FINAL REPORT

INHALATION TOXICITY STUDY OF NITROUS OXIDE AND HALOTHANE IN RATS

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16. Abstracts

The mutagenic potential of long-term exposure of 120 male Sprague-Dawley rats to nitrous oxide is measured by observing aberrations in bone marrow cell chromosomes. Forty rats serve as controls, 40 are exposed to 50 ppm nitrous oxide plus 1 ppm halothane, and 40 are exposed to 500 ppm nitrous oxide plus 10 ppm halothane, for 7 hours a day, 5 days a week for 52 weeks. The most -requently found aberration is the chromatid gap. There is a significant increase in the number of gaps and this increase is dose-related. Chromatid breaks are the next most frequent aberrations observed. Chromosomal breaks are comparable among all groups. Chromosomal markers are significantly increased in the group receiving 500 ppm nitrous oxide. The incidence of polyploidy is comparable among control and treated groups. Fifty-nine percent of the animals in the first treatment group show aberrant ce-ls; 68% in the second treatment group. It is concluded that exposure to 500 ppm nitrous oxide plus 10 ppm halothane can cause chromosomal damage to bone marrow cells in the rat.

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MATERIAL: Nitrous Oxide Plus Halothane

SUBJECT: FINAL REPORT
Project No. 785-200
Contract No. CDC-99-74-46

I. OBJECTIVE

The purpose of this study was to assess the effect of chronic inhalation exposure to two concentrations of nitrous oxide (N_2O) and halothane combined, on the incidence of reticuloendothelial malignancies in Fischer 344 rats. The concentrations targeted were 1.0 and 10 ppm halothane with 50 and 500 ppm N_2O, respectively.

II. MATERIALS AND METHODS

A. Animals and Animal Groups

Three hundred Fischer 344 strain rats, approximately 30 days old, 150 males and 150 females, were evenly divided into three groups of 50 of each sex by random selection. The groups are identified below:

Group No.	No. of Rats M	No. of Rats F	Treatment
1	50	50	Air Control
2	50	50	50 ppm N_2O + 1 ppm Halothane
3	50	50	500 ppm N_2O + 10 ppm Halothane

B. Exposure Conditions

The animals in the treatment groups were exposed under dynamic conditions of 1200 liters/minute airflow to the respective N_2O and halothane mixtures in 6000-liter glass and stainless steel Rochester-type inhalation chambers.

Exposures were conducted for seven hours per day, five days a week, for 104 weeks. The control males and females were exposed to filtered room air in a similar chamber with flow characteristics identical to that of the treatment groups.

The animals were individually numbered with ear tags and housed in groups of five of a sex in stainless steel mesh cages with stainless steel top loading feeders and automatic drinking valves. The cages were arranged on one layer throughout the study. Water and basal laboratory diets (Purina Rat Chow) were available ad libitum. The cages were changed and washed weekly.

C. Exposure Method

Input of filtered air was delivered to the chamber via a tangential pipe into a cylindrical turret at the apex of the pyramidal top and the chamber exhausted via a goose-necked duct at the bottom above the central drain pipe.

Nitrous oxide (N_2O) was supplied from pressurized cylinders containing certified 98% pure nitrous oxide anhydride. The gas was passed under positive pressure through a flowrator and critical orifice into a 3-necked mixing flask prior to being inserted via the input duct into the chamber turret.

The halothane used was the Fluothane® brand of 2-bromo-2-chloro-1,1,1,-trifluorothane. Nitrogen was passed via a flowrator and critical orifice through the headspace of a glass flask containing liquid halothane and then passed into the mixing flask to mix with the N_2O prior to delivery into the chamber air input duct.

D. Chamber Monitoring

Chamber concentrations of N_2O and halothane were determined from samples pulled via a standard Teflon® probe located just above the middle cage in the chamber prior to exposure, at hourly intervals during the first five days of exposure, and at least daily during the remainder of the exposure period after chamber equilibration (T_{99} = 23 minutes). Chamber concentrations of nitrous oxide were determined by on-line infrared spectrophotometry validated against gas chromatography (1). Halothane concentrations were determined by peak height analysis on a gas chromatograph with an electron capture detector. Gas samples were taken by syringe from a septum in the sample line and injected into a vacutainer containing a measured volume of 2,2,4 trimethyl pentane, and then a 1.0 µl aliquot of the solution was injected into the gas chromatograph.

E. Body Weights

Individual body weights were recorded at pre-exposure and weekly for the first 24 weeks, biweekly for the succeeding 28 weeks, once every four weeks for the next 36 weeks, and then biweekly thereafter.

F. Observations

Daily observations were made for morbidity and mortality. Records were maintained for gross signs of systemic toxicity and of the incidence, size, and location of tissue masses at the same intervals as body weights were recorded.

G. Clinical Studies

Hematologic studies were conducted on eight male and eight female animals from each group at 13, 26, 52, 78, and 104 weeks. Indices that were measured are as follows:

hematocrit
hemoglobin concentration
erythrocyte count
total leukocyte count
differential leukocyte count

H. Necropsies

At the termination of the study, complete gross necropsies were performed on all surviving animals as well as those animals dying or killed *in extremis* during the study, with the following exceptions where necropsies were not performed on animals which died: Group 1 - one male and one female; Group 2 - one male and three females; and Group 3 - two males and two females. Of these animals, one Group 1 female and one Group 2 female were inadvertently not necropsied. Advanced autolysis precluded necropsies in the remaining cases.

The following tissues from each necropsied animal were excised and preserved in 10% neutral buffered formalin: heart, liver, spleen, kidneys, testes with epididymides, thymus, hepatic lymph node, mesenteric lymph node, thoracic lymph node, thymic lymph node, cervical lymph node, axillary lymph node, inguinal lymph node, bone marrow, unusual lesions, and any tissue masses.

Organ weights were recorded for the following organs: heart, liver, spleen, kidneys, and testes with epididymis. These were used to calculate organ weight/body weight ratios.

I. Histopathology

Following fixation in 10% neutral buffered formalin, the following tissues from each necropsied animal (except for one Group 1 male which died and was necropsied, but the tissues were not subsequently located) were embedded in Paraplast®, sectioned at five microns, slide-mounted, and stained with hematoxylin and eosin: tumors (excluding interstitial cell tumors of the testes which are common in the Fischer rat and are well documented in the literature), liver, thymus, spleen, kidney, hepatic lymph node, mesenteric lymph node, thoracic lymph node, cervical lymph node, axillary lymph node, and inguinal lymph node.

Bone marrow smears were stained with Wright-Giemsa. All slides were examined under a light microscope by a veterinary pathologist.

Though necropsies were performed on most rats which died, autolysis was too far advanced for diagnostic observations to be made on any tissues in one or two animals in each group and on one or more tissues from a few other animals in each group. Because many regional lymph nodes were undetectable at necropsy, this tissue survey was incomplete in the majority of animals. However, the large majority of animals yielded at least three regional lymph nodes for microscopic evaluation. Femoral bone marrow plugs were usually examined but when this was not feasible, decalcified sections of femur and/or sternebrae were examined.

J. Statistical Analysis

Data for terminal body weight, organ weights, and organ weight/body weight ratios were analyzed by a single classification analysis of variance or F-test.

For all analyses, the level of probability chosen for rejecting the null hypothesis was ≤ 0.05.

III. RESULTS

A. Chamber Analyses

The grand means ±S.D. of N_2O and halothane generated for all analytical determinations during the 104-week exposure period were 49.0 ppm N_2O ±5.0 plus 1.1 ppm halothane ±.4 and 501.5 ppm N_2O ±49.4 plus 9.8 ppm halothane ±2.6 for Groups 2 and 3, respectively. During the first month of exposure, N_2O concentration ranges were 31.5 ppm - 67.5 ppm and 477 ppm - 560 ppm for Groups 2 and 3, respectively, while the halothane concentration ranges were .3 ppm - 2.7 ppm and 5.9 ppm - 14.0 ppm for Groups 2 and 3, respectively. From the second month of exposure to the termination of the study, N_2O concentrations ranged from 20.3 ppm - 61 ppm in Group 2 and from 425 ppm - 580 ppm in Group 3, while the halothane concentrations ranged from .5 ppm - 2.9 ppm in Group 2 and 4.6 ppm - 16.4 ppm in Group 3.

B. Body Weights

Mean body weights ±S.D. for each group of animals measured prior to exposure (Week 0) and at Weeks 13, 26, 52, 76, and 104 are presented in Table 1.

Table 1 - Group mean values ±(S.D.)
for body weight (g).

MALES

Group	WEEK					
	0	13	26	52	76	104
1	112.1 (6.2)	313.7 (18.4)	376.7 (22.1)	431.0 (24.7)	438.5 (27.8)	427.6 (28.3)
2	113.1 (6.2)	312.1 (18.6)	375.0 (23.0)	421.0 (26.3)	441.9 (25.8)	438.4 (41.5)
3	106.6 (9.9)	310.6 (16.3)	381.7 (21.1)	436.8 (27.4)	444.7 (29.0)	427.6 (29.6)

FEMALES

1	90.3 (4.2)	179.3 (7.7)	200.6 (13.5)	220.5 (11.4)	239.3 (16.0)	267.7 (19.0)
2	95.5 (5.0)	177.2 (8.1)	197.9 (9.6)	245.3 (11.3)	250.4 (21.1)	274.3 (27.8)
3	83.0 (10.9)	173.2 (9.3)	195.7 (10.3)	222.9 (15.8)	246.5 (18.8)	276.0 (20.9)

Plots of the mean body weights measured throughout the 104-week study are presented in appended Figures 1, 2, and 3 for the male rats and in appended Figures 4, 5, and 6 for the female rats. Evaluation of these data indicated that the body weight growth for the males and females in Groups 2 and 3 was similar to the growth rate for the control males and females.

C. Clinical Observations and Survival

Throughout the study, there was no evidence of an exposure-related effect with regard to the physical appearance and behavior of those animals exposed to a mixture of N_2O and halothane. Incidental findings commonly noted in laboratory rats of this strain were observed at comparable rates in the control and exposed animals of both sexes and increased in frequency as the animals aged. These signs included sores on the body (particularly on the tail), nasal or eye discharges, staining of the fur, occasional soft feces, and alopecia.

Tissue masses and wart-like lesions consistently observed throughout the study were located in the ears, nose, axilla, back, and legs and were present at comparable incidences in the control and exposed groups.

Survival at 104 weeks among the males was 74% (13/50) for Group 1, 76% (12/50) for Group 2, and 72% (14/50) for Group 3. Survival among the females was 86% (7/50) for Group 1, 78% (11/50) for Group 2, and 84% (8/50) for Group 3. Of these non-surviving animals, five males and six females in Group 1, three males and five females in Group 2, and five males and six females in Group 3 were sacrificed in a moribund condition.

D. Hematology

Group mean values and standard deviations for the hematological determinations made at Weeks 13, 26, 52, 78, and 104 are presented in Table 2. Large variations in monocyte percentages were observed among the groups in both males and females at Weeks 78 and 104. However, no consistent trends were indicated.

Due to faulty differential slide preparation and the subsequent inability to read the slides, the mean values for the 104-week differential leukocyte count for the Group 1 males is based on a sample size of four, while the mean values for the Group 1 females are based on a sample size of two. In the case of the Group 1 females, one of the two animal's slides read was artifactually distorted and yielded a metamyclocyte count of 56%, while the other slide yielded 0%; thus the mean value is abnormally high. Repeat samples for the Group 1 males and females could not be obtained because all of the rats had been sacrificed.

All other hematological parameters were within normal limits for this species.

Table 2 - Group mean values ± (S.D.) for various hematological determinations.

MALES WEEK: 13

GROUP	HCT %	HGB g/dl	RBC 10^6/cc	WBC 10^3/cc	DIFFERENTIAL META %	BAND %	SEG %	LYMPH %	MONO %	EOSIN %	BASO %
1	52.3 (2.4)	14.8 (.7)	9.93 (.95)	15.0 (2.8)	0 (0)	0 (0)	23.5 (6.3)	73.8 (6.9)	1.8 (1.4)	1.0 (1.1)	0 (0)
2	50.8 (3.0)	16.0 (1.1)	9.00 (.71)	13.9 (2.3)	0 (0)	0 (0)	26.5 (4.8)	69.4 (4.3)	2.0 (1.8)	2.1 (1.5)	0 (0)
3	51.3 (2.7)	16.5 (1.0)	9.10 (.69)	14.1 (2.6)	0 (0)	0 (0)	26.1 (6.8)	72.6 (7.2)	.9 (.6)	.4 (.5)	0 (0)

FEMALES WEEK: 13

GROUP	HCT %	HGB g/dl	RBC 10^6/cc	WBC 10^3/cc	DIFFERENTIAL META %	BAND %	SEG %	LYMPH %	MONO %	EOSIN %	BASO %
1	50.5 (3.5)	15.5 (1.0)	9.20 (.80)	16.3 (2.9)	0 (0)	0 (0)	26.5 (6.7)	71.0 (7.4)	1.5 (1.2)	1.0 (1.1)	0 (0)
2	53.8 (3.9)	16.6 (.6)	8.70 (1.65)	11.9 (1.8)	0 (0)	0 (0)	24.6 (9.5)	73.1 (9.9)	1.1 (1.2)	1.3 (.9)	0 (0)
3	51.6 (1.7)	16.6 (1.3)	8.76 (.63)	13.0 (2.1)	0 (0)	0 (0)	30.5 (8.1)	67.3 (9.2)	1.3 (.9)	1.3 (1.0)	0 (0)

Table 2 - Group mean values ± (S.D.) for various hematological determinations.

MALES WEEK: 26

GROUP	HCT %	HGB g/dl	RBC 10^6/cc	WBC 10^3/cc	DIFFERENTIAL META %	BAND %	SEG %	LYMPH %	MONO %	EOSIN %	BASO %
1	51.4 (1.4)	18.4 (.9)	9.86 (.43)	10.1 (2.8)	0 (0)	0 (0)	24.8 (5.9)	73.8 (5.9)	0 (0)	1.5 (.5)	0 (0)
2	49.9 (1.1)	17.1 (1.4)	9.19 (.50)	9.6 (2.9)	0 (0)	0 (0)	33.0 (6.8)	64.6 (7.2)	.6 (.7)	1.8 (1.8)	0 (0)
3	50.7 (1.2)	15.9 (.7)	8.25 (1.00)	14.9 (3.5)	0 (0)	0 (0)	27.9 (3.9)	71.8 (3.7)	0 (0)	.4 (.7)	0 (0)

FEMALES WEEK: 26

1	50.9 (1.5)	17.3 (1.5)	8.46 (.70)	8.9 (3.8)	0 (0)	0 (0)	29 (7.7)	70.9 (8.0)	0 (0)	.1 (.4)	0 (0)
2	50.3 (1.7)	16.5 (.7)	8.11 (.33)	10.3 (1.9)	0 (0)	0 (0)	25.3 (11.5)	73.8 (8.9)	0 (0)	2.3 (2.4)	0 (0)
3	48.1 (3.0)	16.1 (1.6)	7.86 (.69)	13.7 (2.9)	0 (0)	0 (0)	34.1 (12.3)	62.1 (13.2)	1.4 (1.2)	2.4 (1.2)	0 (0)

Table 2 - Group mean values ± (S.D.) for various hematological determinations.

MALES WEEK: 52

GROUP	HCT %	HGB g/dl	RBC 10^6/cc	WBC 10^3/cc	DIFFERENTIAL META %	BAND %	SEG %	LYMPH %	MONO %	EOSIN %	BASO %
1	50.0 (1.2)	16.1 (.4)	8.91 (.25)	8.9 (4.76)	0 (0)	0 (0)	29.9 (11.8)	69.1 (11.9)	0 (0)	1 (.8)	0 (0)
2	48.6 (2.1)	15.9 (1.4)	8.86 (.61)	9.1 (2.2)	0 (0)	0 (0)	34.1 (8.0)	65.1 (7.8)	0 (0)	.8 (.9)	0 (0)
3	49.7* (1.3)	15.9* (.9)	8.91* (1.23)	12.0* (3.4)	0* (0)	.1* (.3)	30.8* (10.4)	68.0* (10.1)	.2* (.6)	.8* (.8)	0* (0)

FEMALES WEEK: 52

1	47.9 (1.3)	15.3 (.7)	7.74 (.58)	6.3 (1.9)	0 (0)	0 (0)	29 (8.1)	70.0 (8.8)	0 (0)	1.0 (1.1)	0 (0)
2	47.0 (1.1)	15.4 (.8)	7.76 (1.38)	7.8 (2.1)	0 (0)	0 (0)	29.5 (11.6)	69.6 (12.0)	0 (0)	.9 (.8)	0 (0)
3	47.0 (1.8)	15.3 (.5)	8.09 (.62)	6.4 (1.8)	0 (0)	0 (0)	33.1 (6.6)	65.8 (7.1)	.5 (1.4)	.6 (.7)	0 (0)

*Mean based on a sample size of 9.

Table 2 - Group mean values ± (S.D.) for various hematological determinations.

MALES WEEK: 78

GROUP	HCT %	HGB g/dl	RBC 10^6/cc	WBC 10^3/cc	DIFFERENTIAL META %	BAND %	SEG %	LYMPH %	MONO %	EOSIN %	BASO %
1	48.5 (1.0)	16.3 (.4)	8.88 (.99)	7.5 (2.7)	0 (0)	0 (0)	42.5 (13.8)	50.0 (12.8)	5.9 (2.0)	1.6 (1.1)	0 (0)
2	49.8 (2.9)	16.6 (.6)	8.67 (.43)	10.4 (2.0)	0 (0)	.4 (1.1)	45.5 (8.3)	53.0 (8.6)	0 (0)	1.1 (1.1)	0 (0)
3	49.5 (3.3)	16.6 (.6)	8.89 (.99)	11.6 (3.2)	0 (0)	0 (0)	42.6 (13.2)	50.9 (15.3)	5.8 (3.0)	.8 (.9)	0 (0)

FEMALES WEEK: 78

1	47.7 (1.1)	16.3 (.6)	8.17 (.39)	7.0 (.9)	0 (0)	0 (0)	34.1 (5.9)	60.0 (6.2)	4.4 (1.8)	1.5 (1.2)	0 (0)
2	50.5 (3.7)	16.0 (.9)	8.16 (.78)	6.7 (4.6)	0 (0)	0 (0)	39.6 (5.4)	57.8 (6.4)	0 (0)	2.6 (1.5)	0 (0)
3	48.4 (1.2)	16.0 (.9)	8.56 (.58)	9.9 (2.1)	0 (0)	0 (0)	33.1 (5.8)	58.9 (6.6)	6.5 (3.6)	1.5 (1.7)	0 (0)

Table 2 - Group mean values ± (S.D.) for various hematological determinations.

MALES WEEK: 104

GROUP	HCT %	HGB g/dl	RBC 10^6/cc	WBC 10^3/cc	DIFFERENTIAL META %	BAND %	SEG %	LYMPH %	MONO %	EOSIN %	BASO %
1	51.9 (6.7)	17.5 (1.9)	8.96 (1.2)	13.4 (3.3)	0* (0)	0* (0)	49.8* (11.0)	47.0* (9.9)	2.3* (3.2)	1.0* (0)	0* (0)
2	55.5 (8.2)	18.8 (2.7)	9.35 (1.38)	13.6 (4.3)	.1φ (.4)	.3φ (.5)	54.1φ (6.7)	40.4φ (8.9)	4.9φ (3.5)	1.4φ (1.1)	.1φ (.4)
3	51.4 (7.9)	16.9 (2.8)	8.76 (1.88)	19.5 (15.6)	0 (0)	.8 (1.8)	52.1 (16.8)	44.6 (15.4)	1.5 (1.7)	1.0 (1.4)	0 (0)

FEMALES WEEK: 104

1	45.4 (6.3)	15.8 (2.7)	7.63 (1.74)	15.8 (13.6)	28.0† (39.6)	3.0† (2.8)	33.5† (10.6)	34.5† (30.4)	1.0† (1.4)	0† (0)	0† (0)
2	47.7 (2.0)	16.8 (.6)	9.50 (.85)	10.9 (4.2)	0 (0)	.3 (.7)	43.9 (9.9)	46.9 (11.2)	7.8 (3.3)	1.3 (.9)	0 (0)
3	49.9 (1.6)	17.2 (.8)	8.58 (.47)	7.4 (1.8)	0 (0)	.4 (.7)	46.8 (7.4)	49.8 (7.0)	1.1 (1.5)	2.0 (1.3)	0 (0)

* Mean based on sample size of 4.
φ Mean based on sample size of 7.
† Mean based on sample size of 2.

E. Terminal Body Weight, Organ Weights, and Organ/Body Weights Ratios

Group means and standard deviations of terminal body weights, organ weights, and organ/body weight ratios are presented in Table 3. Analysis of variance indicated that the heart weight, kidney weight, and the kidney weight/body weight ratio for the Group 3 males was significantly lower than the values obtained for the control group males. The spleen weights for the Group 2 and 3 females and the spleen ratio for the Group 2 females were significantly lower than the values for the control females. No other significant differences were indicated among the control and exposed animals although the spleen ratio for Group 3 females was markedly lower than in Group 1 females.

F. Gross Pathology

Gross necropsy findings indicated no gross lesions in three Group 1 males, 13 Group 1 and Group 3 females, and 19 Group 2 females. No exposure-related lesions were apparent. Incidental findings were observed at a comparable incidence in the control and exposed animals. These findings included areas of discoloration on the lungs; pale, mottled, or granular livers; discolored, granular, or cystic kidneys; enlarged and/or granular spleens; discolored, enlarged, cystic testes; and cystic ovaries. A variety of tissue masses or nodules were observed at a comparable incidence in all groups of males and females.

G. Histopathological Evaluation

A variety of non-neoplastic lesions were observed in all groups without relationship to the experimental regimen. Most commonly observed were chronic renal disease, chronic lymphadenopathy, cholangiofibrosis, and biliary hyperplasia.

Table 3 - Mean values ±(S.D.) for terminal body weight, organ weights, and organ/body weight ratios.

MALES

Group	Terminal Body Weight g	Heart Weight g	Heart Ratio %	Liver Weight g	Liver Ratio %	Spleen Weight g	Spleen Ratio %	Kidney Weight g	Kidney Ratio %	Testes Weight g	Testes Ratio %
1	408.2 (26.7)	1.26 (.16)	.31 (.04)	10.54 (1.02)	2.59 (.25)	1.27 (1.01)	.32 (.27)	2.81 (.26)	.69 (.06)	6.55 (1.12)	1.61 (.26)
2	415.6 (37.6)	1.24 (.14)	.30 (.04)	11.06 (1.68)	2.67 (.37)	1.30 (.89)	.32 (.23)	2.72 (.29)	.66 (.07)	6.63 (1.58)	1.61 (.41)
3	402.0 (27.7)	1.16* (.20)	.29 (.05)	10.22 (1.71)	2.54 (.40)	1.24 (1.21)	.31 (.30)	2.59* (.33)	.65* (.09)	6.21 (1.79)	1.54 (.44)

*Significantly lower than control at $p \leq 0.05$.

Table 3 - Continued

FEMALES

Group	Terminal Body Weight g	Heart Weight g	Heart Ratio %	Liver Weight g	Liver Ratio %	Spleen Weight g	Spleen Ratio %	Kidney Weight g	Kidney Ratio %
1	253.6 (20.3)	.90 (.16)	.36 (.07)	6.76 (1.33)	2.68 (.61)	1.03 (1.58)	.42 (.68)	1.81 (.15)	.72 (.08)
2	255.1 (23.8)	.85 (.09)	.34 (.07)	6.61 (.83)	2.61 (.32)	.54* (.40)	.22* (.18)	1.81 (.28)	.72 (.16)
3	245.7 (36.2)	.86 (.12)	.36 (.09)	6.48 (.67)	2.71 (.59)	.54* (.26)	.23 (.13)	1.77 (.15)	.74 (.16)

* Significantly lower than control at $p \leq 0.05$.

Lymphadenopathy was characterized by lymphoid atrophy, pigment and plasmocytosis of medullary cords. Only neoplastic lesions will be discussed.

Group 1:

Six male and seven female rats had monocytic leukemia. Usually multiple organs were involved but most constantly affected were the spleen and liver. Bone marrow was variably affected; however, since only a small portion of the total bone marrow mass was actually examined microscopically, this may reflect failure to section neoplastic foci. Microscopically in the spleen and liver, the disease was characterized by diffuse cellular infiltration of the sinusoids of the splenic red pulp and liver. The infiltrating cells were mononuclear with round, oval, or indented nuclei showing hyperchromasia and often in mitoses. The cytoplasm was usually abundant and faintly eosinophilic. In sections of lymph nodes, the diagnosis of monocytic leukemia was made when neoplastic monocytes could be demonstrated in sinuses, lymphatic and/or vascular channels. Cellular infiltrations of medulla or cortex were not always observed. In the kidney, neoplastic monocytes occasionally could be demonstrated within renal vessels.

Sections of liver revealed one male animal with a neoplastic nodule. This lesion was characterized by the proliferation of small hepatocytes in a trabecular pattern and showing distinct compression of adjacent hepatic parenchyma. One female rat was observed to have a coelomic mesothelioma which involved the surfaces of the kidney, small and large intestine, ovary, abdominal fat, mesentery and was metastatic to the thoracic lymph node.

Characteristically, this neoplasm was composed of proliferating mesothelial cells forming numerous papillary structures supported on a fibrous stroma. Two male rats also had mesotheliomas, one involving abdominal fat, parietal and visceral peritoneum and scrotal fat and one involving scrotal fat only. The morphology of these neoplasms was similar to that observed in the female.

One female had a lymphocytic lymphoma involving the thoracic and axillary lymph nodes only. This was characterized by a diffuse destructive infiltration of relatively mature appearing lymphocytes throughout the node obliterating normal architecture.

A well-differentiated pheochromocytoma was present in the adrenal of one female while another female rat had an alveolar adenoma in a section of lung. Sections of ovary revealed an arrhenoblastoma in one female. This neoplasm was characterized by proliferating sheets of epithelial cells which in some areas formed small tubular structures filled with pale staining cells. An endometrial stromal sarcoma occurred in the uterus of one female rat. Evidence of malignancy was based on the fact that early invasion of uterine wall was demonstrated at one focus. Fibroadenomas of the mammary gland were found in two females and one male rat. These benign growths usually had an abundance of collagenous stroma which separated varying amounts of glandular structures.

A single islet cell adenoma of the pancreas was observed in one male animal. One male rat also had an osteogenic sarcoma involving multiple organs of the thoracic and abdominal cavity.

This neoplasm was well-differentiated and composed of proliferating spindle cells which formed areas of osteoid and trabecular bone. The heart, lung, mediastinum, surface of the urinary bladder, mesentery, mesenteric lymph node, and adrenals were involved.

Sections of skin and subcutaneous tissues revealed three males with fibromas composed of mature collagenous tissues, one male with a fibrosarcoma, one female with a squamous cell carcinoma, one male with a cutaneous papilloma, one male with a trichofollicular adenoma, and one female with a malignant hemangiopericytoma.

Group 2

The incidence and variety of neoplasms present in this group appeared to be unrelated to the exposure of N_2O and halothane. Seven male rats and four female rats had monocytic leukemia. The morphology and organ distribution of this proliferative disease appeared similar to that observed in the reference control group. In sections of liver, three male rats were observed to have neoplastic nodules. These nodules characteristically were composed of small proliferating hepatocytes growing in trabecular pattern and always showed compression of adjacent hepatic parenchyma. One male rat also had a biliary carcinoma which metastasized to the kidney and lung. An alveolar adenoma of the lung was present in one male and an alveolar/bronchiolar adenoma occurred in the lung of one female rat.

A single male rat was observed to have an islet cell carcinoma of the pancreas. This neoplasm was composed of proliferating pale staining epithelial cells growing in sheets and intersected by delicate fibrovascular stroma and in some areas having a sinusoidal pattern of growth.

The entire mass was encapsulated with fibrous tissue and at one focus, invasion of the capsule and adjacent tissues was observed. Numerous mitotic figures were observed in the proliferating epithelial cells. One male rat also had an islet cell adenoma of the pancreas.

One male had a squamous cell carcinoma of the preputial gland. A pheochromocytoma was present in the adrenal of one female and one female also had a papillary adenocarcinoma of the uterus. Sections of mammary gland showed that five females had fibroadenomas and one female also had a mammary adenoma.

In sections of skin, two females and one male had subcutaneous fibrosarcomas, three males had subcutaneous fibromas and one male had an anaplastic subcutaneous fibrosarcoma.

In summary, there appeared to be no association between the exposure to nitrous oxide and halothane and the incidence and variety of neoplasms observed.

Group 3

The variety and incidence of neoplasms observed in this group were unrelated to exposure to nitrous oxide and halothane. Six male rats and three female rats had monocytic leukemia. This proliferative disease appeared similar to that observed in the reference control group. In addition, one female rat also had a histiocytic lymphoma involving the spleen, liver, kidney, hepatic lymph node, mesenteric lymph node, and inguinal lymph node. Neoplastic nodules were present in the hepatic parenchyma of two male rats.

One male rat was observed to have a coelomic mesothelioma involving the splenic capsule, kidney, small intestine, mesenteric fat, and scrotal fat. One male also had a mesothelioma which only involved omentum. An osteoma occurred in the skull of one male rat. This neoplasm was characterized by the presence of relatively mature appearing trabecular bone and the growth was well-demarcated. One male rat also had a squamous cell carcinoma of the non-glandular stomach.

Sections of mammary gland revealed mammary fibroadenomas in five females and five males. One female had a mammary adenoma.

Sections of skin and subcutaneous tissues revealed subcutaneous fibromas in two females and three males. These neoplasms were composed almost entirely of mature collagenous fibrous tissue. One male rat also had an anaplastic subcutaneous fibrosarcoma.

A single male animal had a pulmonary alveolar adenoma.

Detailed histopathology incidence tables are presented in Table 4 for all groups of animals.

Conclusions

Under the conditions of this experiment, long term administration of nitrous oxide and halothane at two exposure levels was not associated with an increase in the incidence of neoplasia in general and specifically an increase in the incidence of reticuloendothelial tumors. The wide variety of tumors observed in the control (and exposed) animals have been observed previously in this strain of rat. The overall incidence of monocytic leukemia in this study (13.8% in the males and 9.7% in the females yielding

Table 4
Individual Histopathology Findings

GROUP 1 MALES

Organ	Anim. No./Cage	1/10	2/10	3/10	4/10	5/10	1/11	2/11	3/11	4/11	5/11	1/12	2/12	3/12	4/12	5/12	1/13	2/13	3/13	4/13	5/13	1/14	2/14	3/14	4/14	5/14
SPLEEN		X	X		X			X	X		X	X	X	X	X	X	A	X		X		0	X	X		X
Monocytic Leukemia				P		P	P			P											P				P	
LIVER		X	X	X	X			X	X		X	X	X	X	X	X	A	X		X		X	X	X		X
Monocytic Leukemia						P	P			P											P				P	
KIDNEY		X	X	X	X	X	X	X	X		X	X	X	X	X	X	A	X		X	X	X	X	X		X
Monocytic Leukemia										P															P	
BONE MARROW		X	X		X	X	X	0	X		X	0	X	X	X	X	A	X		X		0	X	X	X	X
Monocytic Leukemia				P						P											P					
THYMUS		X	X	X	X	X	X	X	X		0	X	X	X	0	X	A	X		X	X	0	X	X		X
Monocytic Leukemia										P															P	
THORACIC LYMPH NODE		X	X	X	X	X		X	X		X	0	X	X	X	X	A	X		X	X	0	X	X		X
Monocytic Leukemia							P			P															P	
HEPATIC LYMPH NODE		X	X	0	X			X	X		X	0	X	X	X	X	A	X		X	0	0	X	X		X
Monocytic Leukemia						P	P			P															P	
MESENTERIC LYMPH NODE		X	X	X	X			X	X	X	X	X	X	X	X	X	A	X	NO NECROPSY	X		0	X	X		X
Monocytic Leukemia						P	P														P				P	
CERVICAL LYMPH NODE		X	X	X	X	X		X	X	X	X	X	X	X	X	X	A	X		X	X	0	X	X		X
Monocytic Leukemia							P																		P	
AXILLARY LYMPH NODE		X	X	0	X	X		X	0	X	0	0	X	X	X	X	A	X		0	X	0	X	X		0
Monocytic Leukemia							P																		P	
INGUINAL LYMPH NODE		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	A	X		0	0	0	0	0	0	0
UNUSUAL LESIONS																										
Abdominal Fat																										
Mesothelioma				P																						
Perietal Peritoneum																										
Mesothelioma				P																						
Visceral Peritoneum																										
Mesothelioma				P																						
Testes																										
Mesothelioma (scrotal fat)				P																						
Interstitial Cell Tumor												P														
Mammary Gland																										
Fibroadenoma																P										
Subcutaneous Fibroma												P	P							P						
Heart																										
Osteogenic Sarcoma																						P				
Lung																										
Osteogenic Sarcoma																						P				
Mediastinum																										
Osteogenic Sarcoma																						P				
Urinary Bladder																										
Osteogenic Sarcoma																						P				
Mesenteric Lymph Node																										
Osteogenic Sarcoma																						P				
Adrenal																										
Osteogenic Sarcoma																						P				
Mesentery																										
Osteogenic Sarcoma																						P				

Table 4
Individual Histopathology Findings

GROUP 1 MALES

Organ	Anim. No./Cage																									
	1/15	2/15	3/15	4/15	5/15	1/16	2/16	3/16	4/16	5/16	1/17	2/17	3/17	4/17	5/17	1/18	2/18	3/18	4/18	5/18	1/19	2/19	3/19	4/19	5/19
SPLEEN	X	X	A		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
LIVER	X	X	A		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Neoplastic Nodule																						P			
KIDNEY	X	X	A		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BONE MARROW	X	X	0		X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
THYMUS	X	X	A		X	X	X	X	X	X	X	X	X	X	0	X	0	0	X	X	X	X	X	X	X
THORACIC LYMPH NODE	X	X	A		X	X	X	X	X	X	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HEPATIC LYMPH NODE	X	0	A		X	0	0	X	X	X	0	X	X	X	0	X	X	X	X	X	X	X	X	X	0
MESENTERIC LYMPH NODE	X	X	A		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CERVICAL LYMPH NODE	X	X	A		X	X	X	X	X	X	X	X	0	X	X	X	X	X	X	X	X	X	X	X	X
AXILLARY LYMPH NODE	X	0	A		0	X	0	X	0	0	X	X	0	X	0	X	X	0	X	X	0	X	X	X	0
INGUINAL LYMPH NODE	0	0	A		X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
UNUSUAL LESION																									
Pancreas																									
Islet Cell Adenoma																					P				
Testes																									
Mesothelioma (scrotal fat)										P															
Interstitial Cell Tumor											P														
Subcutaneous Fibrosarcoma																					p				
Cutaneous Papilloma											P														
Subcutaneous Trichofollicular Adenoma						P																			

TISSUES TAKEN BUT NOT LOCATED

Table 4
Individual Histopathology Findings

GROUP 1 FEMALES

Organ	Anim. No./Cage	1/20	2/20	3/20	4/20	5/20	1/21	2/21	3/21	4/21	5/21	1/22	2/22	3/22	4/22	5/22	1/23	2/23	3/23	4/23	5/23	1/24	2/24	3/24	4/24	5/24
SPLEEN			X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X
Monocytic Leukemia													P	P												
LIVER			X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X
Monocytic Leukemia													P	P												
KIDNEY			X	X	X	X	X	X	X	X		X			X	X	X	X	X	X	X	X	X	X	X	X
Monocytic Leukemia													P	P												
Mesothelioma											P															
BONE MARROW			X	X	0	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X
Monocytic Leukemia													P	P												
THYMUS			X	X	X	X	X	X	0	X	X	X	X	X	X	X	X	X	X	X	X	0	X	X	X	X
THORACIC LYMPH NODE			X	X	X	X	X	0	X	X		X			X	X	X	X	0	X	X	X	X	X	X	X
Monocytic Leukemia													P	P												
Mesothelioma											P															
HEPATIC LYMPH NODE			0	X	0	X	X	X	X	X	0	X			X	X	X	X	X	X	X	X	0	X	0	X
Monocytic Leukemia													P	P												
MESENTERIC LYMPH NODE			X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X
Monocytic Leukemia														P												
CERVICAL LYMPH NODE			X	X	X	X	X	X	X	X	X	X	X		X	X	X	0	X	X	X	X	X	X	X	X
Monocytic Leukemia														P												
AXILLARY LYMPH NODE			X	X	X	0	0	X	0	0	0	X	X	0	X	X	0	0	0	X	X	0	X	X	X	X
INGUINAL LYMPH NODE			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0
UNUSUAL LESION																										
Small Intestine																										
Mesothelioma											P															
Abdominal Fat																										
Mesothelioma											P															
Adrenal																										
Pheochromocytoma																				P						
Ovary																										
Mesothelioma											P															
Large Intestine																										
Mesothelioma											P															
Mesentery																										
Mesothelioma											P															
Malignant Hemangiopericytoma					P																					

NO NECROPSY

Table 4
Individual Histopathology Findings

GROUP 1 FEMALES

Organ	Anim. No./Cage	1/25	2/25	3/25	4/25	5/25	1/26	2/26	3/26	4/26	5/26	1/27	2/27	3/27	4/27	5/27	1/28	2/28	3/28	4/28	5/28	1/29	2/29	3/29	4/29	5/29
SPLEEN		X		X	X	X	X	X	X		X	X	X		X	X	X		X	X		X	X	X	X	X	X
Monocytic Leukemia			P							P				P				P			P					
LIVER		0		X	X	X	X	X	X		X	X	X		X	X	X		X	X		X	X	X	X	X	X
Monocytic Leukemia			P							P				P				P			P					
KIDNEY		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BONE MARROW		X	0	X	X	X	0	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X
Monocytic Leukemia																		P								
THYMUS		0	X	X	X	0	X	X	X	0	0	0	X	X	X	X	X		X	X	X	X	X	X	X	X	X
THORACIC LYMPH NODE		X		X	X	0	0	X	X	0	X	X	X		X	X	X		X	X		X		X	X	X	X
Monocytic Leukemia			P											P				P			P					
Lymphocytic Lymphoma																							P			
HEPATIC LYMPH NODE		X	0	X	X	0	0	X	X	X	X	0	X		0	0	X		X	X	0	0	X	X	X	X
Monocytic Leukemia														P				P								
MESENTERIC LYMPH NODE		X		X	X	X	X	X	X		X	X	X		X	X	X		X	X		X	X	X	X	X	X
Monocytic Leukemia			P							P				P				P			P					
CERVICAL LYMPH NODE		X		X	X	X	X	0	X	X	X	X	X		X	0	X		X	X		X	X	X	X	X	X
Monocytic Leukemia			P											P				P			P					
AXILLARY LYMPH NODE		0		0	0	X	0	X	X	0	X	X	X		X	0	0		X	X	0	0		X	X	X
Monocytic Leukemia			P											P				P								
Lymphocytic Lymphoma																							P			
INGUINAL LYMPH NODE		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	X	0	0	X	0
Monocytic Leukemia																		P								
THYMIC LYMPH NODE																										
Monocytic Leukemia			P																							
UNUSUAL LESION																										
Lung																										
Alveolar Adenoma															P											
Uterus																										
Endometrial Stromal Sarcoma								P																		
Ovary																										
Arrhenoblastoma																										P
Mammary Gland																										
Fibroadenoma								P					P													
Squamous Cell Carcinoma			P																							

Table 4
Individual Histopathology Findings

GROUP 2 MALES

Organ	Anim. No./Cage	1/30	2/30	3/30	4/30	5/30	1/31	2/31	3/31	4/31	5/31	1/32	2/32	3/32	4/32	5/32	1/33	2/33	3/33	4/33	5/33	1/34	2/34	3/34	4/34	5/34
SPLEEN		X	X	X	X		X	X	X	X	X	X			X	X	X	X	X	X	X	X		X		X
Monocytic Leukemia													P	P									P		P	
LIVER		X	X	X	X		X		X	X	X	X			X	X	X	X	X	X	X	X		X		X
Monocytic Leukemia													P	P									P		P	
Neoplastic Nodule								P																		
KIDNEY		X	X	X	X		X	X	X	X	X	X	A		X	X	X	X	X	X	X	X		X	X	X
Monocytic Leukemia														P									P			
BONE MARROW		X	X	X	X		X	X	X	X	X	X	A	0	X	X	X	X	X	X	X	X		X	X	X
Monocytic Leukemia																							P			
THYMUS		X	X	X	X		X	X	X	X	X	X		X	X	X	X	X	X	X	X	0		X	X	X
Monocytic Leukemia													P										P			
THORACIC LYMPH NODE		X	X	X	X		X	X	X	X	X	X		X	X	X	X	X	X	X	X	0		X		X
Monocytic Leukemia													P										P		P	
HEPATIC LYMPH NODE		X	X	X	X		0	X	X	X	X	X	0		0	0	0	X	X	X	X	X	X	X		0
Monocytic Leukemia														P											P	
MESENTERIC LYMPH NODE		X	X	X	X		X	X	X	0	X	X			X	X	X	X	X	X	X	X		X		X
Monocytic Leukemia													P	P									P		P	
CERVICAL LYMPH NODE		X	X	X	0		X	0	X	X	X	X			X	X	X	X	X	X	X	X		X		X
Monocytic Leukemia													P	P									P		P	
AXILLARY LYMPH NODE		X	X	X	X		X	X	X	X	X	X			X	X	X	X	X	X	X	X		X	X	X
Monocytic Leukemia													P	P									P			
INGUINAL LYMPH NODE		0	0	0	0		0	0	0	0	0	0	A	0	0	0	0	0	0	0	0	0	0	0	0	0
UNUSUAL LESION																										
Skull																										
Osteogenic Sarcoma												P														
Pancreas																										
Islet Cell Carcinoma																	P									
Testes																										
Interstitial Cell Tumor														P												
Subcutaneous Fibroma							P																			
Subcutaneous Fibrosarcoma					P																					

NO NECROPSY

Table 4
Individual Histopathology Findings

GROUP 2 MALES

Organ	Anim. No./Cage	1/35	2/35	3/35	4/35	5/35	1/36	2/36	3/36	4/36	5/36	1/37	2/37	3/37	4/37	5/37	1/38	2/38	3/38	4/38	5/38	1/39	2/39	3/39	4/39	5/39
SPLEEN		X	X	X	X	X	X	A	A		X	X	X	X	X	X	X		X	X	X		X	X	X	X
Monocytic Leukemia										P								P				P				
LIVER			X	X	X	X	X	A	A		X	X	X	X	X	X	X		X	X			X	X	X	X
Monocytic Leukemia										P								P				P				
Biliary Carcinoma		P																								
Neoplastic Nodule										P											P					
KIDNEY			X	X	X	X	X	A	A	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X
Monocytic Leukemia																						P				
Biliary Carcinoma, Metastatic		P																								
BONE MARROW		X	X	X	O	X	X	A	A	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	O
Monocytic Leukemia																						P				
THYMUS		O	X	X	X	O	X	A	A	X	X	X	X	X	X	X	X	X	X	X	X	A	X	X	O	X
THORACIC LYMPH NODE		X	X	X	X	X	X	A	A	X	X	X	X	X	X	X	X		O	X	X	A	X	X	O	X
Monocytic Leukemia																		P								
HEPATIC LYMPH NODE		O	X	X	X	O	O	A	A	X	X	X	X	X	X	X	X	X	X	X	X	O	X	O	X	O
MESENTERIC LYMPH NODE		X	X	X	X	X	O	A	A		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Monocytic Leukemia										P																
CERVICAL LYMPH NODE		X	X	X	X	X	O	A	A	X	X	X	X	X	X	X	X	X	X	X	X	X	O	X	X	X
AXILLARY LYMPH NODE		X	X	X	O	O	X	A	A	O	O	X	X	X	X	X	X		X	X	X	X	X	X	X	X
Monocytic Leukemia																		P								
INGUINAL LYMPH NODE		O	O	O	O	O	O	A	A	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
UNUSUAL LESION																										
Lung																										
Biliary Carcinoma		P																								
Alveolar Adenoma			P																							
Pancreas																										
Islet Cell Adenoma																							P			
Testes																										
Interstitial Cell Tumor					P																					P
Preputial Gland																										
Squamous Cell Carcinoma					P																					
Subcutaneous Fibroma													P						P							
Subcutaneous Anaplastic Fibrosarcoma																									P	

Table 4
Individual Histopathology Findings

GROUP 2 FEMALES

Organ	Anim. No./Cage																									
	1/40	2/40	3/40	4/40	5/40	1/41	2/41	3/41	4/41	5/41	1/42	2/42	3/42	4/42	5/42	1/43	2/43	3/43	4/43	5/43	1/44	2/44	3/44	4/44	5/44
SPLEEN	X	X	X	X	X	X	X	X	X	X	X	X	X		X			X	X	X	X	X	X	X	X
Monocytic Leukemia																	P								
LIVER	XX		X	X	X	X	X	X	X	X	X	X	X		X			X	X	X	X	X	X	X	X
Monocytic Leukemia																	P								
KIDNEY	X	X	X	X	X	X	X	A	X	X	X	X	X		X		X	X	X	X	X	X	X	X	X
BONE MARROW	X	X	X	X	X	X	X	X	X	X	X	X	X		X			X	X	X	X	X	0	X	X
Monocytic Leukemia																	P								
THYMUS	X	X	X	X	X	X	X	0	X	X	0	X	X		X		X	X	X	X	X	X	X	X	X
THORACIC LYMPH NODE	X	X	X	X	X	X	X	X	0	X	X	X	X		X			X	X	X	X	X	X	X	X
Monocytic Leukemia																	P								
HEPATIC LYMPH NODE	X	X	X	X	X	X	X	X	0	X	0	X	X		X			X	X	X	0	X	0	X	0
Monocytic Leukemia																	P								
MESENTERIC LYMPH NODE	X	X	X	X	X	X	X	0	X	X	X	X	X		X			X	X	X	X	X	X	X	X
Monocytic Leukemia																	P								
CERVICAL LYMPH NODE	X	X	X	X	X	X	X	X	X	X	X	X	X		X			X	X	X	X	X	X	X	X
Monocytic Leukemia																	P								
AXILLARY LYMPH NODE	X	0	X	X	X	X	0	X	X	0	X	X	X		X			0	X	X	0	X	X	X	0
Monocytic Leukemia																	P								
INGUINAL LYMPH NODE	0	0	0	0	0	0	0	0	0	0	0	0	0		0		0	0	0	0	X	0	0	0	0
THYMIC LYMPH NODE											0														
UNUSUAL LESIONS																									
Uterus																									
Papillary Adenocarcinoma											P														
Mammary Gland																									
Fibroadenoma		P									P														P
Adenoma						p																			
Subcutaneous Fibrosarcoma								P																	

NO NECROPSY

Table 4
Individual Histopathology Findings

GROUP 2 FEMALES

Organ	Anim. No./Cage	1/45	2/45	3/45	4/45	5/45	1/46	2/46	3/46	4/46	5/46	1/47	2/47	3/47	4/47	5/47	1/48	2/48	3/48	4/48	5/48	1/49	2/49	3/49	4/49	5/49
SPLEEN		X	X		X	X	X		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X		X
Monocytic Leukemia								P										P							P	
LIVER		X	X		X	X	X		0	X	X	X	X	X	X	X	X		X	X	X	X	X	X		X
Monocytic Leukemia								P										P							P	
KIDNEY		X	X		X	X	X	X	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BONE MARROW		X	X		X	X	X	X	X	X	X	X	X	A	X	X	X		X	X	X	X	X	X	X	X
Monocytic Leukemia																		P								
THYMUS		X	0		X	X	X	0	X	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0
THORACIC LYMPH NODE		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	0	X	X	X	X	X	X	X
HEPATIC LYMPH NODE		X	X		X	X	X		0	X	0	X	X	0	X	X	X	X	0	0	0	X	X	X	X	X
Monocytic Leukemia								P																		
MESENTERIC LYMPH NODE		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0	X	X	X	X	X	X
CERVICAL LYMPH NODE		X	X		0	X	X	X	X	X	X	X	X	0	X	X	X	0	X	0	X	0	X	X	X	X
AXILLARY LYMPH NODE		X	0		X	0	0	X	X	X	0	X	0	X	X	X	X	X	0	X	X	0	X	X	0	X
INGUINAL LYMPH NODE		0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	X	0	0	0	0	0	0	0
UNUSUAL LESIONS																										
Lung																										
Alveolar/Bronchiolar Adenoma																									P	
Adrenal																										
Pheochromocytoma																						P				
Mammary Gland																										
Fibroadenoma										P								P								
Subcutaneous Fibrosarcoma																						P				

NO NECROPSY

Table 4

Individual Histopathology Findings

Organ	Anim. No./Cage	GROUP 3 MALES																									
		1/50	2/50	3/50	4/50	5/50	1/51	2/51	3/51	4/51	5/51	1/52	2/52	3/52	4/52	5/52	1/53	2/53	3/53	4/53	5/53	1/54	2/54	3/54	4/54	5/54
SPLEEN		X		X	X		X	X	X	X	X	X	X	X		X	X	X	X			X	X	X	X	X
Monocytic Leukemia			P			P									P						P					
Mesothelioma																				P						
LIVER		X			X		X	X		X	X	X	X	X		X	X	X	X	X		X	X	X	X	X
Monocytic Leukemia			P			P			P						P						P					
Neoplastic Nodule				P																						
KIDNEY		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X
Mesothelioma																				P						
BONE MARROW		X		X	0		X	X	X	X	A	X	X	X		X	X	X	X	X	X	X	X	X	X	X
Monocytic Leukemia			P			P									P											
THYMUS		0	X	X	X	X	0	0	X	X	X	X	X	X		0	X	X	X	X	X	X	X	X	0	X
Monocytic Leukemia															P											
THORACIC LYMPH NODE		X	X	X	X	X	X	X	X	X	X	X	X	X	X	0	X	X	X	X		X	X	X	X	X
Monocytic Leukemia																					P					
HEPATIC LYMPH NODE		X	0	X	0	X	X	X	X	X	X	X	0	0		0	0	0	X	X		X	X	X	X	X
Monocytic Leukemia															P						P					
MESENTERIC LYMPH NODE		X		X	X	X	X	X	X	0	X	X	X	X		X	0	0	X	X	X	X	X	X	X	X
Monocytic Leukemia			P												P											
CERVICAL LYMPH NODE		X		X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X
Monocytic Leukemia			P												P											
AXILLARY LYMPH NODE		0	X	X	X	X	X	X	X	X	X	X	X	0		0	X	X	X	0	X	X	X	X	X	X
Monocytic Leukemia															P											
INGUINAL LYMPH NODE		0	X	0	0	0	0	0	0	0	0	0	0	0	0	X	0	0	0	0	0	0	0	0	0	0
UNUSUAL LESIONS																										
Skull																										
Osteoma											P															
Stomach																										
Squamous Cell Carcinoma												P														
Small Intestine																										
Mesothelioma																				P						
Omentum																										
Mesothelioma					P																					
Mesenteric Fat																										
Mesothelioma																				P						
Testes																										
Mesothelioma (scrotal fat)																				P						
Interstitial Cell Tumor					P																					
Mammary Gland																										
Fibroadenoma				P		P										P			P							
Subcutaneous Fibroma					P										P	P										

Table 4
Individual Histopathology Findings

GROUP 3 MALES

Organ	Anim. No./Cage	1/55	2/55	3/55	4/55	5/55	1/56	2/56	3/56	4/56	5/56	1/57	2/57	3/57	4/57	5/57	1/58	2/58	3/58	4/58	5/58	1/59	2/59	3/59	4/59	5/59
SPLEEN		X	X	X	X	X	X	X	X			X	A	X	X	X		A	X	X	X	X	X	A	X	X
Monocytic Leukemia											P															
LIVER		X	X	X	X	X	X	X	X			X	A	X	X	X		A	X		X	X	X	A	X	X
Monocytic Leukemia											P															
Neoplastic Nodule																				P						
KIDNEY		X	X	X	X	X	X	X	X		X	X	A	X	X	X		A	X	X	X	X	X	A	X	X
BONE MARROW		X	X	X	X	X	X	A	0		X	X	A	X	X	X		A	X	X	X	X	X	A	X	X
THYMUS		X	X	X	X	X	0	X	X		0	X	A	0	X	0		A	X	X	X	X	X	A	X	0
THORACIC LYMPH NODE		X	X	X	X	X	X	X	0		0	X	A	X	X	X		A	X	X	X	X	X	A	X	X
HEPATIC LYMPH NODE		X	X	X	X	X	0	0	0		0	X	A	0	0	X		A	X	X	X	X	X	A	X	X
MESENTERIC LYMPH NODE		X	X	X	X	X	X	X	X			X	A	X	X	X		A	X	X	X	X	X	A	X	X
Monocytic Leukemia											P															
CERVICAL LYMPH NODE		X	X	X	X	X	X	X	X			X	A	X	X	X		A	X	X	X	X	X	A	X	X
Monocytic Leukemia											P															
AXILLARY LYMPH NODE		X	0	X	0	0	X	0	X			X	A	X	X	X		A	X	X	X	X	X	A	X	X
Monocytic Leukemia											P															
INGUINAL LYMPH NODE		0	0	0	0	0	0	0	0		0	0	A	0	0	0		A	0	0	0	0	0	A	0	0
UNUSUAL LESIONS								0																		
Lung																										
Alveolar Adenoma			P																							
Testes																										
Interstitial Cell Tumor											P															
Mammary Gland																										
Fibroadenoma												P														
Subcutaneous Anaplastic																										
Fibrosarcoma					P																					

NO NECROPSY

Table 4

Individual Histopathology Findings

Organ	Anim. No./Cage	GROUP 3 FEMALES																								
		1/60	2/60	3/60	4/60	5/60	1/61	2/61	3/61	4/61	5/61	1/62	2/62	3/62	4/62	5/62	1/63	2/63	3/63	4/63	5/63	1/64	2/64	3/64	4/64	5/64
SPLEEN		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X		X	X
Monocytic Leukemia																								P		
LIVER		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X		X	X
Monocytic Leukemia																								P		
KIDNEY		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X
BONE MARROW		X		X	X	X	X	X	X	0	X	X	0	X	X	X	X	X	X	X		X	X	X	X	X
THYMUS		X		X	X	X	X	X	X	X	0	0	0	X	X	X	0	0	X	X		X	X	X	X	X
THORACIC LYMPH NODE		X		X	X	X	X	X	0	0	X	X	X	X	0	X	X	X	X	X		X	X	X	X	X
HEPATIC LYMPH NODE		X		X	X	0	0	0	X	0	X	0	X	X	X	0	0	X	X	X		X	X	0	X	X
MESENTERIC LYMPH NODE		X		X	X	X	X	X	X	X	X	0	X	X	X	X	X	X	X	X		X	X		X	0
Monocytic Leukemia																								P		
CERVICAL LYMPH NODE		X		X	X	X	X	X	X	X	X	X	X	0	X	0	X	0	X	X		X	X	0	X	X
AXILLARY LYMPH NODE		X		X	X	0	X	X	X	X	X	X	X	X	X	X	X	X	0	X		X	X	X	X	0
INGUINAL LYMPH NODE		0		0	0	0	0	0	0	0	0	0	0	0	0	X	0	0	0	0		0	0	0	0	0
THYMIC LYMPH NODE												0	0													
UNUSUAL LESIONS																										
Mammary Gland																										
Fibroadenoma										P		P					P									
Adenoma								P																		
Subcutaneous Fibroma		P											P													

NO NECROPSY

Table 4
Individual Histopathology Findings

GROUP 3 FEMALES

Organ	Anim. No./Cage	1/65	2/65	3/65	4/65	5/65	1/66	2/66	3/66	4/66	5/66	1/67	2/67	3/67	4/67	5/67	1/68	2/68	3/68	4/68	5/68	1/69	2/69	3/69	4/69	5/69
SPLEEN		X	X	X	X	X	X	X	X	X		X	X	X	X		X	X	X	X	X	X	X	X	X	X
Monocytic Leukemia																P										
Histiocytic Lymphoma											P															
LIVER		X	X	X	X	X	X	X	X	X		X	X	X	X			X	X	X	X	X	X	X	X	X
Monocytic Leukemia																P	P									
Histiocytic Lymphoma											P															
KIDNEY		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Histiocytic Lymphoma											P															
BONE MARROW		0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	0	X	X	X	X	X	X
THYMUS		X	0	X	X	X	X	0	X	X	X	X	X	0	X	X	X	X	X	0	X	X	X	X	X	X
THORACIC LYMPH NODE		X	X	X	X	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HEPATIC LYMPH NODE		X	X	X	X	0	0	X	X	X		X	X	X	0	X		X	0	0	X	X	X	X	X	X
Monocytic Leukemia																	P									
Histiocytic Lymphoma											P															
MESENTERIC LYMPH NODE		X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Histiocytic Lymphoma											P															
CERVICAL LYMPH NODE		X	X	X	0	X	X	X	X	X	X	X	X	0	X	X	X	X	X	X	X	X	X	0	X	X
AXILLARY LYMPH NODE		X	0	X	X	X	X	X	X	X	0	X	X	0	X	X	X	0	X	0	X	X	X	X	0	X
INGUINAL LYMPH NODE		0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Histiocytic Lymphoma											P															
THYMIC LYMPH NODE																				0						
UNUSUAL LESIONS																										
Mammary Gland																										
Fibroadenoma										P																P

tissues for evaluation) was considerably lower than the ≈25% incidence reported by Jacobs and Huseby (2) in older Fischer 344 rats.

IV. SUMMARY AND CONCLUSIONS

Three hundred Fischer 344 rats, 150 males and 150 females, were evenly divided into three groups of 50 of each sex by random selection. Two groups were each exposed via inhalation to a mixture of nitrous oxide (N_2O) and halothane for seven hours per day, five days a week, for 104 weeks. A third group was similarly exposed to filtered room air and served as controls. Group designations for this study are presented below:

Group No.	No. of Rats		Analytical Concentration ppm ± S.D.	
	M	F	N_2O	Halothane
1 (Control)	50	50	------	------
2	50	50	49.0 ± 5.0	1.1 ± .4
3	50	50	501.5 ±49.4	9.8 ± 2.6

Evaluation of body weight data indicated that the body weight growth for the males and females in Groups 2 and 3 was similar to the growth rate for the control males and females. No evidence of an exposure-related effect with regard to physical appearance and behavior was indicated for the animals exposed to the mixtures of N_2O and halothane. Survival at 104 weeks among the males was 74% for Group 1, 76% for Group 2, and 72% for Group 3. Survival among the females was 86% for Group 1, 78% for Group 2, and 84% for Group 3.

Routine hematology determinations measured after 13, 26, 52, 78, and 104 weeks of exposure did not indicate any exposure-related effects.

Similarly, no compound-effect was inferred from measurements of terminal body weight, organ weights, and organ weight to body weight ratios. The significantly lower spleen ratios in exposed females were due to high control values in those animals with monocytic leukemia.

Histopathological evaluation of selected tissues indicated that long-term inhalation exposures of rats to mixtures of N_2O and halothane did not result in an increase in the incidence of neoplasia in general nor, specifically, in an increase in the incidence of reticuloendothelial tumors. The wide variety of tumors observed in the control (and exposed) animals have been previously observed in this strain of rat.

Submitted by W B Coate

WILLIAM B. COATE, PH.D.
Director
Inhalation Toxicology Department

Histopathology: Ulland
Report Preparation: Noble
Supervision: Hardy and Noble
:ew

V. REFERENCES

1. Leithe, W.: The Analysis of Air Pollutants, Ann Arbor-Humphrey Science, Ann Arbor, 1970.

2. Jacobs, B.B. and Huseby, R.A.: Neoplasma occurring in aged Fischer rats with special reference to testicular, uterine, and thyroid tumors. J. Nati. Cancer Inst. Vol. 39, No. 2: 303-309, 1967.

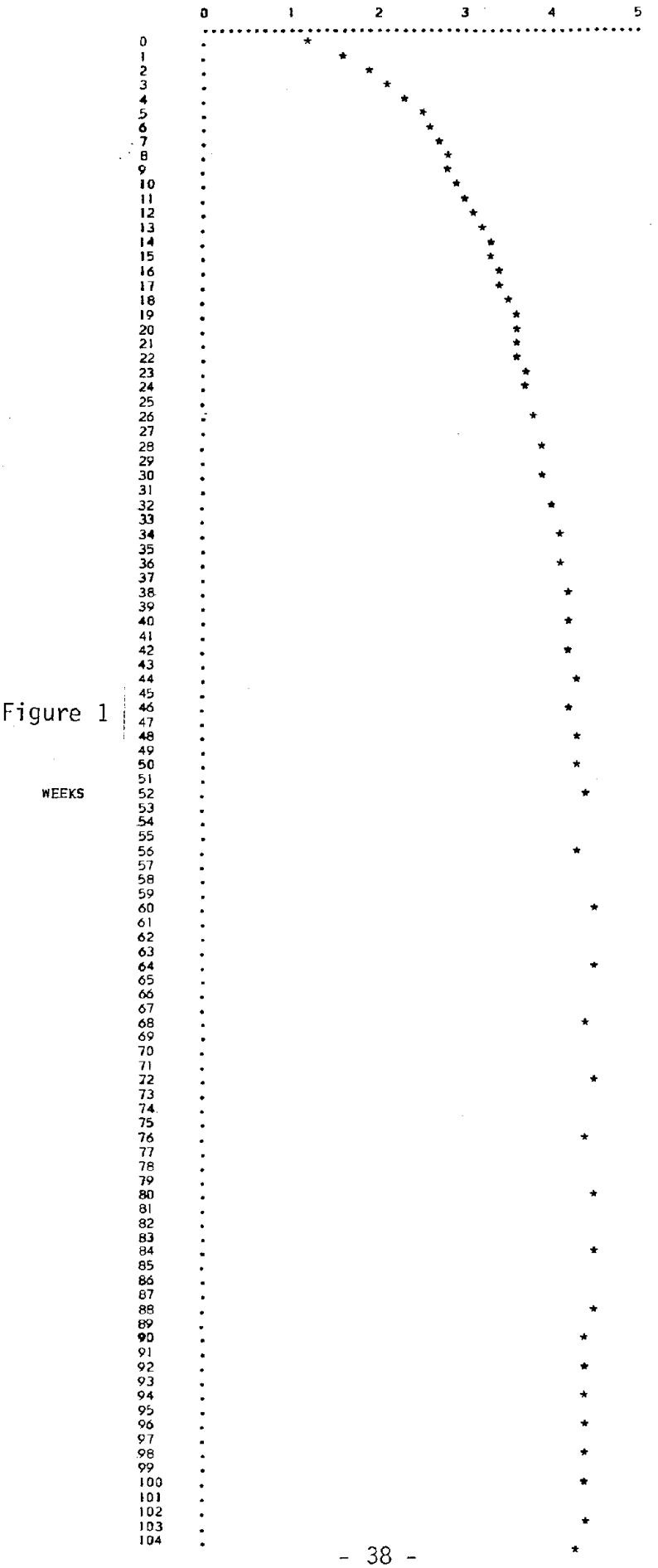

Figure 1

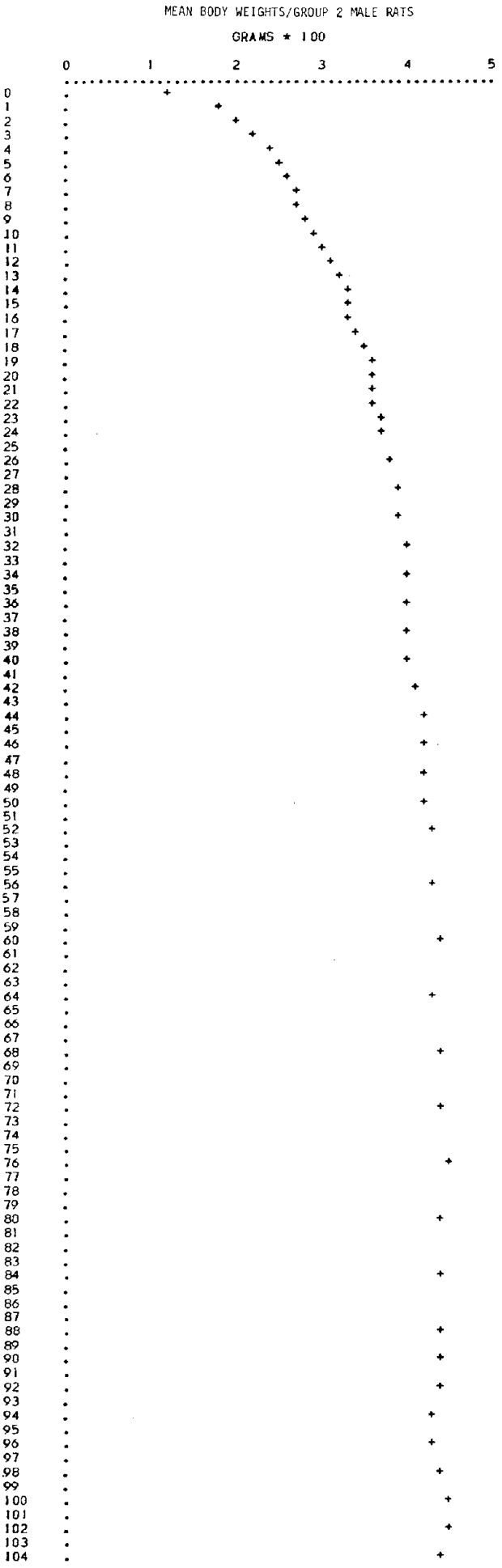

Figure 2

MEAN BODY WEIGHTS/GROUP 3 MALE RATS

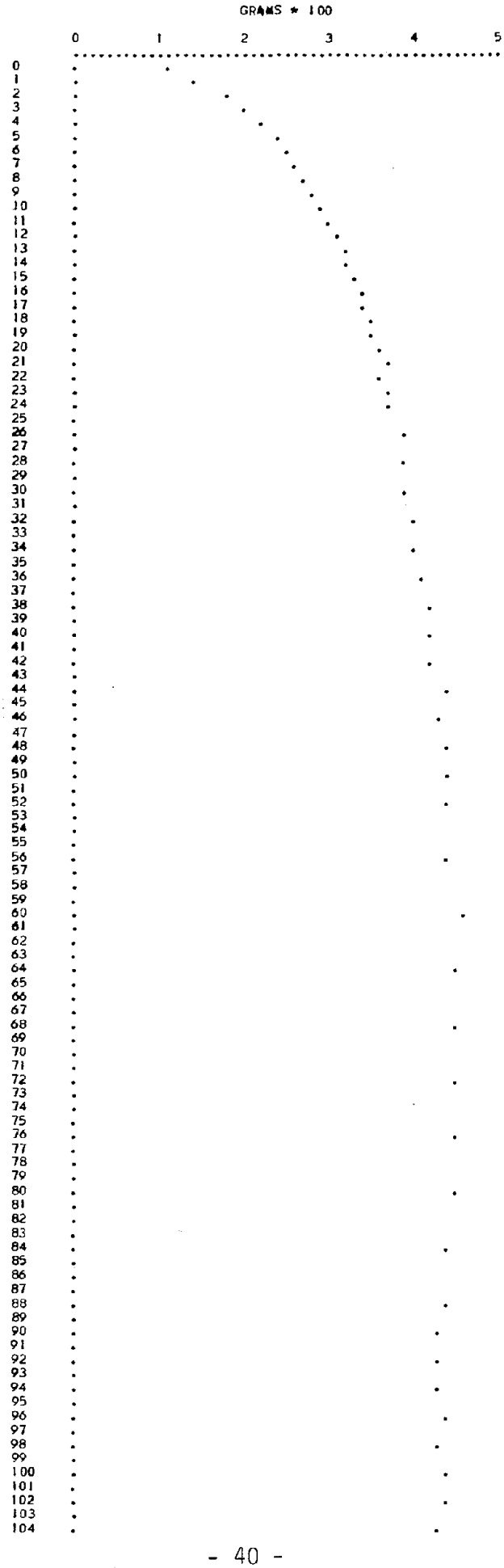

Figure 3

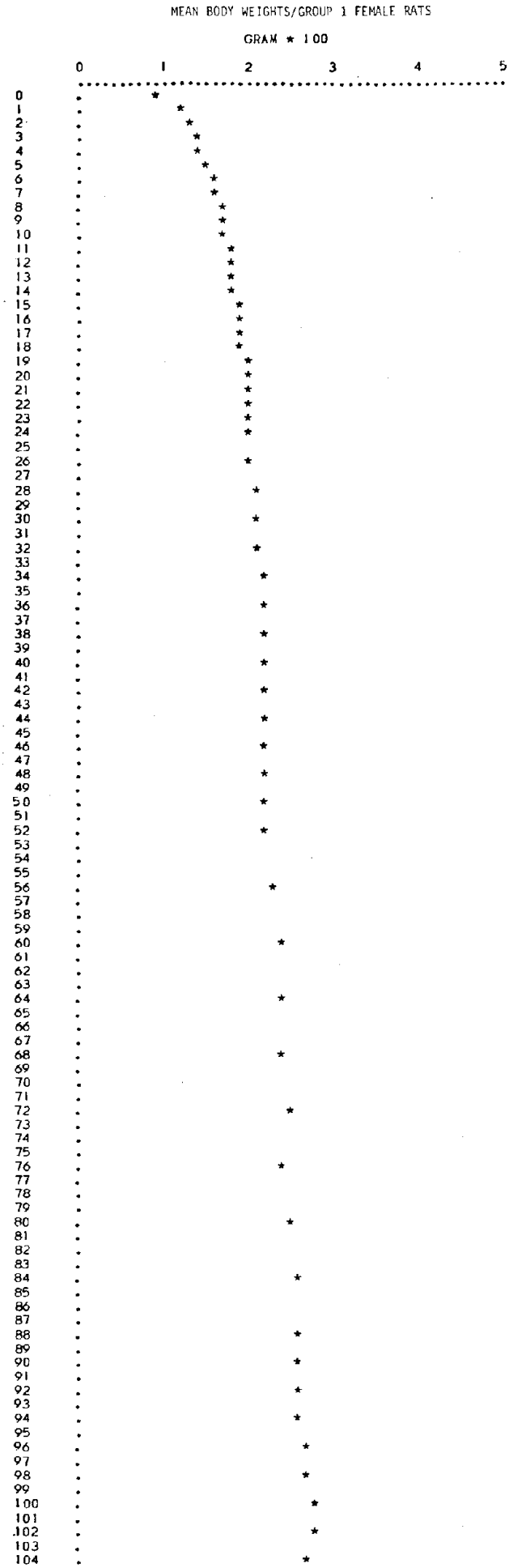

Figure 4

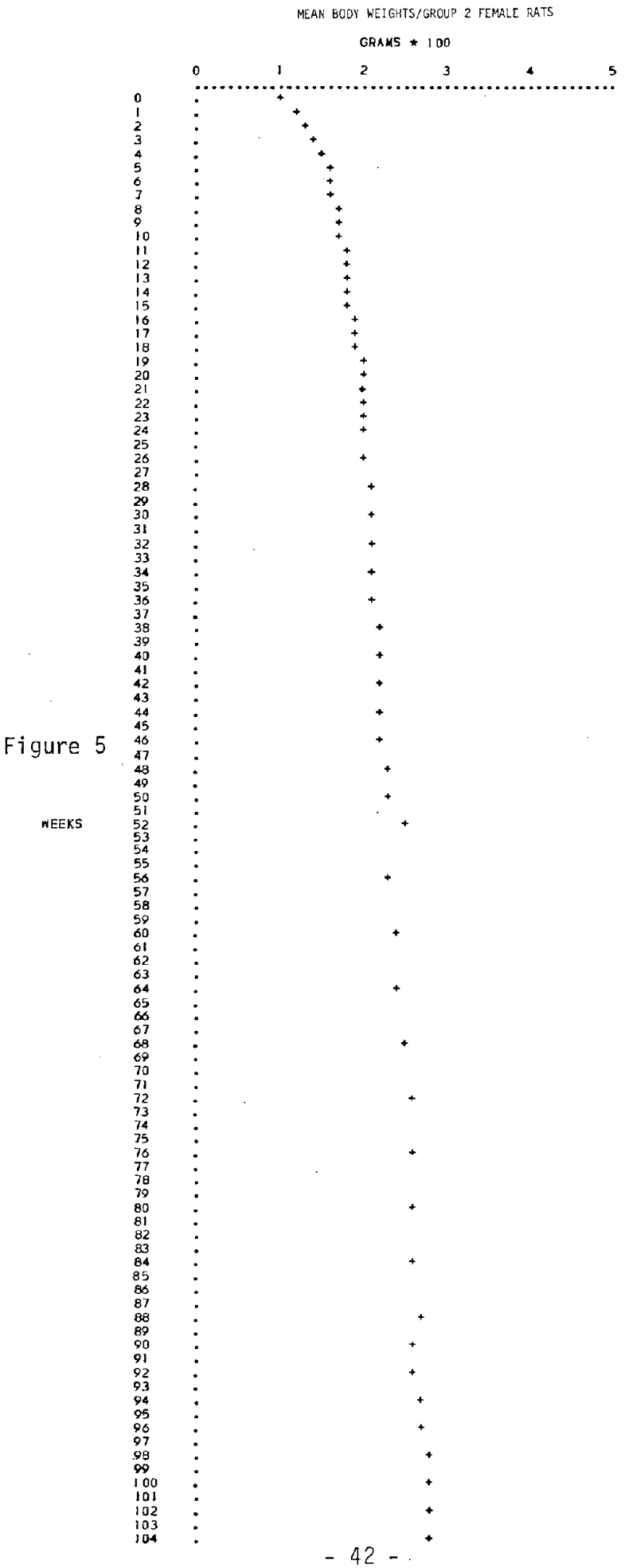
MEAN BODY WEIGHTS/GROUP 2 FEMALE RATS
GRAMS * 100
0
1
2
3
4
5
WEEKS

Figure 5

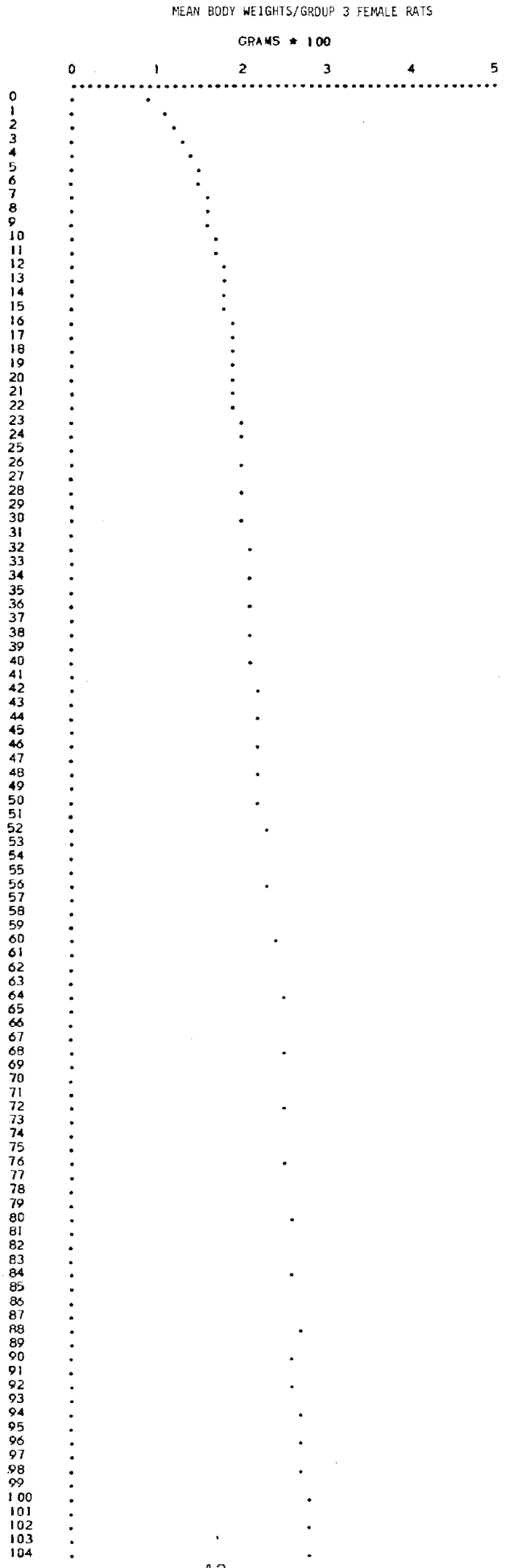
MEAN BODY WEIGHTS/GROUP 3 FEMALE RATS
GRAMS * 100
0
1
2
3
4
5
WEEKS

Figure 6