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The Dow Chemical Company

2030 Dow Center
October 25, 1991

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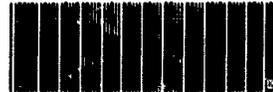
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BEHQ-1091-1404 INIT
8892000050

Attn: 8(e) Coordinator



8892000050

Re: METHYL CHLORIDE
CAS No. 74-87-3
CAP Agreement No. CAP-0111

Dear Sir/Madam:

The Dow Chemical Co. submits the enclosed document titled:

REPRODUCTION IN F-344 RATS EXPOSED TO METHYL CHLORIDE BY
INHALATION FOR TWO GENERATIONS

pursuant to TSCA Section 8(e) Compliance Audit Program.

The document contains information which may reasonably support the conclusion that the referenced chemical may present a substantial risk of injury to human health or the environment, as indicated in the Reporting Guide provided by EPA in connection with the CAP. The information is summarized below:

METHYL CHLORIDE HAD AN EFFECT ON REPRODUCTION IN THE FIRST GENERATION GROUPS EXPOSED TO 475 PPM AND 1500 PPM. AT 475 PPM THERE WAS A REDUCED NUMBER OF LITTERS AND FEWER MALES WERE PROVEN FERTILE BUT THIS EFFECT WAS COMPLETELY REVERSIBLE AFTER 10 WEEKS OF NO EXPOSURE. AT 1500 PPM THERE WAS COMPLETE STERILITY WITH LESIONS IN THE TESTICLE. THE STERILITY WAS REVERSED IN A SMALL NUMBER OF THE 1500 PPM GROUP MALES AFTER A 10 WEEK PERIOD OF NO-EXPOSURE BUT THE LESIONS IN THE TESTICLE PERSISTED.

Dow requests guidance from EPA whether the Agency believes the information contained in this document satisfies the criteria in the CAP Reporting Guide. Any correspondence relating to this submission should reference document number CAP00015 .

Sincerely,

Paul A. Wright

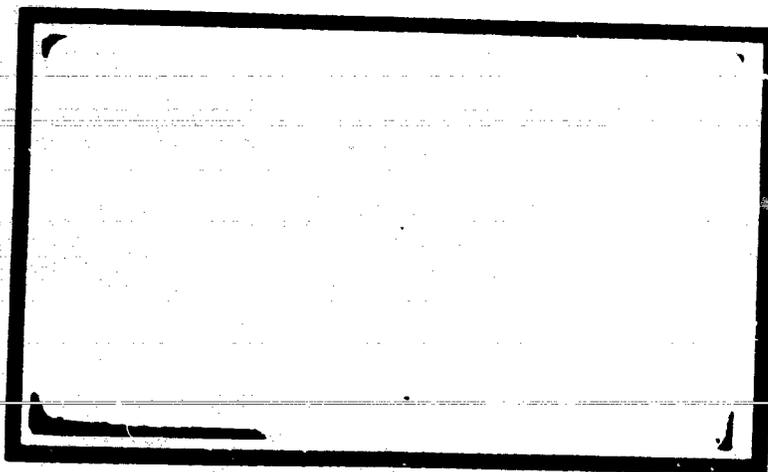
Paul A. Wright
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Photo through references
on page 3. A copy of
the entire report is on
file.

CIIT

Chemical Industry Institute of Toxicology



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Science in the Public Interest

0 0 0 5

Docket #11244

CHEMICAL INDUSTRY INSTITUTE OF TOXICOLOGY

P. O. BOX 12137

RESEARCH TRIANGLE PARK, NC 27709

REPRODUCTION IN F-344 RATS
EXPOSED TO METHYL CHLORIDE BY INHALATION
FOR TWO GENERATIONS

Report Issued:
April 13, 1984

Exposures

First Generation

Start: 4/5/82

Completed: 8/15/82

Second Generation

Start: 9/17/82

Completed: 1/20/83

Submitted by:

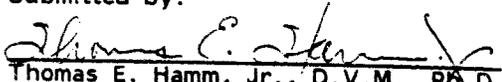

Thomas E. Hamm, Jr., D.V.M., Ph.D.
Study Director
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- F. BODY WEIGHT DATA
- G. CLINICAL OBSERVATION SUMMARY DATA
- H. DATES OF QUALITY ASSURANCE INSPECTIONS AND REPORTS
- I. PATHOLOGY REPORT F₀ MALES NECROPSY DATE 28 JUNE 1982
- J. PATHOLOGY REPORT F₀ MALES NECROPSY DATE 8 NOV 1982
- K. PATHOLOGY REPORT F₀ MALES NECROPSY DATE 26 JAN 1983
- L. PATHOLOGY REPORT F₀ FEMALES NECROPSY DATE 23 AUG 1982
- M. PATHOLOGY REPORT F₁ PUPS NECROPSY DATE 24 AUG 1982
- N. PATHOLOGY REPORT F₁ ADULT MALES NECROPSY DATE 8 NOV 1982
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EFFECTS OF REPRODUCTION IN FISCHER-344 RATS
EXPOSED TO METHYL CHLORIDE BY INHALATION
SPONSORED BY AND PERFORMED AT:
CHEMICAL INDUSTRY INSTITUTE OF TOXICOLOGY
5 X DAVIS DRIVE

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QUALITY ASSURANCE STATEMENT

The conduct of this study was inspected periodically in keeping with CIIT Quality Assurance (QA) procedures for monitoring intramural testing program studies for compliance with the U.S.F.D.A. Good Laboratory Practice Regulations (GLPs). Inspection reports were submitted periodically to the Study Director and management. Inspection and report dates are given in Appendix H.

report has been reviewed under the GLP requirements set forth by the U.S. F.D.A. and E.P.A. and accurately reflects the study raw data, methods and procedures. All specimens, raw data, and reports are stored in the archives at CIIT.


Garis D. Parker, Jr., M.M.Sc. 4/1/84 Date
Quality Assurance Officer
Manager, Laboratory Compliance

SUMMARY

Male and female Fischer-344 rats were exposed to methyl chloride by inhalation (0, 150, 475 or 1500 ppm, 6 hours/day, 5 days/week, 40 males and 80 females per group). The only treatment related clinical signs were a 10 to 20% body weight gain depression (BWGD) in both males and females exposed to 1500 ppm at all weekly weighings after two weeks of exposure and a 5-7% BWGD in 475 ppm exposed animals after day 57. After 10 weeks the exposure schedule was changed to 6 hours/day 7 days/week and each male was mated to four unexposed females. The mating period lasted two weeks and then ten males/group were necropsied. The only treatment related lesions found were severe testicular degeneration (10/10) and granulomas in the epididymis (3/10) in the 1500 ppm males. The remaining 30 males per group were then mated during a two week period with 60 unexposed females. The exposed females were continued on exposure from the start of mating to day 18 of gestation (6 hours/day, 7 days/week). The females were not exposed from gestation day 18 to postnatal day 4, but exposure (6 hours/day, 7 days/week) of these females was resumed from postnatal day 4 to postnatal day 28. There were no significant differences between groups in the number of exposed or unexposed females that mated as evidenced by copulation plugs. No litters were born to exposed or unexposed females mated to the 1500 ppm males (0 litters/87 exposed plus unexposed females). There was no significant difference in the number of litters produced by the 150 ppm groups when compared to the control groups. Fewer litters were born in the 475 ppm groups than in the control groups. No differences in litter size, sex ratio, pup viability or pup growth were found among the 475 ppm, 150 ppm or control F₀ groups. When bred 10 weeks after the cessation of exposures,

5 of 20 1500 ppm F_0 males had regained the ability to sire normal litters. The same number of 475 ppm F_0 males were proven fertile (15/20) as control F_0 males (13/20). After weaning, F_1 pups from the 475, 150 and 0 ppm groups were exposed to the same concentrations of methyl chloride for 10 weeks and then mated. A trend toward decreased fertility was found in the 475 ppm group.

In conclusion, methyl chloride by inhalation had no effect on reproduction in two generations of F-344 rats at 150 ppm. Methyl chloride had an effect on reproduction in the first generation groups exposed to 475 ppm and 1500 ppm. At 475 ppm there was a reduced number of litters and fewer males were proven fertile but this effect was completely reversible after 10 weeks of no exposure. At 1500 ppm there was complete sterility with lesions in the testicle. The sterility was reversed in a small number of the 1500 ppm group males after a 10 week period of no-exposure but the lesions in the testicle persisted.

INTRODUCTION

Methyl chloride (chloromethane, CH_3Cl , CAS #074-87-3) is a colorless, nearly odorless, non-corrosive gas at room temperature. The current threshold limit value-time weighted average is 50 ppm (105 mg/m^3) and the threshold limit value-short term exposure limit is 100 ppm (205 mg/m^3) (ACGIH, 1981). Methyl chloride is used mainly in the production of silicones. It is also used as a blowing agent in molding polystyrene and polyurethane foams; as an aerosol propellant for insecticides; as a catalyst, solvent, and chemical intermediate in methylation reactions in the oil and rubber industries; and as a dewaxing solvent (EPA, 1980). In 1979, U.S. production capacity was approximately 625 million pounds, produced by nine manufacturers at eleven sites (CMR, 1979).

In a CIIT supported two year bioassay of methyl chloride (Pavkov et al., 1982; CIIT, 1982) Fischer-344 (F-344) rats were exposed by inhalation to 0, 51, 224, or 997 ppm methyl chloride 6 hours per day, 5 days per week for 24 months. The only methyl chloride related effects in F-344 rats were testicular lesions in 997 ppm males and significant body weight gain reductions in both male and female 997 ppm animals. The testicular lesions, testicular tubular degeneration and atrophy, were first found after 6 months of exposure. The reproduction study reported here was done to determine if methyl chloride exposure by inhalation for 10 weeks had an effect on reproduction in the F-344 rat. If reproduction was affected, we wished to also determine with this initial study, what doses affected reproduction after a 10 week exposure, was reproduction affected at doses that did not cause testicular lesions, was the effect on the males only or on both sexes, and was the effect reversible.

MATERIALS & METHODS

Figure 1 contains a flow chart of the experimental design.

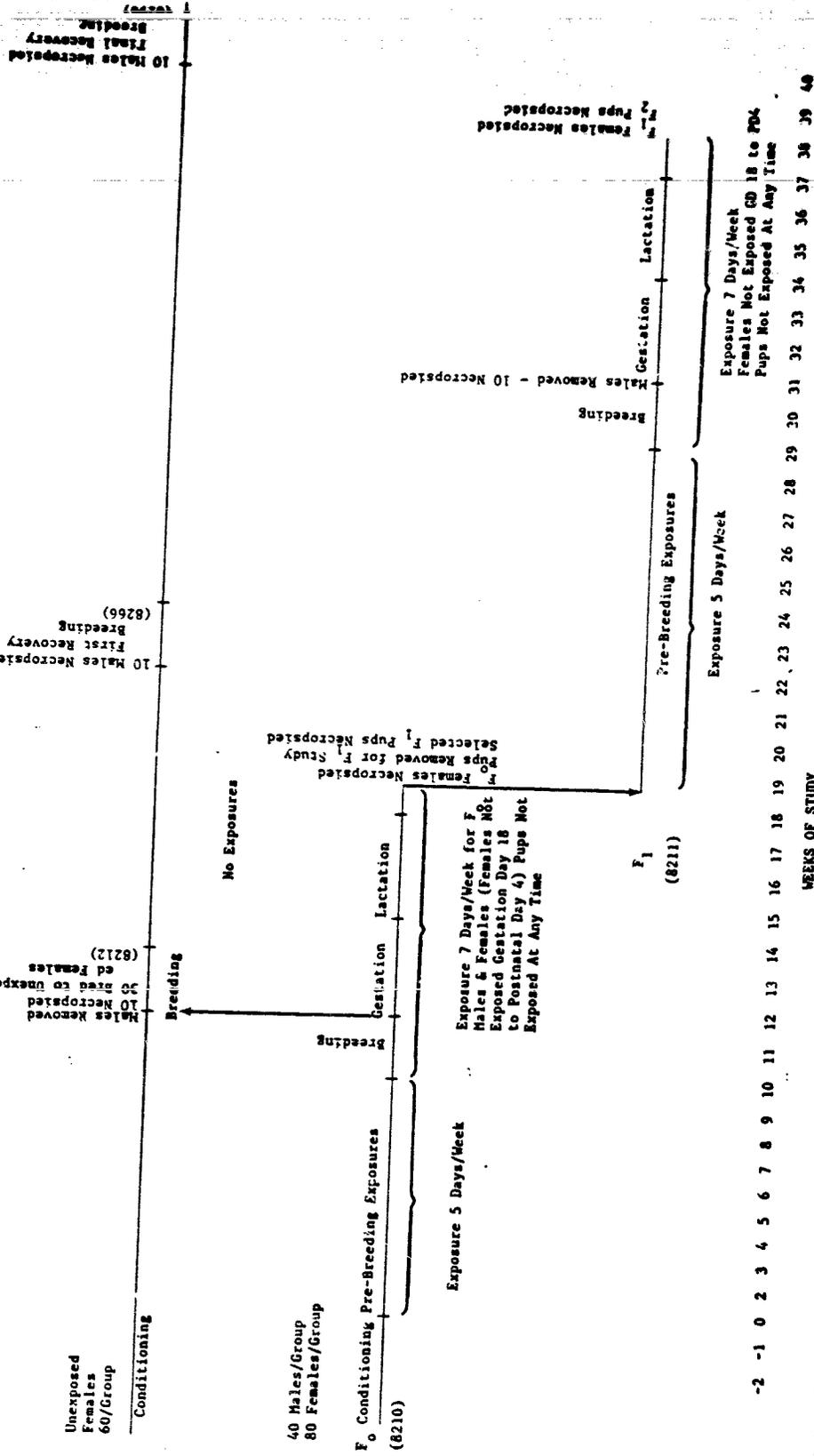
a) Test Chemical

Twenty cylinders containing methyl chloride (lot #L24-0095) were purchased from Matheson Gas Products, Morrow, GA. All gas used for the study was from the same batch, stored in new cylinders. Two analyses were done by Matheson Gas Products personnel. The first was done on a sample drawn prior to shipment and the second was done on a sample drawn after the study was completed. At both times the gas was 99.98% pure methyl chloride; the only detected impurity was water. (Purity analyses in Appendix C).

b) Animals

Two hundred male (50-55 gm) and 650 female (45-50 gm) weanling (4-5 weeks old) Fischer-344 rats [CDF[®] (F-344)/CrIBR] were obtained from Charles River Breeding Laboratories (Kingston, NY). Upon arrival all animals were individually housed in hanging stainless steel mesh cages (Hazelton Systems, Inc., Vienna, VA) in a mass air displacement animal room (Bioclean, Hazelton Systems, Inc., Vienna, VA). Animals were given NIH-07 open formula pelleted chow (Ziegler Bros., Gardner, PA) ad libitum except during exposure. The diet was analyzed for nutrients and contaminants (Lancaster Labs, York, PA) (analyses in Appendix D). They were given purified tap water (Raynor et al., 1982) ad libitum, except during exposure, using an automatic watering system. Lights were controlled by an automatic timer set for 12 hours of darkness.

FIG. 1. Design for the Study of Reproductive Effects in Fischer 344 Rats Exposed to Methyl Chloride for Two Generations



Study #
 8210 = Exposure and breeding of F₀ animals
 8211 = Exposure and breeding of F₁ animals
 8212 = Breeding of 30 F₀ males per group with unexposed females the two weeks immediately after exposures stopped for the males
 8266 = Recovery breeding 12 weeks after exposure stopped for the males
 8296 = Final recovery breeding 180 days after exposure stopped for the males

Animal room temperature was set for $72 \pm 2^\circ\text{F}$ and humidity was set for $50 \pm 5\%$. Randomly selected animals necropsied on arrival were free of known rodent pathogens (standard screening necropsy, Microbiological Associates, Bethesda, MD). Sentinel animals bled at monthly intervals throughout the study were free of virus titers using the standard rat murine viral antibody screening procedure (Microbiological Associates, Bethesda, MD). (Serological Test Results in Appendix B).

After nineteen days of acclimation, the animals were randomized into four groups of 40 males each, four groups of 80 females each and four groups of 60 females each. Groups of 40 males and 80 females, housed in stainless steel mesh cages, were moved into each of 4 inhalation chambers. The 4 groups of 60 females remained in the mass air displacement room. Throughout the experiment body weights were taken on adult animals weekly except for females during pregnancy. All animals were observed twice daily for signs of toxicity and clinical observations were recorded weekly.

c) Inhalation Procedures

Exposures were conducted in eight cubic meter, glass and stainless steel inhalation chambers. Chamber air supply was filtered (HEPA, Type-A) prior to entering the chamber. Methyl chloride calibrated flowmeters were used to meter the CH_3Cl gas into the chambers. The CH_3Cl was diluted using the filtered chamber supply air to achieve the target concentrations. Chamber air flow calibrations were measured by the pressure drop across an orifice plate. A 2000 liter/minute chamber air flow was maintained throughout the study. Methyl chloride analysis

was done using an infrared gas analyzer (Miran, Model 801, Foxboro Analytical). Analytical parameters were chosen after comparing the infrared spectra of humidified ultra-zero air (Matheson Gas Products, Morrow, GA) and methyl chloride (1760 ppm). The following parameters were used throughout the study: pathlength was 20.25 meters, analytical wavelength was 9.991 microns, and the slit opening was 1 mm. A mixture of approximately 350 ppm carbon dioxide with ultra-zero air was used daily (at 2700 ml/minute) to zero the analytical instrument. This mixture was split with approximately 2400 ml/minute being bubbled through distilled water. Analytical readings were taken hourly from each chamber during the six-hour exposure. A time-weighted average for each six hour exposure was reported as the daily chamber concentration. Chamber temperature and humidity readings were recorded each hour during the six hour exposure using a scanning tele-thermometer (Model 47, Yellow Springs Instrument Co., Inc., Yellow Springs, OH). Temperature data from dry and wet bulb thermometers were converted to relative humidity (Appendix E).

d) Breeding Procedures

The rats in the inhalation chambers were exposed to target concentrations of 0, 150, 475 and 1500 ppm of methyl chloride for 6 hours per day five days per week for 10 weeks. Then, exposures were conducted 6 hours per day seven days a week and each male was mated to a female from the same group until either a copulation plug was observed in the pan under the cage or one week had passed. Then, a second female was placed in the cage and given a similar opportunity to breed. The

day that a pen plug was found was noted as gestational day (GD) 0. Females that did not have a copulatory plug were eliminated from the study since the pregnant animals had to be moved to a polycarbonate cage for littering on a specific date relative to GD 0. Eighty females per group were used to provide a minimum of twenty or more litters in the control group to compensate for the loss of data from females eliminated from the study.

e) Litter Evaluations

Females were exposed until GD 18 when they were placed in a polycarbonate cage (Health Guard[®], Laboratory Products, Inc., Maywood, NJ) with heat-treated, hardwood chip bedding (Beta Chip[®], Laboratory Products, Inc., Maywood, NJ). From GD 20 until parturition the females were observed every hour from 7 a.m. to 7 p.m. Observations were not made during the dark cycle to avoid disturbing the animals. At parturition the following were recorded: date of delivery (postnatal day 0), number and sex of pups, number and sex of live pups, combined weight of male pups, and combined weight of female pups. On postnatal day (PND) 4 the following were recorded: number and sex of live pups, and combined weight of pups by sex. At this time the litter was culled using a computer generated randomization program to eight pups while attempting to achieve an equal sex ratio. Then the adult females were placed in the inhalation chambers and exposed to methyl chloride 6 hours per day 7 days per week (the pups remained in the polycarbonate cages and were not directly exposed to methyl chloride) until PND 28 when the pups were weaned. On PND 14, 21, and 28 the number and sex of the pups and the combined weight by sex were recorded.

f) Necropsy Procedures

At the end of the breeding period 10 males per dose group were randomly selected using a computer generated randomization program and necropsied. Necropsies included external examination of the carcass, weighing the animal, the brain, the liver, and the testes; and gross examination. The following tissues were collected in neutral buffered 10% formalin: brain, pituitary, eyes, spinal cord, salivary glands, peripheral nerve, urinary bladder, nasal turbinates, thyroid, parathyroid, thymus, esophagus, lungs, heart, liver, spleen, prostate, adrenals, colon, pancreas, stomach, duodenum, jejunum, ileum, skin, bone marrow, kidneys, seminal vesicles, lymph nodes, aorta, and any gross lesions. Testes and epididymides were collected, and fixed in Bouin's solution. Hematoxylin and eosin stained tissue sections were prepared and examined from three sections of brain (cerebrum, cerebellum and brain stem), two sections of liver, one section of each kidney, one section of each adrenal, a section of each epididymis (head, body and tail) and gross lesions of all 1500 ppm and control F_0 males. Testes and epididymal sections were also prepared and examined from all 475 ppm and 150 ppm F_0 males. Other grossly normal tissues were not examined since methyl chloride has not been reported to affect these tissues (Pavkov et al 1982; Morgan et al 1982; CIIT, 1982). Fifteen male and 15 female F_1 pups and 25 F_0 females from each dose group were randomly selected and necropsied after weaning. Necropsies were similar to those already described except one section of ovary, oviduct, uterine horn and cervix was examined histopathologically for each female instead of testicle and epididymis.

g) Recovery Evaluations

The remaining 30 F_0 males per group were moved to a mass air displacement animal room. They were each bred to two unexposed females and all data recorded. All methods were as described for the preceding mating (see d Breeding Procedures). Then these males were housed for 10 additional weeks when ten per group were necropsied and the remaining 20 males per group bred to two unexposed females of the same age. The remaining 20 males per group were housed an additional 18 weeks when 10 per group were necropsied and the other 10 per group bred to 20 unexposed females of the same age.

h) F_1 Generation Matings and Evaluations

Groups of 40 males and 80 females were randomly selected from the control and 150 ppm groups of F_1 pups allowing equal chance for each litter to be represented. Only twenty three male and 46 female F_1 rats were selected from the 475 ppm group. Larger numbers of F_1 rats from the 475 ppm group were not available since fewer litters were born at this concentration in the F_0 breeding. All procedures used for the F_1 generation study were the same as those described for the F_0 generation study except no recovery evaluations were done.

i) Statistical Analysis

The number of females with copulation plugs which produced litters, the number of males which were proven fertile and the number of live litters in each dose group were compared to control using a one tailed Fisher exact test (Bradley, 1968) with the Bonferroni correction (Miller, 1981). Some litter statistics (total number of fetuses/litter, live fetuses/

litter, growth of pups by sex from 0 to 4 days, growth of pups by sex from 4 to 14 days, growth of pups by sex from 14 to 21 days and growth of pups by sex from 21 to 28 days) were compared using an analysis of variance. The remaining litter statistics (percent live at birth, percent males, viability from 0 to 4 days, viability from 4 to 14 days, viability from 14 to 21 days, and viability from 21 to 28 days) were subjected to an Arcsin square root transformation and then compared using an analysis of variance. In all cases a Kolmogorov-Smirnov test (when $N > 50$) or a Wilk-Shapiro test (when $N \leq 50$) for normality was applied to the combined data from all groups (Daniel, 1978). If the distribution was not normal ($p \leq 0.05$), then the data were ranked and transformed prior to analysis. If the analysis of variance was significant, Dunnett's test (Steel and Torrie, 1960) was used to compare the dose groups to the control groups. In all cases, a probability level of $p < 0.05$ was preselected as the significance level.

RESULTS

a) F_0 Generation Breeding

The mean time-weighted average (TWA) analytical chamber concentrations derived from daily TWA concentrations were: 1502 \pm 19 ppm (1462-1559); 472 \pm 7 (427-510); 151 \pm 4 (135-164) and 0. Temperature was 71 \pm 1°F (63-74) for all four chambers and humidity was 59 \pm 3 (50-66); 53 \pm 5 (42-75); 54 \pm 4 (46-64) and 54 \pm 7 (39-71), respectively.

Figures 2 and 3 are the body weight curves for the F_0 exposed animals. First generation (F_0) males weighed 100-186 gms and females weighed 73-127 gms at the start of exposures. Females had a statistically significant body weight gain depression (BWGD) in the 1500 ppm group when compared to controls after 7 days of exposure. Males in the 1500 ppm group had a statistically significant BWGD compared to the control group after 15 days. The BWGD reached 10% in males by 29 days and in females by 15 days and remained between 10-20% for both sexes of the 1500 ppm group throughout the exposure period. The mean body weights for the 475 ppm males and females were significantly lower than the controls after day 57 (males had a 7% and females had a 5% BWGD). The 150 ppm group males did not have a significant BWGD at any time during the study. The 150 ppm females had a 3% BWGD after day 57.

One female from the 150 ppm F_0 group died during the exposure period. Two males from the control F_0 group and one female from the 1500 ppm F_0 group died just after the completion of exposures. All 4 apparently died from dehydration caused by faulty values in the automatic watering system. No treatment related clinical signs other than effects on body weights were found. A complete listing of clinical observations is in Appendix G.

Table 1 contains a summary of the results of breeding the F₀ generation males to exposed females and Table 2 contains a summary of the results of breeding the males to unexposed females. There were no significant differences among groups in the numbers of exposed or unexposed females that had copulation plugs. No litters were born to exposed or unexposed females mated to the 1500 ppm males. Significantly fewer litters were born to exposed or unexposed females mated to the 475 ppm males (Table 1). There was no significant difference in the number of litters born to exposed or unexposed females mated to 150 ppm males compared to control males. Table 3 contains a summary of male breeding success. The 1500 ppm males were sterile. The number of 475 ppm males proven fertile after two matings to either two exposed or two unexposed females was lower than the number of control males proven fertile but the decrease in fertility was not statistically significant. Table 4 contains a summary of male breeding results comparing the males that were each bred to two exposed and two unexposed females. The number of males proven fertile after 4 breedings was statistically lower in the 475 ppm group. No consistent significant differences in litter size, sex ratio, pup viability, pup survival or pup growth were found.

Treatment-related lesions were found only in the 1500 ppm F₀ males and consisted of minimal to severe atrophy of 60 to 80% of the seminiferous tubules (10/10) and granulomas in the epididymis (3/10). The 1500 ppm F₀ male testes weighed approximately one-half as much as testes from the other groups (control, mean 1.39 ± 0.06 g; 150 ppm, 1.47 ± 0.11 g; 475 ppm, 1.47 ± 0.13 g; and 1500 ppm, 0.73 ± 0.21 g). Severely affected tubules were lined by Sertoli's cells and by occasional stem cell spermatogonia. Less severely affected tubules contained a decreased number of spermatogonia, primary

spermatocytes, and/or secondary spermatocytes. Minimally involved and normal tubules contained spermatids and sperm. The epididymes of 1500 ppm males contained only a few sperm and sloughed spherical cells in their lumen. All other organs examined were normal.

b) Recovery Breedings

One week after exposures were stopped, the mean body weights of the 475 ppm males had increased and were not different from control. The 1500 ppm males had a significantly decreased mean body weight for 9 weeks after exposures were stopped, when they returned to the same as control. Table 5 contains the results of the first recovery breeding. At this time the fertility of the 475 ppm group was not different from control and the 1500 ppm group had a partial recovery of fertility. The testicles of the 1500 ppm group males were still small (control, 1.55 ± 0.07 gms; 475 ppm group, 1.54 ± 0.07 gms and 1500 ppm group, 0.78 ± 0.19). Histopathologically there was no difference in the testicular lesions from those observed in animals necropsied after the exposure period. Most of the epididymides of 1500 ppm males contained sperm; but the number appeared to be decreased compared to controls, and more sloughed cells were found among the sperm. No epididymal granulomas were found. No further improvement in fertility occurred after an additional 12 weeks of recovery (5/10 control males, 5/10 475 ppm males and 1/10 1500 ppm males were proven fertile).

c) F₁ Generation Breedings

Figures 4 and 5 are body weight curves for F₁ animals and Table 6 contains a summary of the results of breeding the F₁ animals. No statistically significant effect of methyl chloride exposure on fertility was found in the

second generation although 475 ppm animals had fewer litters than 150 ppm or control groups. Litters in the 475 ppm group had a significantly decreased percentage of males. Male and female pup growth 14-21 days was also significantly less than control. No other statistically significant effects were found.

FIGURE 2

BODY WEIGHT CURVES FOR F₀ MALES

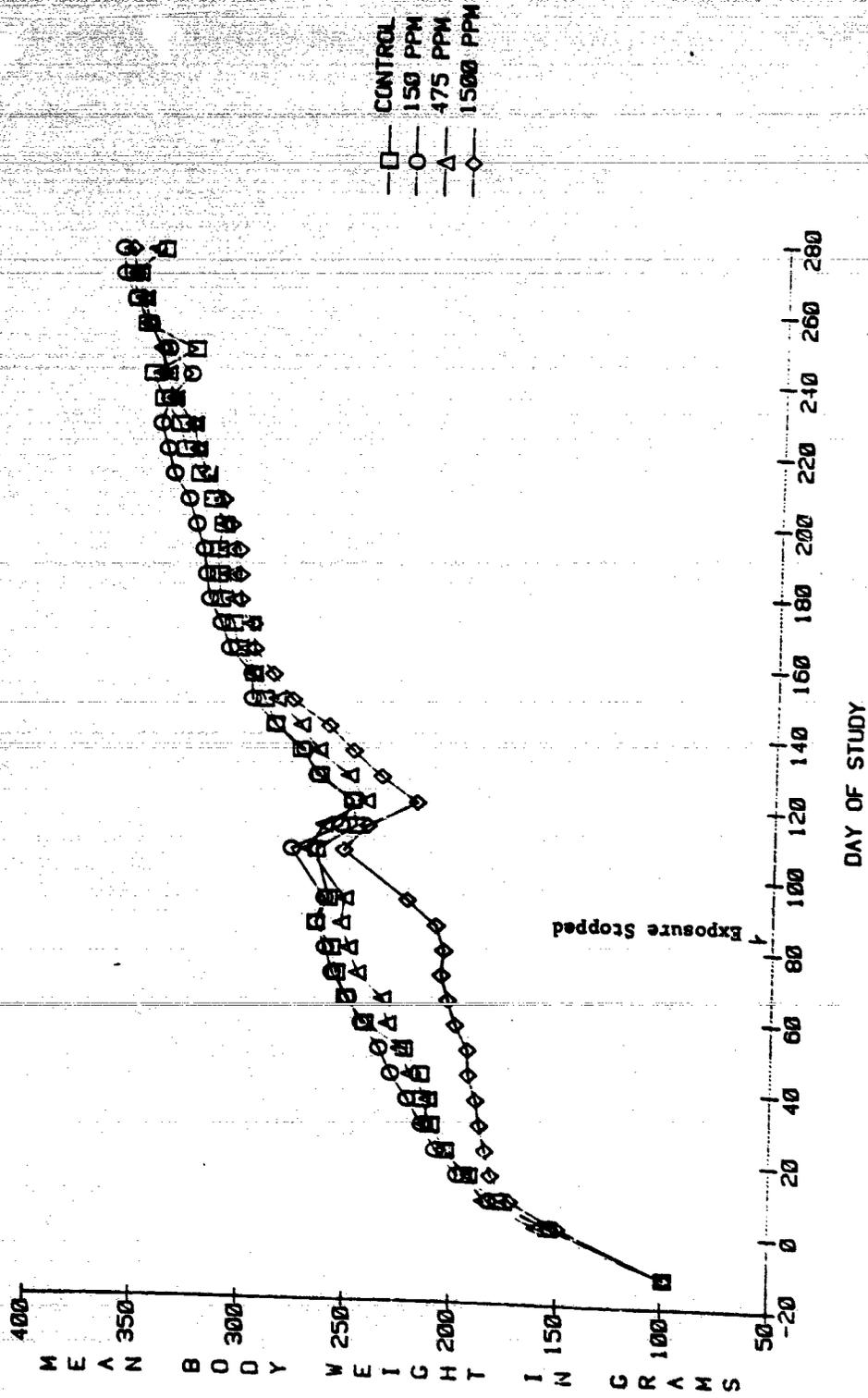


FIGURE 3

BODY WEIGHT CURVES FOR F₀ FEMALES

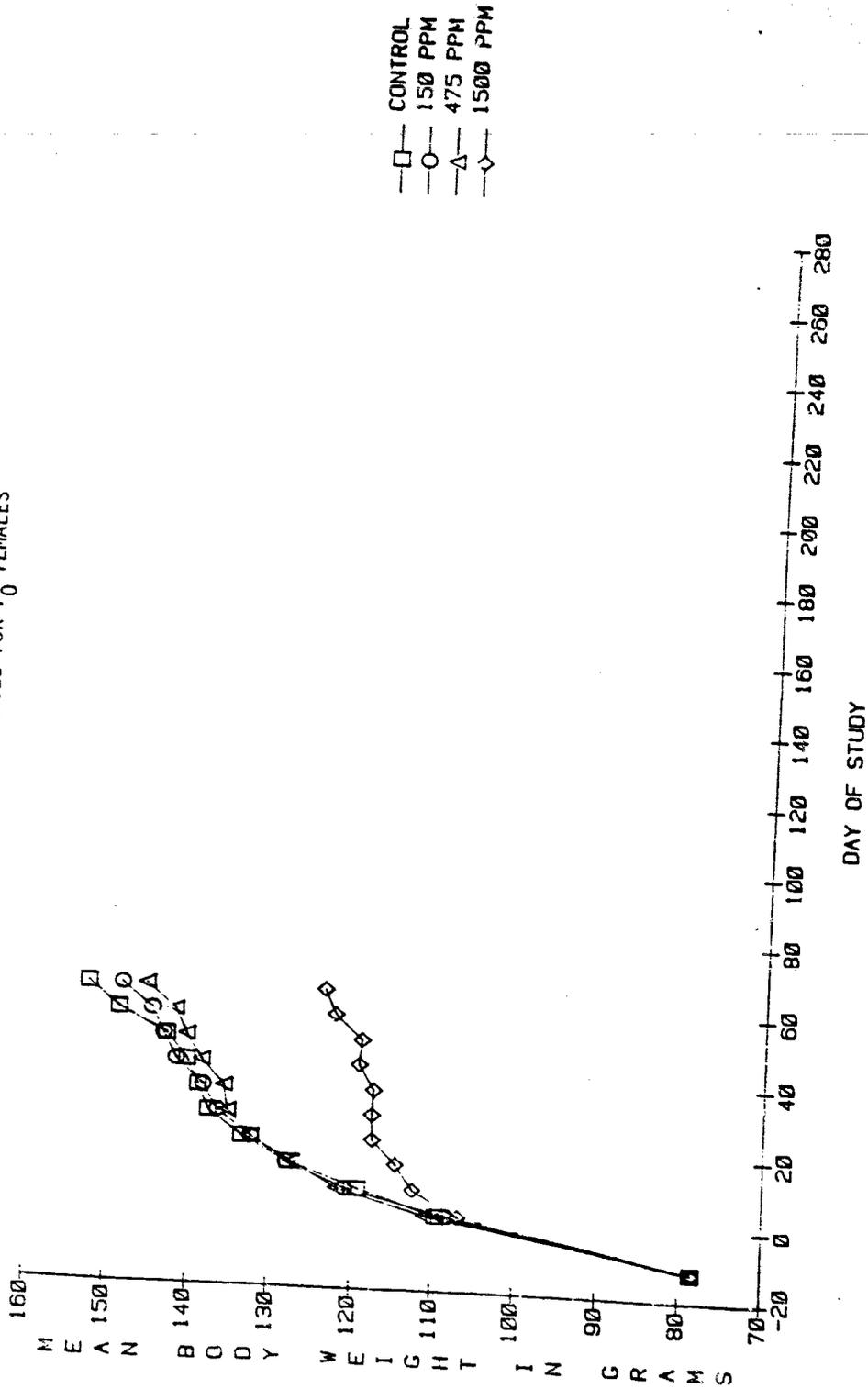


Table 1. Reproductive Parameters for F₀ F-344 Rats, Both Sexes Exposed by Inhalation to Methyl Chloride for 10 Weeks Prior to Mating.

	Exposure Group			
	0 ppm	150 ppm	475 ppm	1500 ppm
Number of males	40	39	40	40
Number of females	80	79	80	80
Females with copulation plugs	37 (46%)	50 (63%)	45 (56%)	52 (65%)
Females with copulation plugs producing litters	22 (59%)	27 (54%)	14* (31%)	0*
Number of males proven fertile	18 (45%)	20 (51%)	12 (30%)	0*
Number of pups per litter	8.8 ± 2.4	9.6 ± 2.3	9.1 ± 1.2	0*
Percentage of male pups	48.2 ± 19.1	51.2 ± 18.4	49.3 ± 18.1	
Percentage of pups live at birth	99.6 ± 1.9	98.4 ± 6.2	97.2 ± 8.3	
Percentage of pups surviving at day 4	97.8 ± 4.7	95.3 ± 20.9	85.2 ± 33.8	
Percentage of 4 day old pups surviving to day 14	100 ± 0	98.96 ± 3.5	100 ± 0	
Percentage of 21 day old pups surviving to day 21	100 ± 0	97.84 ± 6.2	100 ± 0	
Percentage of 28 day old pups surviving to day 28	100 ± 0	100 ± 0	100 ± 0	
Mean growth of 4 day old pups surviving to day 4 (gms)	100 ± 0	96.9 ± 7.6	100 ± 0	
Mean growth of 14 day old male pups to day 14 (gms)	2.0 ± 0.6	2.2 ± 0.7	2.6 ± 0.4	
Mean growth of 21 day old male pups to day 21 (gms)	6.6 ± 1.5	7.2 ± 1.6	6.7 ± 1.4	
Mean growth of 28 day old male pups to day 28 (gms)	7.9 ± 1.2	8.0 ± 1.7	7.2 ± 0.5	
Mean growth of 4 days old female pups to day 4 (gms)	16.2 ± 5.4	19.5 ± 5.6	18.0 ± 3.1	
Mean growth of 14 days old female pups to day 14 (gms)	2.1 ± 0.5	2.2 ± 0.8	2.3 ± 0.5	
Mean growth of 21 days old female pups to day 21 (gms)	6.4 ± 1.2	7.2 ± 1.6	6.4 ± 1.2	
Mean growth of 28 days old female pups to day 28 (gms)	7.6 ± 1.2	7.7 ± 1.2	7.0 ± 0.4	
	16.6 ± 2.7	19.4 ± 4.0*	17.0 ± 2.0	

*Significantly different from control, p < .05.

Table 2. Reproductive Parameters for F₀ F-344 Rats, Males Exposed to Methyl Chloride by Inhalation for 12 Weeks and Females Unexposed.

	Exposure Group			
	0 ppm	150 ppm	475 ppm	1500 ppm
Number of males	28	28	28	26
Number of females	58	58	58	56
Number of females with copulation plug	43 (74%)	44 (76%)	36 (62%)	35 (63%)
Females with copulation plugs producing litters	37 (86%)	32 (73%)	21* (58%)	0*
Number of males proven fertile	23 (82%)	21 (75%)	12 (43%)	0*
Number of pups per litter	9.5 ± 2.7	9.8 ± 2.1	9.6 ± 2.0	0*
Percentage of male pups	53.9 ± 15.8	53.3 ± 14.3	46.7 ± 17.8	
Percentage of pups live at birth	97.4 ± 10.6	99.4 ± 2.2	100 ± 0	
Percentage of pups surviving at day 4	100 ± 0	96.3 ± 11.7	100 ± 0	
Percentage of 14 day old pups surviving to day 14	98.8 ± 3.8	97.2 ± 10.0	96.1 ± 12.7	
Percentage of 21 day old pups surviving to day 21	98.5 ± 6.4	100 ± 0	100 ± 0	
Percentage of 28 day old pups surviving to day 28	100 ± 0	100 ± 0	100 ± 0	
Mean growth of 4 day old pups surviving to day 4	97.2 ± 7.2	97.2 ± 10.0	96.1 ± 12.7	
Mean growth of 4 day old male pups to day 4 (gms)	2.6 ± 1.1	3.0 ± 0.7	2.3 ± 1.3	
Mean growth of 14 day old male pups to day 14 (gms)	8.9 ± 2.1	8.5 ± 2.7	7.6 ± 3.6	
Mean growth of 21 day old male pups to day 21 (gms)	10.0 ± 3.5	9.5 ± 2.1	10.2 ± 2.6	
Mean growth of 28 day old male pups to day 28 (gms)	22.0 ± 4.9	23.5 ± 3.4	21.3 ± 5.6	
Mean growth of female pups to day 4 (gms)	2.5 ± 1.1	2.9 ± 0.7	2.3 ± 1.3	
Mean growth of 4 days old female pups to day 14 (gms)	8.5 ± 2.8	7.9 ± 2.5	7.2 ± 3.3	
Mean growth of 14 day old female pups to day 21 (gms)	9.1 ± 3.8	9.3 ± 2.6	10.2 ± 2.6	
Mean growth of 21 day old female pups to day 28 (gms)	21.9 ± 4.1	22.3 ± 3.1	20.5 ± 5.3	

*Significantly different from control, p < .05.

Table 3. Number of F₀ F-344 Males Exposed to Methyl Chloride by Inhalation for 10 Weeks Prior to Breeding that Sired Litters When Bred to Exposed or Unexposed F-344 Female Rats.

	2 Exposed Females Dosage Group		2 Unexposed Females Dosage Group	
	150 ppm	475 ppm	150 ppm	475 ppm
Males siring 0 litters	22/40	28/40	40/40	26/26
Males siring 1 litter	14/40	10/40	0/40	0/26
Males siring 2 litters	4/40	2/40	0/40	0/26
Total males proven fertile	18/40	20/39	40/40*	26/26*

*Total males proven fertile that were significantly different from control, $p < .05$.

Table 4. Number of F₀ F-344 Males Exposed to Methyl Chloride by Inhalation Prior to Breeding that Sired Litters When Bred to 2 Exposed and 2 Unexposed F₀ F-344 Female Rats.

	0 ppm	Dosage Group		
		150 ppm	475 ppm	1500 ppm
Males siring 0 litters	3/28	5/27	11/28	26/26
Males siring 1 litters	8/28	4/27	10/28	0/26
Males siring 2 litter	8/28	11/27	5/28	0/26
Males siring 3 litters	8/28	6/27	2/28	0/26
Males siring 4 litters	1/28	1/27	0/28	0/26
Total males proven fertile	25/28	22/27	17/28*	0/26*

*Total males proven fertile that were significantly different from control, $p < .05$.

FIGURE 4

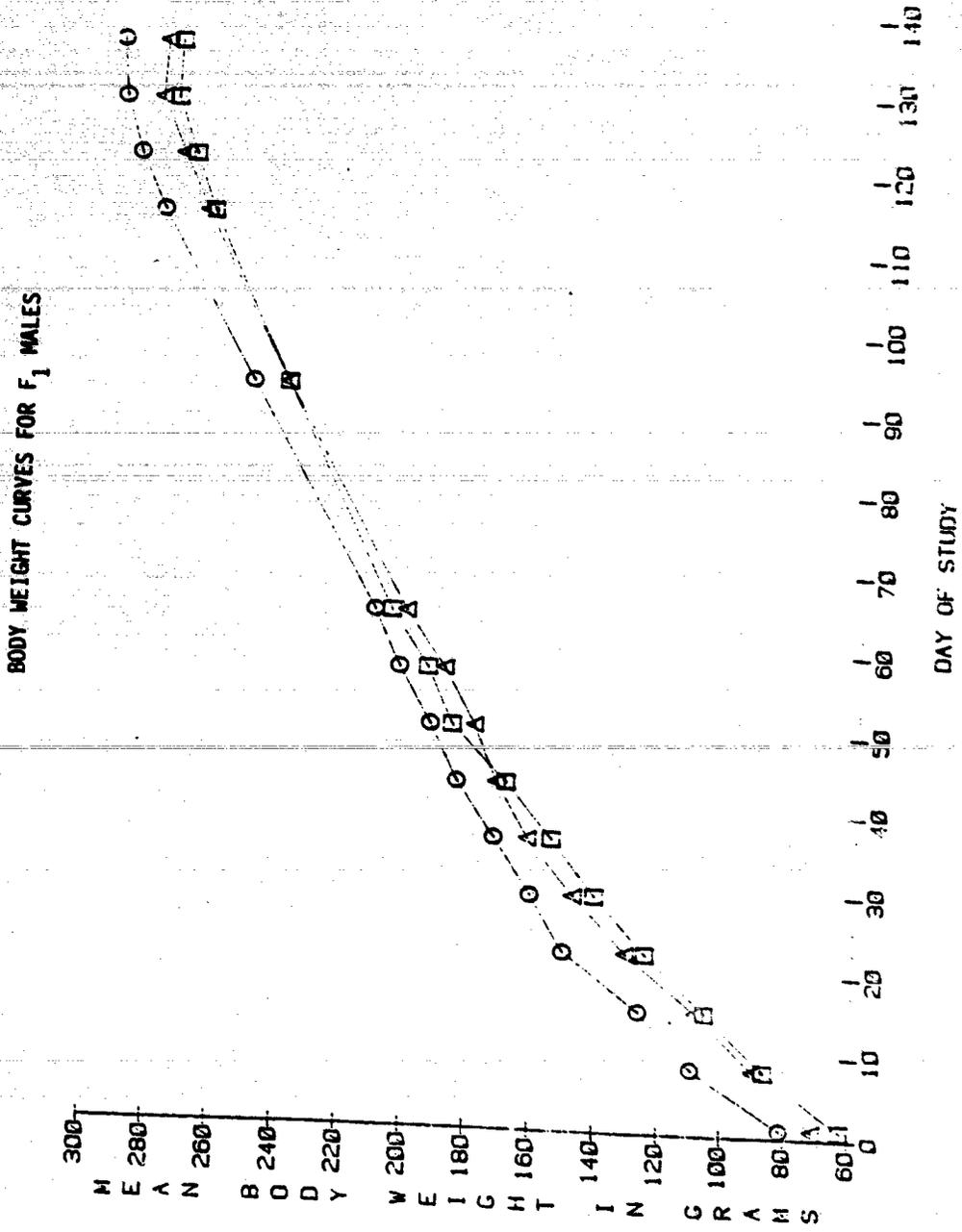


FIGURE 5

BODY WEIGHT CURVES FOR F₁ FEMALES

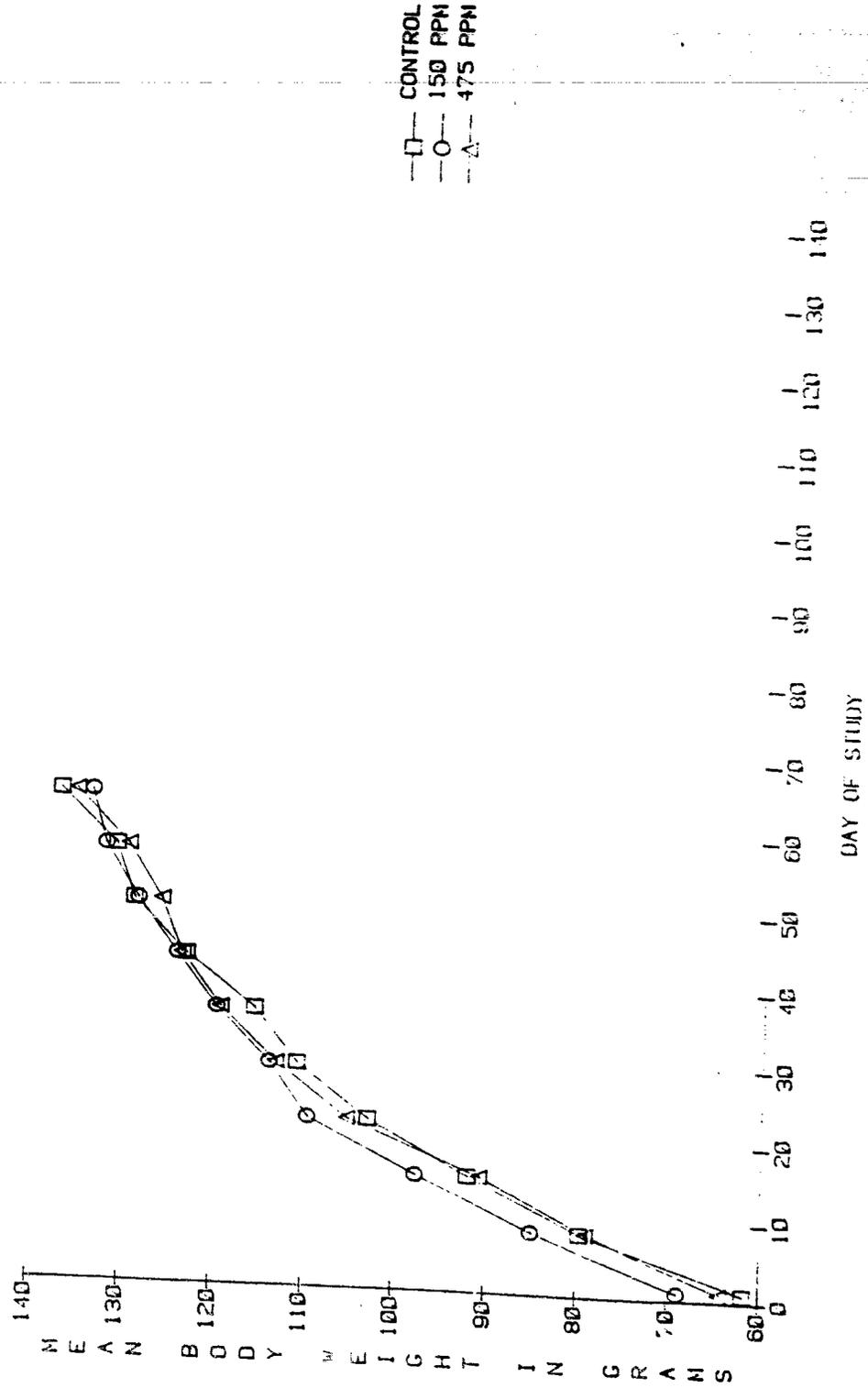


Table 5. Reproductive Parameters for F₀ F-344 Male Rats Exposed to Methyl Chloride by Inhalation for Twelve Weeks Followed by 10 Weeks of N₂ Exposure Prior to Mating with Unexposed Females.

	Exposure Group			
	0 ppm	475 ppm	1500 ppm	
Number of males	20	20	20	
Number of females	40	40	40	
Females with copulation plug	25 (63%)	27 (68%)	26 (70%)	
Number of copulation plugs producing litters	21 (84%)	26 (74%)	8* (25%)	
Number of males proven fertile	15 (75%)	13 (65%)	5* (25%)	
Number of pups per litter	8.7 ± 2.9	8.7 ± 3.9	8.0 ± 2.3	
Percentage of male pups	50.6 ± 17.4	50.8 ± 22.8	52.1 ± 12.6	
Percentage of pups live at birth	95.1 ± 17.1	92.5 ± 22.4	97.5 ± 7.1	
Percentage of 4 day old pups surviving to day 4	93.9 ± 22.0	92.4 ± 22.3	97.4 ± 4.9	
Percentage of 14 day old pups surviving to day 14	98.2 ± 5.7	99.2 ± 3.4	100 ± 0	
Percentage of 21 day old pups surviving to day 21	100 ± 0	98.2 ± 5.4	100 ± 0	
Percentage of 4 day old pups surviving to day 28	100 ± 0	100 ± 0	100 ± 0	
Mean growth of male pups to day 4 (gms)	98.2 ± 5.7	97.5 ± 7.6	96.9 ± 8.9	
Mean growth of 4 day old male pups to day 14 (gms)	2.8 ± 1.0	3.1 ± 0.9	3.2 ± 0.7	
Mean growth of 14 day old male pups to day 21 (gms)	10.1 ± 4.4	11.0 ± 2.1	11.6 ± 2.5	
Mean growth of 21 day old male pups to day 28 (gms)	12.5 ± 4.8	10.4 ± 2.1	11.7 ± 2.1	
Mean growth of female pups to day 4 (gms)	25.7 ± 1.4	24.7 ± 2.2	25.6 ± 2.4	
Mean growth of 4 days old female pups to day 14 (gms)	2.8 ± 1.0	2.9 ± 0.8	3.6 ± 0.6*	
Mean growth of 14 day old female pups to day 21 (gms)	9.7 ± 4.3	10.9 ± 1.7	10.7 ± 2.1	
Mean growth of 21 day old female pups to day 28 (gms)	11.9 ± 4.9	10.6 ± 2.1	10.8 ± 2.0	
	23.4 ± 1.5	22.8 ± 2.3	22.2 ± 2.0	

*Significantly different from control, p < .05.

Table 6. Reproductive Parameters for F₁ F-344 Rats Both Sexes Exposed by Inhalation to Methyl Chloride for Ten Weeks Prior to Mating.

	Exposure Group		
	0 ppm	150 ppm	475 ppm
Number of males	40	40	23
Number of females	80	80	46
Number of females with copulation plug	64 (80%)	51 (64%)	30 (65%)
Females with copulation plugs producing litters	46 (72%)	36 (71%)	18 (60%)
Number of males proven fertile	31 (78%)	26 (65%)	14 (61%)
Number of pups per litter	9.2 ± 1.8	9.3 ± 1.6	8.9 ± 1.6
Percentage of male pups	51.1 ± 17.8	52.8 ± 12.9	41.0 ± 15.6*
Percentage of pups live at birth	100.0 ± 0	100 ± 0	99.0 ± 4.0
Percentage of 4 day old pups surviving at day 4	98.2 ± 8.7	93.1 ± 23.5	98.7 ± 3.8
Percentage of 14 day old pups surviving to day 14	97.8 ± 14.9	91.9 ± 25.7	99.3 ± 3.0
Percentage of 21 day old pups surviving to day 21	99.4 ± 2.9	100 ± 0	100 ± 0
Percentage of 28 day old pups surviving to day 28	98.9 ± 3.6	100 ± 0	100 ± 0
Mean growth of 4 day old pups surviving to day 4	97.1 ± 15.2	91.9 ± 25.7	99.3 ± 3.0
Mean growth of 4 day old male pups to day 4 (gms)	1.8 ± 0.7	1.8 ± 0.5	2.2 ± 1.2
Mean growth of 14 day old male pups to day 14 (gms)	6.9 ± 1.5	6.4 ± 1.8	6.2 ± 1.0
Mean growth of 21 day old male pups to day 21 (gms)	7.9 ± 1.3	7.4 ± 1.3	6.6 ± 1.0*
Mean growth of 28 day old male pups to day 28 (gms)	18.0 ± 5.3	16.0 ± 6.1	18.7 ± 3.5
Mean growth of female pups to day 4 (gms)	1.9 ± 0.6	1.8 ± 0.5	2.1 ± 1.0
Mean growth of 4 day old female pups to day 4 (gms)	6.9 ± 1.3	6.3 ± 1.7	6.2 ± 1.1
Mean growth of 14 day old female pups to day 14 (gms)	8.2 ± 2.0	7.6 ± 1.1	6.0 ± 2.4*
Mean growth of 21 day old female pups to day 21 (gms)	17.9 ± 3.7	17.0 ± 2.5	18.5 ± 3.8

*Significantly different from control, p < .05.

DISCUSSION

In our facility approximately 75% of inexperienced pairs of F-344 rats successfully breed as evidenced by a copulation plug or sperm in the vagina. The F₀ breeding resulted in a lower breeding frequency in all groups (45% in controls, 63% in 150 ppm, 56% in 475 ppm and 65% in 1500 ppm) for unknown reasons. We looked for copulation plugs in the fecal pan as an indicator of breeding and did not look for copulation plugs or sperm in the vagina. This cannot explain the reduction since we have found that fewer than 4% of female rats that are pregnant do not have a pan plug using our system (unpublished data). Sufficient animals were used to provide enough litters in the control groups that the small percentage of females with litters and the elimination of females that did not have a pan plug did not affect the interpretation of this study. When the exposed males were bred to unexposed females there were similar frequencies of copulation plugs found (controls 74%, 150 ppm 76%, 475 ppm 62% and 1500 ppm 63%). Since no treatment related differences were seen between groups in the number of females with copulation plugs, the decrease in fertility caused by methyl chloride did not appear to result from any effects on libido or ability to breed.

Since there was no difference in fertility between exposed or unexposed females mated to males in the same dose group, it appears that the impairment in fertility affected only the male rats. However, mating of unexposed males and females exposed to 1500 ppm would have to be done to be certain that the profound effect on male fertility at 1500 ppm did not hide an effect on the fertility of females at this high concentration. The epididymal granulomas seen in 3 of 10 1500 ppm F₀ males could contribute to a decrease in fertility especially when these lesions were bilateral. (1/10). However, this lesion

occurred in a low incidence at these concentrations which probably did not significantly affect the overall fertility. Statistically fewer litters were born to females bred to 475 ppm males. The number of 475 ppm males not sireing a litter is on the borderline of statistical significance. When data from males bred to either two exposed or two unexposed females was compared to controls the number of 475 ppm males proven fertile was lower but not significantly different from control. The number of 475 ppm males proven fertile was statistically lower when the 4 matings were combined. It appears therefore that the highest no effect concentration in this study is 150 ppm.

Since F-344 rats exposed to 1500 ppm of methyl chloride have a 10 to 20% body weight gain reduction, we studied (Hamm et al., 1983) F-344 rats fed a restricted diet for 10 weeks to produce a body weight gain depression (BWGD) of 21% for males and 16% for females. In these animals, there was a profound but reversible effect on reproduction. Seventy percent (56) of the control females had copulation plugs and 60% (48) had litters. Only 16% (13) of the females on the restricted diet had plugs and no litters were born. After a 10 week recovery period when both groups were fed ad libitum, 52% (31) of the control females had plugs and 38% (23) had litters while 62% (37) of the BWGD females on the restricted diet had plugs and 45% (27) had litters. Rats exposed to 1500 ppm methyl chloride had a similar BWGD depression, but this effect was not the result of decreased food consumption (data not shown), and there was no decrease in copulation plugs as seen in the restricted diet animals. Interestingly, male rats exposed to 475 ppm methyl chloride had a statistically significant 5-7% mean BWGD at the time of breeding. The mean body weight of 475 ppm males producing litters was significantly higher than the mean body weight of 475 ppm males that did not produce litters (data not

shown). There was no decrease in food consumption or in copulation plugs among these groups of 475 ppm males. More work is required to understand the effect of BWGD on reproduction in the F-344 rat and the potential interaction of the mechanisms of BWGD and methyl chloride toxicity to the testicle. The interpretation of other reproduction studies conducted at doses which cause BWGD may be confounded by this problem.

No statistically significant effect was seen on fertility in the second generation breeding. There was, however, a trend toward decreased litters in the 475 ppm group. A smaller number of males was used at 475 ppm for the second generation breeding which decreased our ability to detect a reproductive effect. The significance of the decrease in pup growth from 14 to 21 days in 475 ppm male and female F₂ pups is not known but is probably not biologically important since the decrease is not present prior to this time or later in the study. The significance of the decreased number of male pups in the 475 ppm group is also not known.

The inclusion of a recovery evaluation provided important additional information for the interpretation of these data. There were no histopathological lesions in the testicles of male rats exposed to 475 ppm and fertility in these animals was normal after a 10 week period of non-exposure. At 1500 ppm there were irreversible lesions in the testicles of all males but fertility was partially restored in some males after the 10 week non-exposure period.

The possible mechanisms of the observed reproductive effect are being studied further in our laboratories. A dominant lethal study which includes sperm morphology and sperm viability studies is in progress.

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Appendix A

Protocols

Study #

- 8210 - Exposure and breeding of F₀ animals
- 8211 - Exposure and breeding of F₁ animals
- 8212 - Breeding of 30 F₀ males per group with unexposed females the two weeks immediately after exposures stopped for the males
- 8216 - Inhalation protocol
- 8266 - First recovery breeding of the F₀ male F-344 rats from 8210 with the unexposed female F-344 rats from 8212. The protocol is not included since the breeding procedures were the same as those used for protocol 8212.
- 8296 - Second recovery breeding of the F₀ male F-344 rats from 8210 with the unexposed female F-344 rats from 8212. The protocol is not included since the breeding procedures were the same as those used for protocol 8212.