Title: CARCINOGEN BIOASSAY OF NITROUS OXIDE IN MICE

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Introduction: Nitrous oxide (N_2O) is the most widely used inhaled anesthetic worldwide. As with similar drugs, there is concern that it poses a carcinogenic risk to operating room personnel. In fact, based on epidemiologic data, many investigators believe that it is the most likely anesthetic to be carcinogenic. In lieu of reliable human data, animal studies provide the most definitive means of establishing a carcinogenic hazard. An accepted protocol for conducting *in vivo* studies is to expose animals for most of their life-span to the test agent using a route of administration similar to that experienced by humans; such a protocol has been used to examine the carcinogenic potential of N_2O.

Methods: Two hundred and fifty-six male and 256 female, nine-week-old Swiss-Webster mice were divided randomly into three groups. Group 1 consisted of 96 males and 96 females, groups 2 and 3 each consisted of 80 males and 80 females. Group 1 (control) was exposed to compressed air, and groups 2 and 3 were exposed to 10% and 40% N_2O, respectively, for four hours per day, five days per week. The high dose selected was the maximum tolerated dose (MTD) of N_2O. After 78 weeks of exposure, a four-week period without treatment was allowed, following which remaining mice were killed by carbon dioxide overdose. All mice killed at the time of scheduled sacrifice or dying or killed in extremis at other times, were subjected to complete autopsy. The only exceptions were mice which died early and were eliminated from the study and those in which cannibalism or advanced autolysis precluded examination. More than 40 tissues were examined *in situ*, and 32 routine sections from different organs and all abnormal tissues were taken for microscopic examination. At the time of necropsy and at microscopic exaimination, examiners were unaware of the treatment groups of the mice. Intergroup comparisons of body and organ weights, and of tumors from each organ were made using analysis of variance and Chi-square test as appropriate; $P < 0.05$ was considered statistically significant.

Results: Mean body weight of mice treated with 40% N_2O was on average about 5% less than those of mice in control and 10% N_2O groups. Gross and microscopic examination of tissues revealed a variety of non-neoplastic lesions including ovarian cysts, lymphadenopathy, bladder stones, and testicular atrophy; however, none was related to the N_2O treatment. Many neoplastic lesions were seen. Most were either lung adenomas or liver tumors (table). Lung adenomas were distributed equally among the groups and were generally of alveolar cell origin. Liver tumors were also equally distributed among the groups and were generally classified as basophilic hepatocellular adenomas. The small numbers of other tumors also were unrelated to N_2O treatment.

Table: Number of mice with tumors

	Scheduled Sacrifice					
	Control		10% N_2O		40% N_2O	
	M* (67)+	F** (63)	M (51)	F (63)	M (37)	F (55)
Lung adenomas	22	21	25	15	11	25
Liver tumors	10	2	5	3	4	2
Others	5	6	5	5	3	4
	Unscheduled Sacrifice					
	Control		10% N_2O		40% N_2O	
	M (18)	F (20)	M (18)	F (14)	M (23)	F (14)
Lung adenomas	3	4	1	1	1	2
Liver tumors	10	2	1	3	-	-
Others	5	6	2	6	7	6

M* = Male; F** = Female; ( )+ = Number of Mice

Discussion: The results of our study do not support the thesis that N_2O is a potential carcinogen. They are in agreement, however, with two previously reported but very limited studies of the carcinogenicity of N_2O.[1,2] Both those studies were negative but results were not very convincing because the protocols used involved either short periods of exposure or low concentrations. In our study, the maximum tolerated dose was administered for a life-time. Thus our negative results should provide some reassurance to both patients and operating room personnel that N_2O is not carcinogenic.

References:

1. Eger EI II, White AE, Brown CL, Biava CG, Corbett TH, Stevens WC: A test of the carcinogenicity of enflurane, halothane, methoxyflurane and nitrous oxide in mice. Anesth. Analg. 57:678-694, 1978.
2. Coate WB, Ulland BM, Lewis TR: Chronic exposure to low concentrations of halothane-nitrous oxide: Lack of carcinogenic effect in the rat. Anesthesiology 50:306-309, 1979.