Title: RECONSIDERATION OF THE MECHANISMS OF NITROUS OXIDE TERATOGENICITY

Authors: M. Fujinaga, M.D., R. I. Mazze, M.D., and J. M. Baden, M.D.

Affiliation: Department of Anesthesia, Stanford University School of Medicine, Stanford, California and Anesthesiology Service, Palo Alto V. A. Medical Center, Palo Alto, California 94304

Introduction: It is well known that 24 hr exposure to nitrous oxide (N_2O) concentrations greater than 50% causes reproductive toxicity, including teratogenicity, in rats. Until recently, it also was widely held that these effects occurred as a consequence of the inhibition of methionine synthase activity, which then led to interference with DNA synthesis.[1] However, it was reported last year that the volatile anesthetics, isoflurane and halothane, administered with N_2O prevented adverse reproductive effects in rats without preventing inhibition of methionine synthase activity.[2] Furthermore, treatment with folinic acid, which should have reversed the effects of N_2O on DNA production, did not prevent adverse reproductive effects.[2] Thus, the mechanism of N_2O teratogenicity is open to question. Additional unknowns relate to the gestational day of maximum sensitivity to the toxic effects of N_2O and the types of defects that occur following exposure on various days. Investigating the latter unknowns is an important step in understanding the mechanism of N_2O toxicity and is the subject of this abstract.

Methods: A total of 170 eleven-week old timed-pregnant Sprague-Dawley rats were studied. (Day 0 of gestation was defined as the day when a copulatory plug was observed in the vagina.) 140 rats divided into seven groups of 20 rats each, were exposed to 60% N_2O for 24 hr on each of days 6-12 of gestation. Another 30 rats were exposed to air on day 9 (control). Food and water were withheld during treatment. On day 20 of gestation, rats were killed by CO_2 inhalation, reproductive indices were determined, and fetuses were subsequently examined for external, visceral and skeletal anomalies. All examinations were made without knowledge of the treatment group. The percentage of abnormal fetuses in each litter of each treatment group was determined and group means were compared with control by ANOVA; Student's *t* test, corrected for multiple comparisons, was used as an *a posteriori* test when differences were found with ANOVA.

Results: Different adverse effects peaked on different days. Pregnancy rate was lowest on day 6. Fetal wastage was elevated on all days (except day 7) but was only significantly increased on days 8 and 11. Major skeletal malformations peaked on day 9. Minor skeletal anomalies peaked on days 8 and 9, with a specific abnormality, cervical rib, peaking only on day 9. Major visceral abnormalities peaked on day 8, including the most common abnormality, right sided aortic arch. There were no differences in the number of implantations and live fetuses, and mean fetal weight was the same among all groups.

Discussion: The present study has yielded new information about the toxic reproductive effects of N_2O. Of great interest were the two distinct peaks in total fetal wastage, on days 8 and 11 of gestation. The reason for the bimodal distribution is not clear but the second peak may relate to failure of the fetal circulation to accomplish the usual switch from the yolk sac to the chorioallantoic placenta on day 11 of gestation. Also notable was the increased incidence of major skeletal malformations (mostly vertebral anomalies) on day 9, whereas the minor anomaly, cervical rib, was present only on day 8. These effects of N_2O on skeletal development may have different mechanisms, since one relates to the aberrant development of structures usually present and the other to formation of new structures. We also found that the incidence of major visceral malformations (mostly right sided aortic arch) was significantly increased only on day 8. In summary, our data strongly suggest that multiple mechanisms are involved in the reproductive toxicity of N_2O; they are yet to be elucidated.

References:

1. Nunn JF: Clinical aspects of the interaction between nitrous oxide and vitamin B_{12}. Br J Anaesth 59:3-13, 1987

2. Fujinaga M, Baden JM, Mazze RI: Halothane and isoflurane prevent the teratogenic effects of nitrous oxide, folinic acid does not. Anesthesiology 67:A456, 1987

Gestational day of N_2O exposure	Control	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
No. of rats studied	30	20	20	20	20	20	20	20
No. of rats died	0	2	2	2	2	0	4	3
No. of pregnant rats	23	10	15	13	16	17	14	13
Reproductive indices								
Pregnancy rate (%)	77	56	83	72	89	85	88	76
No. of implantations	11.7	10.2	10.8	12.2	11.3	9.1	9.8	11.9
No. of live fetuses	11.3	8.8	10.3	7.7	10.3	8.2	6.4	9.5
Fetal wastage (%)	3.4	18.9	5.5	36.8*	11.6	16.5	37.3*	20.1
Mean fetal weight (g)	4.4	4.1	4.0	4.0	4.0	4.3	4.3	3.9
External exam.								
No. of fetuses	261	88	155	100	165	140	90	126
Runt (%)	1.1	0.7	1.4	0.6	1.7	0.0	3.7	5.2
Any anomalies (%)	0.3	0.0	0.9	0.6	0.5	6.3	0.0	0.6
Skeletal exam.								
No. of fetuses	131	44	77	49	82	69	45	64
Major abn. (%)	0.0	0.0	0.0	1.4	11.3*	0.0	0.0	0.0
Minor abn. (%)	0.6	1.6	3.4	48.0*	28.8*	3.6	0.0	1.3
Cervical rib (%)	0.6	0.0	0.0	37.5*	2.3	0.0	0.0	0.0
Dev. variants (%)	23.0	22.3	23.4	21.1	37.8	23.3	21.4	28.2
Bip. centrum (%)	4.6	0.0	4.6	10.8	39.3*	17.4	4.4	9.0
Visceral exam.								
No. of fetuses	130	44	78	51	83	71	45	63
Major abn. (%)	0.0	0.0	3.3	34.2*	8.5	6.3	2.8	2.5
Rt. Aor. arch (%)	0.0	0.0	3.3	22.5*	0.9	0.0	2.8	0.0
Minor abn. (%)	3.6	13.1	7.9	21.2	5.2	9.6	4.2	19.7

* $p < 0.05$ vs. Control (ANOVA)