6, respectively). Evaluation of the reproductive parameters in pregnant mice revealed no indication of any adverse effects on pregnancy rate, resorption rate, litter size, fetal sex ratios or fetal body weights among mice exposed to ethyl chloride (Table 7).

The incidences of fetal alterations are summarized in Table 8. There was no indication of a teratogenic response observed in any of the exposure groups when compared to controls. A low incidence of malformations was observed in all exposure groups, including the control group, with no indication of a consistent pattern of effects. Among the control mice, two fetuses from two separate litters exhibited malformations; one fetus had a cleft palate and the second exhibited microphthalmia. In the 500 ppm exposure group, three fetuses from different litters had cleft palates, with one fetus also exhibiting dilated lateral ventricles of the brain and forked and fused ribs. Among litters in the 1500 ppm exposure group, three fetuses from separate litters had malformations. A single fetus exhibited a cleft palate. Exencephaly was observed in the other two malformed fetuses, one of

which also had an omphalocele and fused cervical vertebrae. At 5000 ppm, a single fetus with cleft palate and micrognathia was the only malformed fetus observed.

A number of minor visceral and skeletal alterations were observed scattered among all exposure groups including the controls. A statistically significant increase was seen in the incidence of foramina (small unossified centers) in the skulls of fetal mice exposed to 5000 ppm of ethyl chloride. There were no other changes to suggest any treatment-related effects in any of the exposed groups.

DISCUSSION

Exposure of pregnant CF-1 mice to inhaled ethyl chloride at vapor concentrations up to 5000 ppm on days 6 through 15 of gestation produced no evidence of teratogenicity. No toxicologically significant effects on maternal body weights, liver weights or feed and water consumption were observed at any exposure level tested. Although no adverse maternal effects were observed in the present study, a recent probe study conducted in this laboratory in which pregnant CF-1 mice were exposed to 5000, 10,000 or 15,000 ppm of ethyl chloride over this same period of gestation revealed slight, but statistically significantly lower maternal body weight gain (14.1 g in controls compared to 11.9 g in the 5000 ppm group) on days 10 through 16 of gestation (Scortichini et al., 1985). These animals also displayed an increase in locomoter activity and stereotypic behavior characterized by highly repetitive running patterns during the first two hours of each exposure period. This increased activity was considered evidence of either irritation from the test chemical, or the possibility that these animals were experiencing stage II of anesthesia. This increased activity was consistent with hyperactivity reported by the NTP (1981) in a 13-week study at exposure concentrations of 10,000 and 19,000 ppm.

No effects on reproductive parameters or fetal body weights were observed in any of the exposure groups when compared to controls. Examination of the fetuses for malformations revealed scattered incidences of malformations in all exposure groups, with no indication of a consistent pattern

of malformations in any of the exposed groups. For comparative purposes, a summary of the historical control incidences of the various malformations observed in the present study is presented in Table 9.

The only statistically significant change in any of the parameters measured in the present study was an increase in the incidence of foramina, a small center of delay in ossification, in the bones of the skulls of fetal mice exposed to 5000 ppm. Historically, the incidence of foramina in the skull bones of CF-1 mice is low (mean = 0.2% of fetuses, range = 0 - 1.2%); however, the toxicologic significance of a minor delay in ossification such as this is questionable. And, while no measurable toxicity was detected in the maternal animals in the present study, a previous study conducted at this same level produced some signs of maternal toxicity.

In conclusion, exposure of pregnant CF-1 mice to ethyl chloride vapors for 6 hours per day on days 6 through 15 of gestation at levels up to 5000 ppm produced no indication of any teratogenic effects.

Written by:

B.H. Scortichini 1/29/86
B. H. Scortichini, B.S.

T.R. Hanley 30 Jan 86
T. R. Hanley, Jr., M.S.
Study Director
Diplomate, American Board of Toxicology

K A Johnson 29 Jan 86
K. A. Johnson, D.V.M., Ph.D.
Diplomate, American College of Veterinary Pathologists

Reviewed by:

J G Momany-Pfruender
J. G. Momany-Pfruender, B.S.
30 Jan 86

T D Landry Jan 29/86
T. D. Landry, Ph.D.
Diplomate, American Board of Toxicology

TITLE OF STUDY: ETHYL CHLORIDE: INHALATION TERATOLOGY STUDY IN CF-1 MICE

This study was conducted in the spirit of FDA and EPA Good Laboratory Practice Regulations. The study phases were inspected by the Quality Assurance Unit and the results of these inspections reported to Management and the Study Director on the dates listed below. The report accurately reflects the data generated in accordance with the standard operating procedures of the laboratory. All data and reports are located at the submitting laboratory.

Study Started: 8 May 1985	Report Issued Date: 30 January 1986
Dates of Inspection: 10 May 1985	Date of Report: 10 May 1985
28 June 1985	1 July 1985
24 January 1986	27 January 1986

D. G. Keyes 30 January 1986

D. G. Keyes, B.S., M.T.(ASCP) Date
Quality Assurance
Mammalian and Environmental
Toxicology Research Laboratory
Health & Environmental Sciences
1803 Building
Dow Chemical U.S.A.
Midland, MI 48674

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TABLE 1

ETHYL CHLORIDE: INHALATION TERATOLOGY STUDY IN CF-1 MICE

Exposure Concentrations and Chamber Conditions

Target Conc. (ppm)	Analytical Concentration			Nominal Concentration		Temp.[c] (°C)		Rel.[c] Hum. (%)
	Mean[a] ± S.D.	Range of Values	Coeff. of[b] Variation	Mean[a] ± S.D.	Range of Values	Max.	Min.	
0	-	-	-	-	-	23±1	21±1	62±6
500	491±37	331-525	8	536±12	515-576	23±1	21±1	56±7
1500	1504±84	1122-1581	6	1546±65	1277-1594	23±1	20±1	57±6
5000	4946±159	4528-5400	3	5398±269	4968-6492	22±1	20±1	57±7

[a]Numbers are mean ± S.D. daily time-weighted average (TWA) values for n = 38 days.

[b]Coefficient of variation is the standard deviation of daily TWA measurements divided by the mean (x 100).

[c]Mean ± S.D. of daily measurements taken while exposures in progress.

TABLE 2

ETHYL CHLORIDE: INHALATION TERATOLOGY STUDY IN CF-1 MICE

BODY WEIGHT VALUES OF PREGNANT MICE

	ETHYL CHLORIDE, PPM			
	0	500	1500	5000
MATERNAL BODY WEIGHT (G) ON GESTATION DAY				
6	32.9[a]	33.5	32.3	33.0
	3.9	3.8	3.3	3.0
	23	25	26	22
9	35.5	36.0	34.9	35.7
	3.8	3.7	3.2	3.2
	23	25	26	22
12	39.3	39.4	38.6	39.7
	3.8	3.5	2.9	2.6
	23	25	26	22
15	46.0	45.7	45.1	45.4
	3.8	4.8	3.5	2.7
	23	25	26	22
18	55.3	56.0	55.1	54.8
	5.2	6.0	5.1	3.2
	23	25	26	22
18 (CORRECTED)[b]	37.4	37.0	36.3	37.1
	3.6	3.2	3.2	2.7
	22	24	25	21

[a] Data shown are means, standard deviations and number of females.
[b] Terminal body weight minus gravid uterine weight.

No values were statistically different from the control values by the appropriate statistical test, $\alpha=0.05$.

TABLE 3

ETHYL CHLORIDE: INHALATION TERATOLOGY STUDY IN CF-1 MICE

BODY WEIGHT GAINS OF PREGNANT MICE

		ETHYL CHLORIDE, PPM			
		0	500	1500	5000
NUMBER OF DAMS		23	25	26	22
MATERNAL BODY WEIGHT GAINS (G) ON GESTATION DAYS					
	6-9	2.6[a] 0.7	2.4 0.8	2.7 0.9	2.7 1.0
	9-12	3.8 0.9	3.4 1.0	3.7 1.0	4.0 1.5
	12-15	6.7 1.6	6.3 2.0	6.5 1.3	5.7 1.4
	15-18	9.3 3.1	10.3 2.2	10.0 2.4	9.4 1.2
	6-15	13.1 2.3	12.2 3.0	12.9 2.1	12.4 2.1
	6-18	22.4 5.1	22.5 4.9	22.9 4.3	21.8 3.1

[a]Data shown are means and standard deviations.

No values were statistically different from the control values by the appropriate statistical test, α=0.05.

TABLE 4

ETHYL CHLORIDE: INHALATION TERATOLOGY STUDY IN CF-1 MICE

ORGAN WEIGHTS OF PREGNANT MICE

	ETHYL CHLORIDE, PPM			
	0	500	1500	5000
NUMBER OF DAMS	23	25	26	22
ABSOLUTE LIVER WEIGHT (G)	3.50[a]	3.43	3.36	3.44
	0.42	0.30	0.29	0.23
RELATIVE LIVER WEIGHT (G/100G)	6.37	6.15	6.12	6.29
	0.91	0.41	0.48	0.38

[a]Data shown are means and standard deviations.

No values were statistically different from the control values by the appropriate statistical test, α=0.05.

TABLE 5

ETHYL CHLORIDE: INHALATION TERATOLOGY STUDY IN CF-1 MICE

FOOD CONSUMPTION OF PREGNANT MICE

		ETHYL CHLORIDE, PPM			
		0	500	1500	5000
FOOD CONSUMPTION (G) ON GESTATION DAYS					
	6-9	7.3[a]	7.6	7.6	7.6
		1.1	1.3	1.5	1.0
		22	25	26	22
	9-12	7.7	7.3	7.5	8.1
		1.6	0.8	0.8	1.4
		21	23	26	22
	12-15	8.7	8.6	8.5	8.7
		1.8	1.3	1.2	1.3
		23	25	25	22
	15-18	9.2	9.7	9.8	9.8
		1.5	1.3	1.6	1.1
		23	25	25	22

[a]Data shown are means, standard deviations and number of females.

TABLE 6

ETHYL CHLORIDE: INHALATION TERATOLOGY STUDY IN CF-1 MICE

WATER CONSUMPTION FOR PREGNANT MICE

	ETHYL CHLORIDE, PPM			
	0	500	1500	5000
WATER CONSUMPTION (G) ON GESTATION DAYS				
6-9	9.2[a]	8.8	8.7	9.3
	1.4	1.2	1.1	1.4
	22	25	25	22
9-12	9.5	8.8	9.1	9.9
	1.7	1.1	1.3	1.3
	23	25	25	22
12-15	10.8	10.5	10.9	11.3
	1.7	0.9	1.5	1.6
	23	24	25	22
15-18	10.9	10.7	11.0	11.5
	1.8	1.1	1.3	1.5
	23	25	25	20

[a]Data shown are means, standard deviations and number of females.

TABLE 7

ETHYL CHLORIDE: INHALATION TERATOLOGY STUDY IN CF-1 MICE

Observations Made at the Time of Cesarean Section of Bred Mice

	Ethyl Chloride, PPM			
	0	500	1500	5000
Number of females bred	30	30	30	30
Number of maternal deaths	0	0	0	0
Pregnancies detected by stain	0	0	0	1
% Pregnant, total	77(23/30)	83(25/30)	87(26/30)	77(23/30)
Gravid uterine weight	18.14±4.67	19.01±4.45	19.14±3.86	17.79±3.30
Number of litters	23	25	26	22
Implantation sites/dam	13±2	14±3	13±2	13±2
Fetuses/litter	11±3	12±3	12±3	11±2
Resorptions/litter	2.0±2.8	1.6±1.6	1.2±1.3	1.5±1.4
% Implantations resorbed	15(47/305)	12(39/338)	9(31/342)	12(32/274)
% Litters with resorptions	91(21/23)	80(20/25)	58(15/26)	73(16/22)
Litters totally resorbed	1	0	0	0
Resorptions/litters with resorptions	2.2(47/21)	2.0(39/20)	2.1(31/15)	2.0(32/16)
Dead fetuses	0	0	0	0
Sex ratio, M:F %	51:49	48:52	56:44	48:52
Fetal body weight, g	1.216±0.068	1.183±0.091	1.198±0.073	1.189±0.070

No values were statistically different from the control values by the appropriate statistical test, $\alpha=0.05$.

TABLE 8

ETHYL CHLORIDE: INHALATION TERATOLOGY STUDY IN CF-1 MICE

Incidence of Fetal Alterations Among Litters of Mice

		Ethyl Chloride, PPM			
		0	500	1500	5000
		Number of Fetuses (Number of Litters) Examined			
External Examination		258 (22)	299 (25)	311 (26)	242 (22)
Soft Tissue Examination		132[b] (22)	156 (25)	164 (26)	126 (22)
Skeletal Examination		257[b] (22)	299 (25)	311 (26)	242 (22)
Bones of the Skull[a]		126 (22)	142 (24)	147 (25)	116 (22)
		Percent Affected (Number Affected)			
External Observations					
Cleft palate[+]	F[c]	0.4(1)	1(3)	0.3(1)	0.4(1)[d]
	L	5(1)	12(3)	4(1)	5(1)
Exencephaly[+]	F	0	0	1(2)[e]	0
	L	0	0	8(2)	0
Omphalocele[+]	F	0	0	0.3(1)[e]	0
	L	0	0	4(1)	0
Micrognathia[+]	F	0	0	0	0.4(1)[d]
	L	0	0	0	5(1)
Microphthalmia[+]	F	0.4(1)	0	0	0
	L	5(1)	0	0	0
Soft Tissue Observations					
Dilated renal pelvis and ureter	F	0	1(1)	0	0
	L	0	4(1)	0	0
Pale spleen	F	0	0	1(1)[e]	0
	L	0	0	4(1)	0
Pale foci on liver	F	1(1)	0	0	0
	L	5(1)	0	0	0
Dilated ventricles of the brain[+]	F	0	1(1)	0	0
	L	0	5(1)	0	0
Intraventricular hemorrhage (lateral ventricles)	F	1(1)	0	0	0
	L	5(1)	0	0	0
Skeletal Observations					
Skull -delayed ossification	F	2(3)	6(9)	4(6)	2(2)
	L	9(2)	29(7)	20(5)	9(2)
-foramen	F	1(1)	1(1)	1(1)	4(5)*
	L	5(1)	4(1)	4(1)	23(5)

TABLE 8 (Cont.)

ETHYL CHLORIDE: INHALATION TERATOLOGY STUDY IN CF-1 MICE

Incidence of Fetal Alterations Among Litters of Mice

		Ethyl Chloride, PPM			
		0	500	1500	5000
-irregular pattern of ossification	F	0	0	0	1(1)
	L	0	0	0	5(1)
Vertebrae					
-delayed ossification	F	1(3)	0.3(1)	1(2)	0
	L	14(3)	4(1)[f]	8(2)[e]	0
-centra, delayed ossification	F	0	0.3(1)[f]	0.3(1)[e]	0
	L	0	4(1)	4(1)	0
-atlas forked	F	1(2)	1(2)	1(2)	1(2)
	L	9(2)	8(2)	8(2)[e]	5(1)
-fused[+]	F	0	0	0.3(1)[e]	0
	L	0	0	4(1)	0
Ribs					
-delayed ossification	F	0	0.3(1)[f]	0	0
	L	0	4(1)[f]	0	0
-forked[+]	F	0	0.3(1)[f]	0	0
	L	0	4(1)[f]	0	0
-fused[+]	F	0	0.3(1)[f]	0	0
	L	0	4(1)	0	0
-cervical[+]	F	1(2)	0.3(1)	2(6)[e]	2(4)
	L	9(2)	4(1)	19(5)	18(4)
Sternebrae					
-delayed ossification	F	4(11)	6(18)	4(12)[e]	2(5)
	L	27(6)	48(12)	23(6)	14(3)
-fused	F	5(12)	6(18)	7(23)[e]	5(13)
	L	46(10)	28(7)	54(14)	36(8)
-staggered	F	0	0.3(1)	0.3(1)[e]	0.4(1)
	L	0	4(1)	4(1)	5(1)
-irregular pattern of ossification	F	1(2)	0	0	0
	L	9(2)	0	0	0
-misshapen	F	0.4(1)	0	0	0
	L	5(1)	0	0	0

[a]In litters containing less than 4 fetuses, all heads were preserved in Bouin's fixative and were not processed for skeletal examination. In some litters additional heads were removed and placed in Bouin's to verify the presence of cleft palate.
[b]One fetus was lost during processing.
[c]F = Fetuses; L = Litters
[d]One fetus at 5000 ppm exhibited cleft palate and micrognathia.
[e]One fetus at 1500 ppm exhibited exencephaly, omphalocele, pale spleen, cervical rib, fused cervical vertebrae, delayed ossification of thoracic centra and sternebrae, and fused and staggered sternebrae.
[f]One fetus at 500 ppm exhibited 2 forked ribs, fused ribs and delayed ossification of ribs and thoracic centra.
[+]Considered to be a malformation.
*Different from control value by a censored Wilcoxon test, α=0.05.

TABLE 9

ETHYL CHLORIDE: INHALATION TERATOLOGY STUDY IN CF-1 MICE

Incidence of Fetal Alterations in Historical Control Mice[a] in The Dow Toxicology Research Laboratory

		Cumulative Total (# Affected/# Examined)	Range of Individual Study Incidences
External Alterations			
Cleft palate+	F[b]	34/9237	0/408 - 4/242
	L	26/817	0/36 - 5/26
Exencephaly+	F	17/9237	0/402 -3/376
	L	15/817	0/34 - 2/26
Micrognathia+	F	1/9237	0/408 - 1/265
	L	1/817	0/36 - 1/22
Microphthalmia+	F	0/9237	-
	L	0/817	-
Omphalocele+	F	7/9237	0/408 - 1/221
	L	7/817	0/36 - 1/20
Visceral Alterations			
Dilated cerebral ventricles of the brain+	F	12/3172	0/165 - 3/93
	L	10/816	0/34 - 3/25
Skeletal Alterations			
Fused vertebrae+	F	5/9157	0/407 - 2/240
	L	5/811	0/36 - 2/23
Forked ribs+	F	0/9157	-
	L	0/811	-
Fused ribs+	F	5/9157	0/402 - 1/221
	L	5/811	0/34 - 1/20
Foramen	F	11/5951	0/269 - 3/245
	L	10/803	0/34 - 2/33

[a] Includes data from vehicle control groups from 31 studies conducted between 1974 and 1985. Routes of exposure include drinking water, diet, oral gavage, and inhalation.

[b] F = fetuses; L = litters.

+ Considered to be a malformation