# CYTOGENETIC EVALUATION OF SPERMATOGONIAL CELLS IN THE RAT FOLLOWING LONG-TERM INHALATION EXPOSURE TO NITROUS OXIDE PLUS HALOTHANE

FINAL REPORT

CDC 99-74-46

Submitted to

National Institute for Occupational
Safety and Health
1014 Broadway
Cincinnati, Ohio

November 17, 1976

HAZLETON LABORATORIES AMERICA, INC.
9200 Leesburg Turnpike • Vienna, Virginia 22180 • U.S.A.

SPONSOR: National Institute for Occupational Safety and Health

DATE: November 17, 1976

MATERIAL: Nitrous Oxide and Halothane

SUBJECT: FINAL REPORT
Cytogenetic Evaluation of Spermatogonial Cells in The Rat Following Long-Term Inhalation Exposure to Nitrous Oxide Plus Halothane
Project 785-200

## I. OBJECTIVE

This study was designed to assess the mutagenic potential of long-term exposure of male rats to nitrous oxide ($N_2O$) plus halothane as measured by aberrations in spermatogonial cell chromosomes.

## II. MATERIALS AND METHODS

### A. Animals and Animal Groups

One hundred twenty sexually mature Sprague-Dawley strain male albino rats (Charles River COBS) were randomly assigned to the following groups:

| Group No. | No. of Male Rats | Treatment |
|---|---|---|
| 1 | 40 | Air Control |
| 2 | 40 | 50 ppm $N_2O$ + 1 ppm halothane |
| 3 | 40 | 500 ppm $N_2O$ + 10 ppm halothane |

### B. Exposure Conditions

The animals in the treatment groups were exposed under dynamic conditions at 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 per week, for a total exposure period of 52 weeks. The control animals were exposed to filtered room air in a similar chamber with flow characteristics identical to that of the treatment groups. After 12 weeks on study, all animals were removed from their exposure cages for use in a 21-day mating period and were then returned to their cages and maintained on the appropriate control or exposure level for the remainder of the 52-week period.

The animals were individually numbered with ear tags and housed on one tier, five to a cage, in stainless steel mesh cages with stainless steel top loading feeders and demand-type water valves. Water and basal laboratory diets (Purina Rat Chow) were available ad libitum. The cages were changed and washed weekly and the water bottles were changed and sterilized twice weekly.

C. Exposure Method

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

Nitrous oxide ($N_2O$) was supplied from cylinders containing certified 98% pure nitrous oxide anhydride. The gas was passed under positive pressure through a flowrater 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,-trifluothane. Nitrogen was passed via a flowrater and critical orifice through the headspace of a brown glass flask containing liquid halothane and then passed into the mixing flask to mix with the $N_2O$ prior to delivery into the chamber 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). Analyses of 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 an aliquot of the solution was injected into the gas chromatograph.

E. Observations

All male rats were observed daily for mortality. Terminal body weight was recorded for each animal prior to sacrifice.

F. Sacrifice

Following the 52-week exposure period, the animals were administered an intraperitoneal injection of Colchicine (2 mg/kg) in distilled water in order to arrest mitosis in dividing cells. Approximately five hours post-injection, the rats were sacrificed via a brief exposure to chloroform followed by cervical dislocation.

G. Preparation of Chromosome Slides

Immediately following sacrifice, the testes of each animal were excised and placed on a watchglass. The tunica albuginea was removed and the seminiferous tubules were thoroughly minced. The minced tubules were suspended in approximately 10 ml of pre-warmed (37°C) hypotonic sodium citrate (1%). The suspension of tubules was then transferred from the watch glass to a 12 ml test tube. The tubules were allowed to settle to the bottom of the tube for 2 minutes. The supernatant was then carefully discarded. The remaining tubules were resuspended in 10 ml of fresh 1% sodium citrate. After a total hypotonic exposure period of 10 minutes, the supernatant was removed via pipette without agitating the tubules. The tubules were subsequently fixed in methanol-acetic acid (3:1) for 24 hours at 4°C.

H. Slide Preparation

Following fixation, the fixed tubules were centrifuged at 1000 rpm for 10 minutes. The fixative was discarded and the tubules were exposed to 5 ml of 60% acetic acid for a minimum of 3 minutes. The solution was then gently mixed to produce a homogeneous cell suspension.

With a Pasteur pipette,approximately 0.2 ml of the cell suspension was introduced onto a slide pre-warmed to 65°C on a hot plate. The cell suspension was immediately withdrawn from the slide. This procedure was repeated until the slide was entirely covered with small areas of dried solution. The slides were numbered chronologically with no reference to test groups, but with the code retained.

I. Stain Preparation

Stock Giemsa stain was prepared from 0.38 Giemsa powder (Polyscience) added to 25 ml of glycerin and 25 ml of absolute methanol.

Four ml of this stock solution was then added to 3.0 ml of acetone and 33 ml of buffered $H_2O$ (1 g Harleco buffer salts per 100 ml distilled water.)

J. Slide Staining

Upon completion of all preparations, the slides were stained for eight minutes in the Giemsa stain described above. Slides were rinsed in tap water, air-dried, and subsequently mounted in Permount®.

K. Chromosome Analysis

At least three slides were prepared for each animal. Twenty-five metaphases were evaluated from each preparation that was analyzable and the vernier location was noted for each metaphase. The technical quality of the slides was recorded at the time of evaluation. Chromatid breaks (involving one chromosomal arm) were scored as either with a visible fragment or without a fragment.

Chromosome breaks (involving both arms of the chromosome) were also scored as with a visible fragment or without a fragment. All markers including exchanges, dicentrics, rings, and other abnormal chromosomes were scored separately. Where more than one type of aberration was observed in any one metaphase, the cell was also scored as having multiple aberration types. Those cells with more than nine aberrations (whether of one or more types) were scored as having 10 or more aberrations. Unique markers were tabulated in these cells as well, since markers are considered to be extremely important in cytogenetic evaluation. An estimate of polyploidy was made for each cell at the time of evaluation.

Representative photographs were made on each slide showing characteristic aberrations as well as normal cells. Karyotype analysis was not made since this type of evaluation is not appropriate in the rat because most of the chromosomes are morphologically similar (telocentric) and pairs are not easily identified without more sophisticated staining procedures.

In addition, the number of gaps (regions of chromatid arms with stained areas less than the width of the chromatid arm) were scored.

All slides were scored blindly with no reference to group identification. Upon completion of all scoring, the data sheets were placed into the appropriate groups and statistical analyses were performed.

K. Statistical Analysis

Each category of aberrations was recorded in two ways: (1) the number of aberrations per animal and (2) the number of cells showing a particular aberration per animal. Thus, it could be determined how wide-spread the effects observed were within each animal as against intensity of effect within the animal. Unless otherwise indicated, all analyses were made comparing the mean number of affected cells or the mean number of aberrations for each treatment group to similar means of the control group by analysis of variance (F-test) and by Student's t-test. When variances differed significantly, Student's t-test was appropriately modified (t') and Cochran's approximation utilized (2). Terminal body weights were similarly analyzed. Finally, the proportions of animals showing aberrations and the proportions of examined cells showing aberrations in each group were analyzed by the Chi-square test. The level of probability chosen for rejecting the null hypothesis was $\leq 0.05$.

## III. RESULTS

A. Chamber Analyses

The means ±S.D. for all analytical determinations of $N_2O$ and halothane generated during the 52-week exposure period were 49.7 ppm $N_2O$ ± 5.1 with 1.1 ppm halothane ± .2, and 498.6 ppm $N_2O$ ± 14.6 with 10.6 ppm halothane ± 1.8 for Groups 2 and 3, respectively.

B. Survival

Of the original 120 male rats, 114 survived the 52-week exposure period. The survivors comprised 39/40 in Group 1 (Control), 37/40 in Group 2, and 38/40 in Group 3.

C. Body Weight

Terminal body weight data are presented in Table No. 1.

Table 1 - Mean terminal body weight data ±S.D.

| Group No. | Mean Terminal Body Weight g ±S.D. | Body Weight Range g |
|---|---|---|
| 1 (Control) | 855 ± 133 | 500 - 1275 |
| 2 | 843 ± 160 | 460 - 1070 |
| 3 | 841 ± 118 | 630 - 1075 |

Although there was considerable weight range variation between groups, the mean terminal body weights were similar among the control and treated groups.

D. Cytogenetic Evaluations

Of the 39 animals in Group 1 (Control), 4 were eliminated from statistical analysis since there were not sufficient metaphases available for evaluation. Therefore, the data presented for Group 1 are based on 35 rats which represent a total of 875 spermatogonial cells analyzed for chromosomal damage.

There were 37 animals to be evaluated in Group 2. Two of these were eliminated from statistical analyses since there were not sufficient metaphases available for analysis. Hence, the data in Group 2 are from 35 animals which also represents 875 spermatogonial cells evaluated.

Of the 38 animals in Group 3, 8 were eliminated from statistical analysis since they did not present an adequate number of metaphases for evaluation. Hence, the data presented in Group 3 are based on 30 animals which represents 750 cells.

The mean number of spermatogonial cells per animal which showed chromatid aberrations is presented in Table No. 2.

Table 2 - Mean number of analyzed rat spermatogonial cells per animal showing chromatid aberrations.

| Group No. | Mean No. of Cells with Gaps ±S.D. | Mean No. of Cells w/ Breaks w/ Fragments ±S.D. | Mean No. of Cells w/ Breaks w/o Fragments ±S.D. |
|---|---|---|---|
| 1 (Control) | 2.06 ± 1.24 | 0.31 ± 0.47 | 0.03 ± 0.17 |
| 2 | 2.51 ± 1.25 | 0.57 ± 0.70 | 0.06 ± 0.24 |
| 3 | 2.47 ± 1.20 | 0.73*± 0.74 | 0.10 ± 0.31 |

*Significantly higher than control at $p < 0.05$.

The data in Table No. 2 indicate that the number of cells with gaps were comparable between the control and test groups. The relevance of gaps in cytogenetic analysis has not yet been established and they are not included in the calculation of total aberrations.

Cells in Group 2 which had chromatid breaks where a fragment was also visible were comparable to the cells in the control group. However, the incidence of these aberrant cells was significantly higher than control in Group 3.

Table No. 3 presents the mean number of chromatid aberrations observed per animal.

Table 3 - Mean number of chromatid aberrations per animal in analyzed spermatogonia cells in the rat.

| Group No. | Mean No. of Cells with Gaps ±S.D. | Mean No. of Cells w/ Breaks w/ Fragments ±S.D. | Mean No. of Cells w/ Breaks w/o Fragments ±S.D. |
|---|---|---|---|
| 1 (Control) | 2.34 ± 1.51 | 0.37 ± 0.60 | 0.03 ± 0.17 |
| 2 | 2.94 ± 1.73 | 0.69 ± 0.83 | 0.06 ± 0.24 |
| 3 | 2.87 ± 1.50 | 0.97*± 1.03 | 0.13 ± 0.43 |

*Significantly higher than control at $p < 0.01$.

Data in Table No. 3 indicate that the total number of gaps was comparable between control and test groups.

Chromatid breaks with fragments in Group 2 were comparable to the breaks in the controls. These chromatid breaks where fragments were visible were significantly increased in the high dose group.

Chromatid breaks which were not associated with visible chromatid fragments were comparable among control and test groups.

Table No. 4 presents the mean number of cells per animals in which chromosomal aberrations were observed.

Table 4 - Mean number of analyzed rat spermatogonia cells per animal showing chromosomal aberrations.

| Group No. | Mean No. of Cells with Breaks with Fragments ± S.D. | Mean No. of Cells with Breaks without Fragments ± S.D. |
|---|---|---|
| 1 (Control) | 0.14 ± 0.36 | 0.00 |
| 2 | 0.23 ± 0.49 | 0.00 |
| 3 | 0.23 ± 0.57 | 0.00 |

The data presented in Table No. 4 indicate that there were no differences between control and test groups of observed chromosomal breaks in which fragments were visible. It should also be noted that there were no spermatogonial cells seen in any group in which a visible fragment was not associated with a chromosomal break.

Table No. 5 presents the mean number of chromosomal aberrations both with and without visible fragments.

Here, as in Table No. 4, there are no significant differences between the control and test groups.

Table 5 - Mean number of chromosomal aberrations per animal in analyzed spermatogonial cells in the rat.

| Group No. | Mean No. of Chromosomal Breaks with Fragments ± S.D. | Mean No. of Chromosomal Breaks without Fragments ± S.D. |
|---|---|---|
| 1 (Control) | 0.14 ± 0.36 | 0.00 |
| 2 | 0.23 ± 0.49 | 0.00 |
| 3 | 0.27 ± 0.69 | 0.00 |

Table No. 6 presents the mean number of cells per animal with 10 or more aberrations as well as the mean number of cells with multiple aberration types for each group.

Table 6 - Mean number of analyzed rat spermatogonial cells per animal showing 10 or more aberrations and/or cells with multiple aberration types.

| Group No. | Mean No. of Cells with >10 Aberrations ± S.D. | Mean No. of Cells with Multiple Aberration Types |
|---|---|---|
| 1 (Control) | 0.03 ± 0.17 | 0.09 ± 0.28 |
| 2 | 0.26*± 0.51 | 0.49**± 0.61 |
| 3 | 0.73**± 0.94 | 1.30**± 1.06 |

* Significantly higher than control at $p < 0.05$.
**Significantly higher than control at $p < 0.01$.

Table No. 6 shows that severely aberrant cells which displayed 10 or more aberrations were significantly elevated in both Groups 2 and 3 when compared to the controls.

In the control group, there was only one cell out of 875 or 0.1% which showed 10 or more aberrations. The low level test group revealed 9 cells out of 875 or 1.03% with 10 or more aberrations. The high level test group had 22 out of 750 cells or 2.9% with 10 or more aberrations.

The mean numbers of cells with multiple aberration types in Groups 2 and 3 were significantly increased over control. Out of 875 cells in the control group, 3 revealed more than one type of aberration (0.34%). Group 2 contained 17 cells out of 875 (1.94%) and Group 3 had 39 out of 750 (5.2%) of the observed cells showing multiple types of aberrations.

Table No. 7 presents the mean number of cells per animal which were observed to have marker chromosomes such as exchanges and metacentric markers.

Table 7 - Mean number of analyzed rat spermatogonial cells per animal showing marker chromosomes (exchange figures, rings, and miscellaneous markers)

| Group No. | Mean No. of Cells with Markers ± S.D. |
|---|---|
| 1 (Control) | 0.29 ± 0.52 |
| 2 | 1.06*± 1.19 |
| 3 | 2.57*± 1.72 |

*Significantly higher than control at $p < 0.01$.

As indicated in Table No. 7, the mean number of cells showing markers in both the low and high level groups are significantly increased. Out of the 875 cells in the control group, 10 (1.14%) possessed marker chromosomes. All of these markers were exchanges. In Group 2, there were 37 cells out of 875 (4.23%) which possessed markers. Group 3 showed 77 cells out of 750 (10.27%) with marker-type aberrations.

Table No. 8 shows the mean number of marker chromosomes per animal.

Table 8 - Mean number of marker chromosomes (exchange figures, rings, and miscellaneous markers) per rat in analyzed spermatogonial cells.

| Group No. | Mean No. of Marker Chromosomes ± S.D. |
|---|---|
| 1 (Control) | 0.34 ± 0.64 |
| 2 | 1.69*± 1.97 |
| 3 | 4.63*± 3.84 |

*Significantly higher than control at $p < 0.01$.

In Table No. 8, both Groups 2 and 3 have significantly increased numbers of marker chromosomes when compared to the control group. The total number of markers observed in 875 cells in the control group was 12 (1.37%). The total in Group 2 was 59 out of 875 cells (6.74%). Group 3 revealed a total of 139 markers in 750 cells (18.53%).

It should also be noted that included in the 139 markers of Group 3 are one ring chromosome and one large metacentric marker.

The relatively high number of marker incidence was expected because of the age of the rats at sacrifice (approx. 65 weeks). However, the values for Groups 2 and 3 clearly indicate there was a significant increase in the formation of markers. Furthermore, it appears that the increase is dose-related. Data in Table Nos. 7 and 8 indicate that there were significant increases in numbers of cells showing markers and in numbers of aberrations produced in both treated groups. The number of animals displaying marker-type aberrations was high in the control group (9/35 or 25.71%) because of the advanced age of the animals.

Group 2 had 20 out of 35 animals (57.14%) showing marker aberrations. Of the 30 animals evaluated in Group 3, 25 showed marker aberrations (83.33%). The effect was clearly wide-spread in both the low and high level groups.

Table No. 9 shows the mean number of cells which contained any type of aberration (except gaps).

Table 9 - Mean number of analyzed rat spermatogonial cells per animal showing any type of aberration (excluding gaps).

| Group No. | Mean No. of Cells per rat with Aberrations ± S.D. |
|---|---|
| 1 (Control) | 0.66 ± 0.76 |
| 2 | 1.69*± 1.39 |
| 3 | 3.17*± 1.84 |

* Significantly higher than control at $p < 0.01$.

Table No. 10 presents the mean number of total aberrations observed excluding gaps.

Table 10 - Mean number of all types of aberrations (excluding gaps) per rat in analyzed spermatogonial cells.

| Group No. | Mean No. of All Aberrations ± S.D. |
|---|---|
| 1 (Control) | 0.89 ± 1.16 |
| 2 | 2.89*± 2.70 |
| 3 | 6.63*± 4.71 |

*Significantly higher than control at $p < 0.01$.

Data in Table Nos. 9 and 10 clearly indicate a dose-related effect on both the number of aberrant cells produced as well as the number of total aberrations produced after long-term exposure to halothane and nitrous oxide.

Table No. 11 presents the dispersion of aberrations within each group by number of animals showing aberrant cells and by number of cells affected.

Table 11 - Incidence of animals and spermatogonial cells showing aberrations (excluding gaps).

| Group No. | No. of Animals with Aberrant Cells | No. of Animals Analyzed | No. of Cells With Aberrations | No. of Cells Analyzed |
|---|---|---|---|---|
| 1 (Control) | 17 | 35 | 23 | 875 |
| 2 | 27* | 35 | 59** | 875 |
| 3 | 26** | 30 | 95** | 750 |

* Significantly higher than control at $p < 0.05$ by Chi-square test.
**Significantly higher than control at $p < 0.01$ by Chi-square test.

The control group exhibited a fairly high background rate of spontaneous aberration. In fact, almost 49% of the 35 animals in this group revealed at least one cell with some type of aberration. Of the 875 cells examined, 23 or 2.6% exhibited some sort of chromosomal aberration. Seventy-seven percent of the low level test animals had aberrant cells. Fifty-nine cells out of the 875 examined in this group displayed some visible aberration (6.7%).

In Group 3 (high level exposure), 26 animals out of 30 examined showed chromosomal aberrations (86.7%). Of the 750 cells examined in this group, 95 or 12.7% exhibited chromosomal abnormalities.

An estimate of polyploidy was made on all analyzed slides in all groups. Of the 875 cells examined in the control group, 14 cells (1.6%) showed polyploidy. Eleven of the 35 animals examined (31.4%) exhibited polyploidic cells. Group 2 showed only 11 cells out of the 875 (1.3%) examined to be polyploidic.

Only 8 (22.9%) of the 35 animals examined had polyploid cells. Group 3 displayed 13 polyploidic cells out of 750 cells (1.7%). In this group, 12 (40%) of the animals had such cells. It is evident from these data that polyploidy was observed at comparable frequencies among the three groups.

In addition to polyploidy, other observations included sporadic non-clonal hypodiploidy which was also observed in equal frequencies among the groups.

E. Photographic Documentation

A minimum of five photographs of spermatogonial cells was taken on each animal that was analyzable. The negatives and contact proofs as well as the vernier location of each photograph are on file at HLA.

Representative photographs from each group have been selected for inclusion in this report.

GROUP 1 - PAGE 18

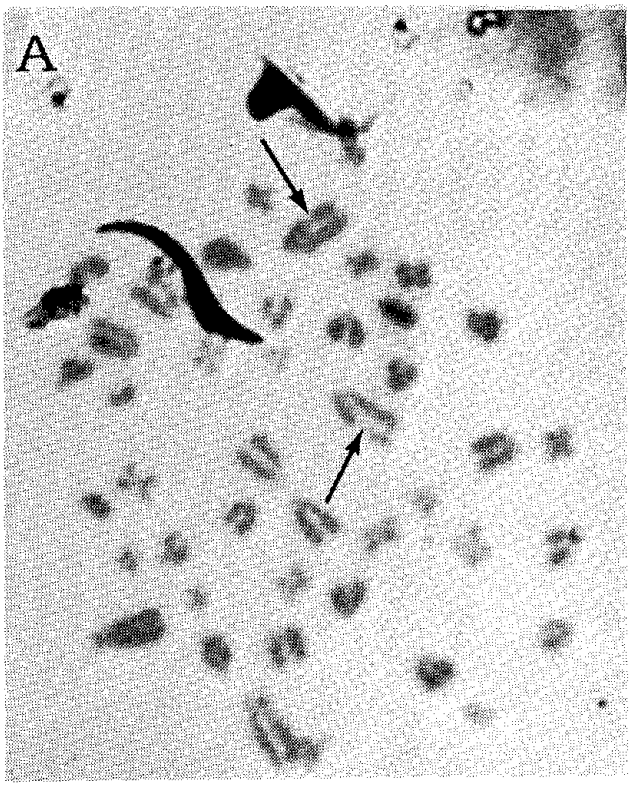
| Slide No. | Vernier Location | Observation |
|---|---|---|
| A Slide G14 | 109.3 X 41.3 | Gap, Chromatid Break |
| B Slide G99 | 116.5 X 51.5 | Normal |
| C Slide G11 | 120.7 X 42.9 | Normal |
| D Slide G109 | 124.3 X 53.2 | Normal |

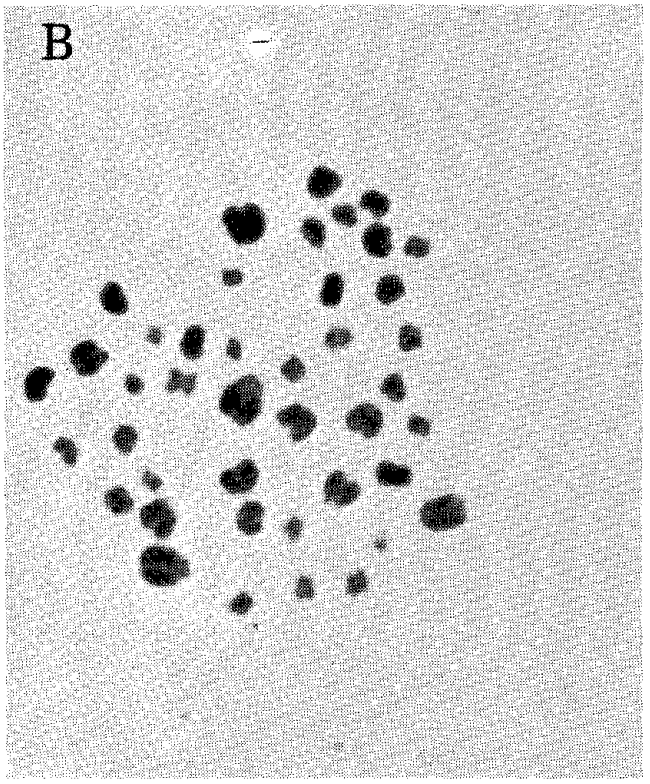
GROUP 2 - PAGE 19

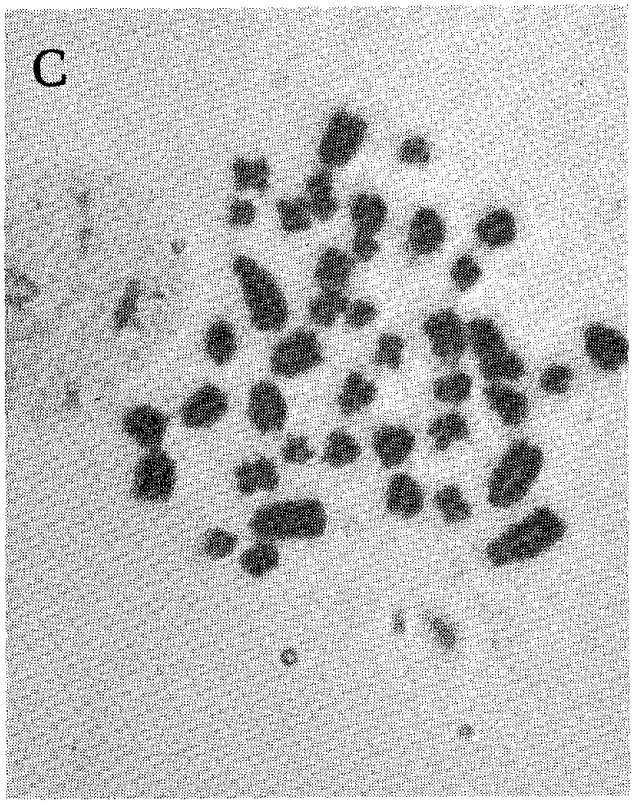
| Slide No. | Vernier Location | Observation |
|---|---|---|
| A Slide G24 | 113.9 X 47.4 | Normal |
| B Slide G22 | 121.1 X 56.5 | Chromosome Break |
| C Slide G21 | 128.4 X 40.8 | Multiple Aberrations |
| D Slide G79 | 119.3 X 45.0 | Breaks, Exchanges |

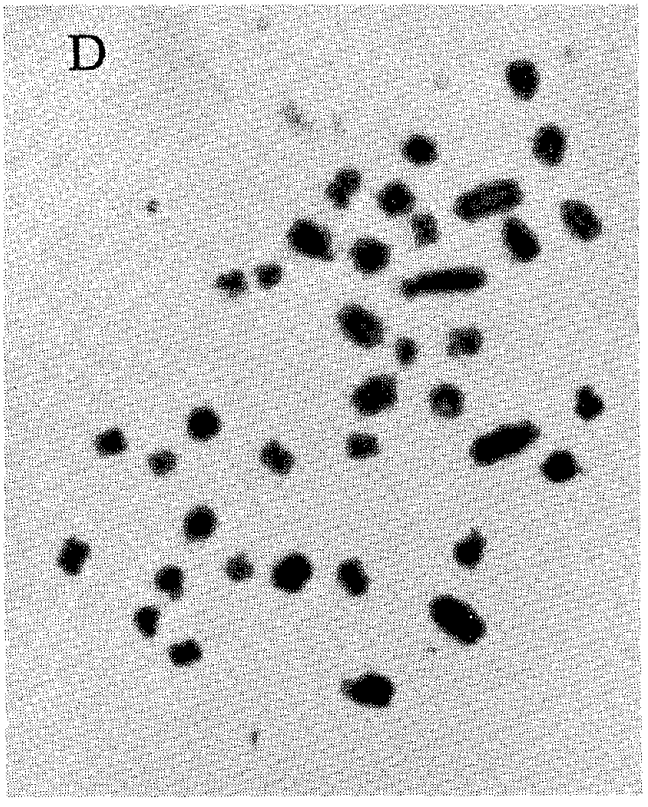
| Slide No. | Vernier Location | Observation |
|---|---|---|
| A Slide G76 | 113.9 X 48.5 | Exchanges |
| B Slide G88 | 128.3 X 50.0 | Exchange and Multiple Aberrations |
| C Slide G35 | 109.6 X 53.7 | Exchanges |
| D Slide G70 | 127.0 X 44.5 | Exchange |

## GROUP 1



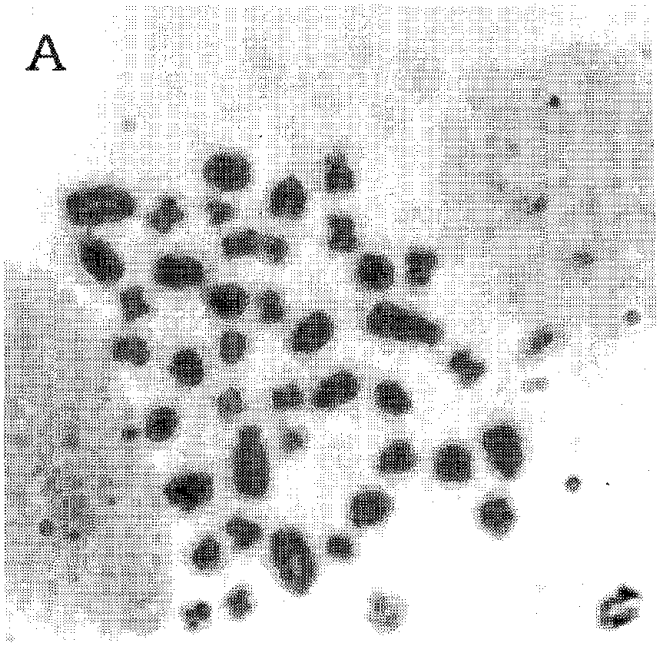



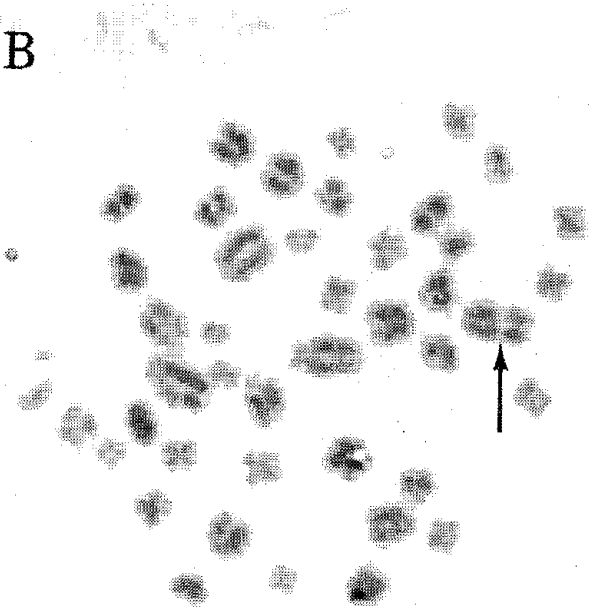



## GROUP 2

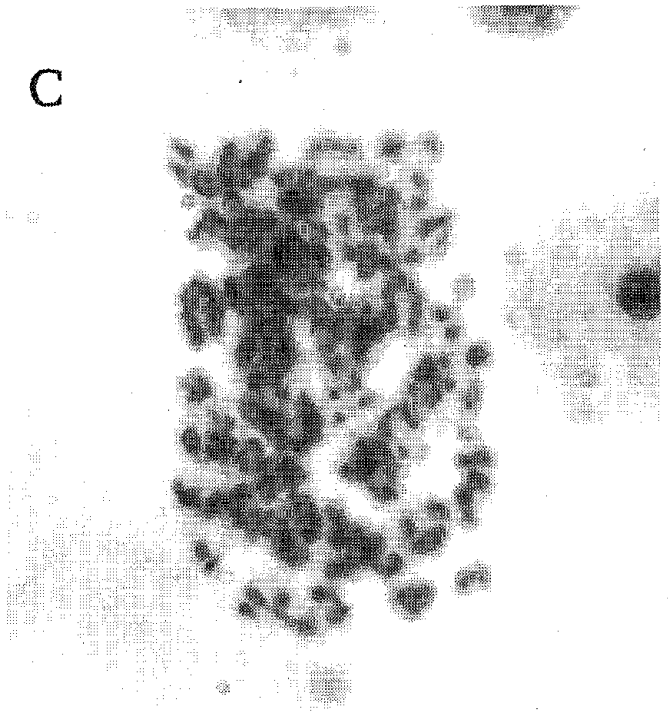
A



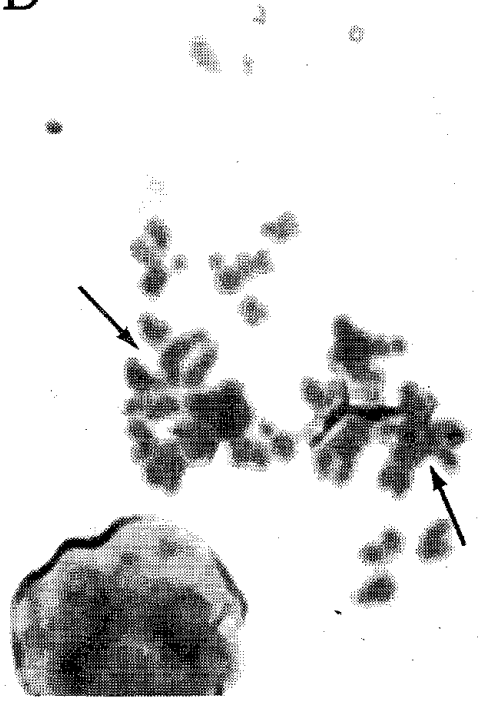
B



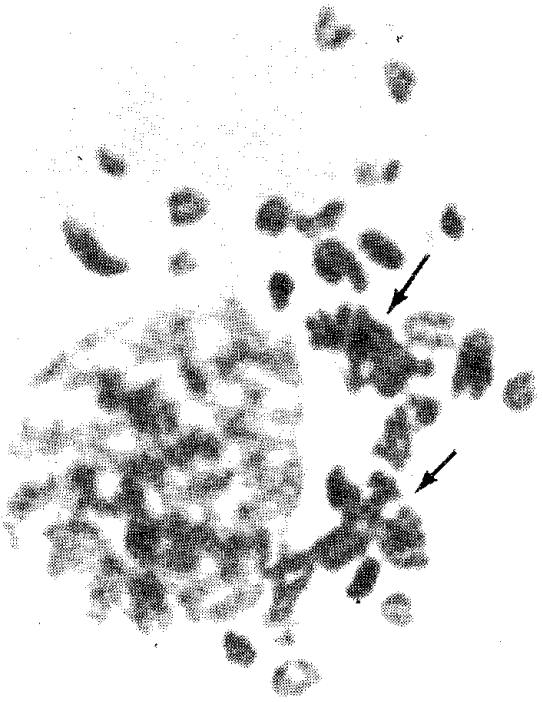
C



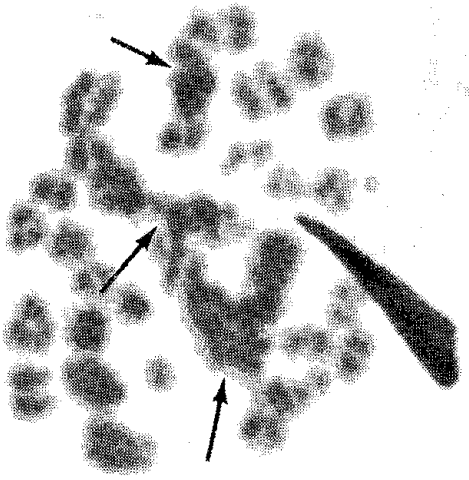
D



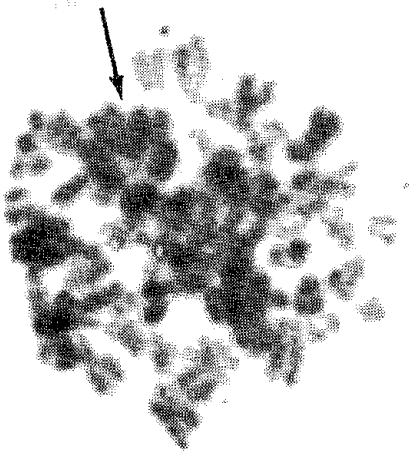
A



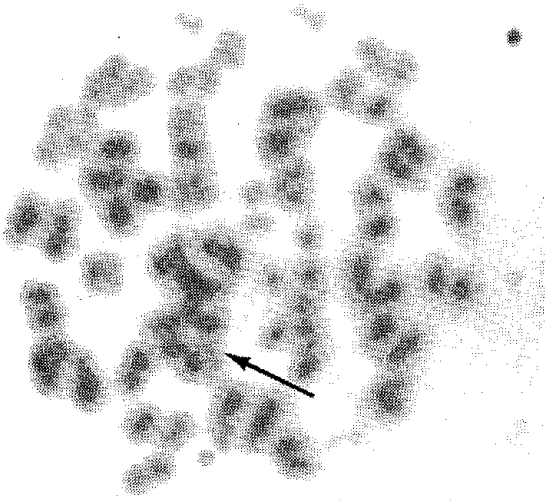
B



C



D



## IV. SUMMARY AND CONCLUSIONS

One hundred twenty sexually mature male albino rats were randomly assigned to three groups of 40 rats each. Two groups were each exposed by inhalation to a mixture of nitrous oxide ($N_2O$) and halothane vapor seven hours per day, five days a week, for 52 weeks. The remaining group was exposed to room air under the same conditions. Following the 52-week exposure period, the surviving animals were sacrificed and subsequent cytogenetic evaluations of spermatogonial cells performed. Group designations for this study are listed below.

| Group No. | No. of Male Rats | Treatment | Mean Analytical Concentration ppm ± S.D. |
|---|---|---|---|
| 1 | 40 | Air Control | --- |
| 2 | 40 | $N_2O$ + halothane | 49.7 ± 5.1<br>1.1 ± 0.2 |
| 3 | 40 | $N_2O$ + halothane | 498.6 ± 14.6<br>10.6 ± 1.8 |

Survival following the exposure period was 39/40, 37/40, and 38/40 for Groups 1, 2, and 3, respectively.

Mean terminal body weight data were similar among the control and treated groups.

The incidence of chromatid gaps was comparable among all three groups.

Chromatid breaks, which represent physical disruption of the continuity of the DNA, were measured in two ways: first, those breaks where fragments were visible and second, those breaks which displayed no fragments. Chromatid breaks with no accompanying fragments were not significantly different than the control group.

Chromatid breaks which were associated with fragments were similar to the controls at the low exposure level but were significantly elevated in the high level group. Chromatid breaks represent a mild form of genetic damage. This type of damage alone would not be sufficient to indicate a heritable genetic event since this type of insult usually occurs after replication and is thought to be lethal to the cell - hence, it is not transmissable.

Chromosomal breaks, which constitute more severe pre-replication damage involving disruption of DNA in both arms, were comparable between control and test groups.

One of the most important cytogenetic indicators of genetic damage is the chromosomal marker which includes exchange figures. These structures are a result of chromosomal and chromatid breakage and subsequent incorrect fusion of these broken ends. The process results in the fusion of two or more chromosomes into a single large abnormally shaped structure which can be identified by its peculiar morphology. The mean number of cells with markers, as well as the mean number of markers observed, were significantly increased in both Groups 2 and 3. The data also indicated that the incidence of markers was dose-related. Concomitantly, the number of cells showing 10 or more aberrations (excluding chromatid gaps) and the number of cells displaying multiple types of aberrations were significantly higher than the controls in both the low and high level exposure groups.

A fairly large proportion (2.6%) of the control group cells showed some kind of genetic damage. This relatively high background rate was probably due to the advanced age (65 weeks) of the rats at sacrifice. Higher percentages (6.7% and 12.7%,respectively) of the low and high level groups were observed to have been genetically damaged. Both test groups were significantly elevated over the control group. It also appeared that the mean number of aberrant cells as well as the mean number of aberrations was related to the exposure concentrations.

Seventy-seven percent of the rats in Group 2 and 86.7% of the rats in Group 3 showed affected cells, whereas only 48.6% of the control rats showed affected cells. In both cases, the proportion of affected animals was significantly greater than in the controls.

Group Nos. 2 and 3 had significant increases in (a) cells showing 10 or more aberrations, (b) cells with multiple types of aberrations (c) cells with marker chromosomes, and (d) cells with total aberrations. Based on these data, it can be concluded that long-term exposure to 50 ppm $N_2O$ plus 1 ppm halothane as well as to 500 ppm $N_2O$ plus 10 ppm halothane caused chromosomal damage to spermatogonial cells in the rat.

Submitted by

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

Cytogenetic Evaluation: Kapp
Report Preparation: Kapp
Supervision: Hardy and Kapp

:ew

## V. REFERENCES

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

2. Snedecor, G.W. and Cochran, W.G.: Statistical Methods, Iowa State University Press, Ames, pp 104-119, 1967.