Title: GERM CELL STUDIES IN MICE AFTER PROLONGED EXPOSURE TO NITROUS OXIDE

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Introduction: Damage to human germ cells could be the common denominator in the reported significant incidences of infertility and spontaneous abortion among workers exposed to waste anesthetic gases and congenital malformations among their offspring. To investigate this possibility in an animal model, we examined germ cells from male and female mice subchronically exposed to N_2O.

Methods: Sixty male and 60 female, 13-14 week old HLA/SW/ICR mice were randomly assigned to 4 groups. Exposures were for 4 hr/day, 5 days/week, for 14 weeks, to: room air, 0.5% N_2O, 5.0% N_2O, or 50% N_2O; O_2 concentration for all treatments was 21-25%. Inhalation exposures were carried out in gas-tight plexiglass chambers of 1000 liters capacity. After 14 weeks, mice were killed. The cauda epididymides were removed and a sperm suspension was prepared for each male. Total sperm count/epididymis was determined and 1000 sperm were examined for morphological abnormalities using criteria defined by Wyrobek and Bruce.[1] As a postive control, sperm were examined from 18 mice administered methyl methanesulfonate (MMS)[1]; control mice received saline. Results are expressed as % abnormal sperm/preparation. Additionally, testes were weighed and processed for histological examination. Ovaries of 6 female mice from the air group and 6 mice from the 50% N_2O group were examined to determine the number of primordial oocytes by the method of Felton et al.[2] As a positive control, ovaries were examined from 5 mice treated with 3-methylcholanthrene (3-MC) in corn oil; 5 control mice received corn oil. Analysis of variance and t tests were used when data were normally distributed.[3] Non-parametric tests (i.e., Mann-Whitney and Kolmogorov-Smiroff) were used when data were not normally distributed.[4] Proportions were compared by the z test. All tests were one sided. $P < 0.05$ was considered significant.

Results: There were no differences among the four exposure groups in weight of testes, % abnormal sperm, sperm count (Table) or histological appearance of the testes. The mean percentage (± SEM) of abnormal sperm ranged from 8.9 ± 2.4 (5.0% N_2O) to 13.5 ± 0.5 (50% N_2O). In contrast, 25.2 ± 4.1 % of sperm from MMS treated mice were abnormal compared with 2.5 ± 0.3 % of sperm from saline treated mice. ($P < 0.05$). There was no significant difference between mean oocyte numbers of mice treated with 50% N_2O (33.3 ± 14.4) and mice treated with air alone (29.8 ± 8.0). However, mice treated with 3-MC had significantly fewer ($P < 0.05$) primordial oocytes, 67.2 ± 19.5 compared with control mice, 222.4 ± 21.9.

Table: Testicular Effect of N_2O

Group	(n)	Sperm 10^6/ Epididymis	%Abnormal Sperm	Testes (mg)
1	(15)	18.4 ± 1.4	10.4 ± 2.3	255 ± 12
2	(14)	21.3 ± 1.7	9.5 ± 3.0	277 ± 10
3	(14)	21.1 ± 1.9	8.9 ± 2.4	271 ± 7
4	(15)	17.1 ± 1.6	13.5 ± 2.5	240 ± 8

Discussion: The present study has shown that although germ cells of HLA/SW/ICR mice are damaged by some chemicals, subchronic exposure to N_2O levels as high as 50% does not result in injury. The results of our study are in agreement with those of Land et al.[5] who found no increase in the percentage of abnormal sperm in (C57BLxC3H)F_1 mice exposed to either 8 or 80% N_2O for 4 hr/day for 5 days. They are at variance, however, with those of Kripke et al.[6] who reported atrophy of seminiferous tubules and decreased testicular weight of LEW/f Mai rats exposed to as little as 20% N_2O for 8 hr/day, for 2 days. A decrease in the number of spermatazoa and an increase in abnormal forms were also reported, although, quantitative determinations were not made. Similar positive results were reported by Gremigni et al.[7] who examined testes of rats exposed to 20% N_2O for 7 days. We do not know whether factors other than a difference in species can explain the positive findings with rats and the negative findings with mice. Nonetheless, The negative findings of the present study for both male and female germ cells should be reassuring for persons working in operating rooms.

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