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Reproductive and Teratogenic Effects of Nitrous Oxide, Halothane, Isoflurane, and Enflurane in Sprague-Dawley Rats

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A total of 305 timed-pregnant Sprague-Dawley rats were exposed for 6 h a day on each of three consecutive days in one of three periods, *i.e.*, pregnancy days 14–16, 11–13, or 8–10, either to 0.55 times the minimum alveolar concentration (MAC) of nitrous oxide (75%) or to 0.75 MAC of halothane (0.8%), isoflurane (1.05%) or enflurane (1.65%); an additional 232 positive-control (retinoic acid) and air control rats were studied. Reproductive indices were determined, and the 5178 offspring delivered at cesarean section were examined for external, internal, and skeletal abnormalities. There were no major or minor teratologic effects in anesthetic treated groups, although several developmental variants were observed in halothane- and enflurane-treated groups. Nitrous oxide exposure on days 14–16 resulted in a three-fold increase in fetal resorptions. The results suggest that the volatile anesthetics are not teratogenic and confirm that nitrous oxide may be associated with increased reproductive loss. (Key words: Anesthetics, gases: nitrous oxide. Anesthetics, volatile: halothane, isoflurane, enflurane. Pregnancy: teratogenicity. Toxicity: fetal, teratogenicity.)

TO DETERMINE WHETHER the inhaled anesthetic agents contribute to adverse reproductive effects, we have in past experiments exposed Swiss Webster mice to trace, subanesthetic, and anesthetic concentrations of halothane, methoxyflurane, enflurane, nitrous oxide, and isoflurane on days 6–15 of pregnancy, the period when they are most susceptible to teratogenic insult.[1–6] The most striking finding in this series of studies was that the highest concentration of isoflurane we studied, 0.6%, caused a 12% incidence of cleft palate. This was six times greater than that which occurred with any of the other agents. However, the biologic significance of the results of this study for humans is uncertain because mice are particularly susceptible to development of cleft palate. Also, our previous studies were carried out sequentially over an 8-yr period and the interstudy comparability of any group of experiments carried out over such an extended time period is open to question. That is because factors such as changes in laboratory methods and personnel, animal husbandry (bedding, diet, light–dark cycle, etc.) and genetic makeup of test animals can confound the results of sequentially performed experiments. Thus, to expand on the design of our previous work, we evaluated the reproductive and teratogenic effects of nitrous oxide, halothane, isoflurane, and enflurane administered during the same time period to Sprague-Dawley rats. Rats are another commonly used rodent model for reproductive and teratogenic studies, but one in which the susceptibility to cleft palate more closely resembles that seen in humans.

Methods and Materials

Upon receipt from the breeder,** timed-pregnant Sprague-Dawley rats were identified with metal ear tags and housed four per cage. Room temperature in the animal facility was maintained at 21–24° C, and artificial lighting was provided from 0600 to 1900 h each day. Rats were bedded on ground corncob†† and fed standard laboratory rodent food‡‡ and tap water *ad libitum:* they were weighed upon arrival and prior to cesarean section. Based on their weight, pregnant dams were divided into six groups of approximately equal mean weight, as follows: 1) treatment control (air); 2) positive control (retinoic acid); 3) 75% nitrous oxide; 4) 0.8% halothane; 5) 1.05% isoflurane; and 6) 1.65% enflurane. We selected these concentrations of the volatile agents, which are equivalent to 0.75 times the minimum alveolar concentration (MAC),[7] because in preliminary studies we determined that these were the maximum equipotent concentrations of the anesthetics that we could administer for 6 h without causing adverse physiologic effects. Nitrous oxide 75%, approximately 0.55 MAC,[8] is the maximum nonhypoxic

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TABLE 1. Classification of fetal abnormalities

External Abnormalities	Skeletal Abnormalities	Internal Abnormalities
Runt	Major malformations	Major malformations
Major malformations	Craniofacial	Minor anomalies
Cleft palate	Ribs or vertebrae	Enlarged brain ventricle
Exencephaly	Other	Hemorrhage
Limb deformity	Minor anomalies	Gonad displacement
Other	Ribs or vertebrae	Bladder distention
Minor anomalies	Sternum	Other
Limb malposition	Other	Developmental variant
Crooked tail	Developmental variant	Increased renal pelvic cavitation
Other	Supernumerary rib	Other
	Asymmetric or bipartite sternum	
	Other	
	Generalized decreased ossification	

concentration of that agent that we could administer. The experiment was performed in three separate exposure periods using a total of 537 timed-pregnant Sprague-Dawley rats. Rats in period I (n = 147) were exposed for 6 h on days 14–16 of pregnancy. In the two subsequent periods, additional groups of rats were exposed to identical treatments on days 11–13 (period II; n = 196) and 8–10 (period III; n = 194) of pregnancy. We chose 3 days of 6 h, day-time exposures in contrast to one 24-h exposure or continuous low-dose exposure throughout pregnancy, as used in some studies,[9–12] because we did not want to interfere with primary rodent feeding and wakefulness patterns that normally occur at night. Also, were adverse effects to occur, three consecutive, 3-day treatment intervals would yield more specific data regarding the acute embryotoxicity of the inhaled anesthetics than would a single exposure throughout the entire period of organogenesis (days 6–15).

Inhalational exposures were performed simultaneously in five gas-tight, Plexiglas® chambers, each of approximately 1,000-l capacity. During treatment, rats remained in their cages, which were placed in the chambers. Halothane, isoflurane, and enflurane were vaporized in copper kettle vaporizers with medical-grade compressed air and delivered to the chambers through Tygon® tubing at total flow rates of 6–10 l/min. The desired concentration of each agent was achieved within 10–15 min by adding measured amounts of liquid anesthetic to the circuit. Small quantities of supplemental oxygen also were added. Medical-grade nitrous oxide and oxygen were mixed to achieve the desired nitrous oxide concentration (75%). Treatment control rats were exposed to compressed air simultaneously in the fifth chamber. Positive control rats remained in their cages and breathed room air. They received 5 mg/kg of retinoic acid in corn oil, by gavage, each day during the treatment period. Retinoic acid is a known teratogen and reproductive toxin. Its teratogenic effects vary, depending on the test species, dose, and time of administration. Its primary use in teratology experiments is for quality control purposes.

Concentrations of each agent were monitored continuously with Miran 1A-1F® infrared analyzers and were recorded on strip-chart recorders; they were maintained within 5% of the desired level. Oxygen concentration was monitored continuously with IL402® analyzers and was maintained at 22–25% in all chambers, including that of the air control group. Carbon dioxide concentration was measured at random times with a Beckman LB-2® infrared analyzer; it was found not to exceed 0.6% and usually was in the range of 0.1–0.2%. Temperature in the chambers ranged from 21–30° C, with a mean value of 24° C; there were no differences in chamber temperatures among the groups.

On day 21 of pregnancy, rats were killed by carbon dioxide inhalation, and cesarean sections were performed. The uterus of each was examined, and the numbers and positions of live and dead fetuses and resorptions were recorded. Weight and sex of each live fetus were determined, and each fetus was examined for external abnormalities. Every other fetus was fixed in 95% ethanol and cleared with potassium hydroxide. The skeleton was then stained with alizarin red S using the method of Staples and Schnell[13] and subsequently examined for skeletal abnormalities. The remainder of the fetuses were preserved in Bouin's fixative solution and subsequently dissected and examined for internal soft-tissue abnormalities as described by Barrow and Taylor.[14] All examinations were done using a dissecting microscope; they were accomplished without knowledge of the treatment group.

Abnormalities were classified by type and severity (table 1). Fetuses weighing 25% less than the mean litter weight were classified as runts. Fetal morphologic abnormalities that would have precluded normal survival were considered major malformations, while abnormalities that were neither severely disfiguring nor incapacitating were classified as minor anomalies. The term developmental variant was used to describe distinct variations in normal development that are common in untreated animals, but which can occur with increased frequency as a consequence of exposure to teratogens, *e.g.*, supernumerary ribs. Decreased ossification was classified separately from other skeletal variants because we wished to distinguish a process that only delayed fetal maturation from one that altered fetal morphology.

For statistical analyses, we computed the percentage of fetuses affected in each litter for each type of abnormality.

TABLE 2. Reproductive Indices (mean ± SD)

	Period	Control	Positive Control	75% N_2O	0.8% Halo	1.05% Iso	1.65% Enf
Dams	I	39	20	19	18	21	20
	II	48	21	29	26	25	21
	III	50	24	25	24	23	23
Dam weight gain (g)	I	84.9 ± 12.7	81.7 ± 9.9	68.5* ± 25.7	76.4 ± 13.8	62.5* ± 12.8	66.5* ± 15.2
	II	95.1 ± 27.9	91.1 ± 40.1	90.9 ± 21.9	74.6 ± 25.7	81.9 ± 22.7	90.1 ± 26.3
	III	113.3 ± 15.6	109.1 ± 19.8	104.2 ± 13.5	96.7* ± 13.9	100.3* ± 12.2	104.0 ± 16.7
Fetuses	I	429	224	198	200	238	214
	II	529	211	319	276	257	241
	III	557	249	272	254	252	258
Fetal weight (g)	I	4.58 ± 0.29	4.65 ± 0.31	4.13* ± 0.46	4.22* ± 0.35	4.17* ± 0.31	4.30* ± 0.33
	II	4.52 ± 0.51	4.27 ± 0.65	4.46 ± 0.66	4.31 ± 0.28	4.35 ± 0.40	4.34 ± 0.73
	III	4.49 ± 0.38	4.38 ± 0.33	4.29 ± 0.28	4.21* ± 0.37	4.13* ± 0.32	4.04* ± 0.47
Implantations/ dam	I	11.4 ± 1.4	11.5 ± 1.7	11.8 ± 2.2	11.8 ± 1.2	11.7 ± 2.1	11.3 ± 2.7
	II	11.7 ± 2.9	12.1 ± 2.5	11.6 ± 2.3	11.0 ± 2.5	10.8 ± 3.2	12.2 ± 2.9
	III	11.8 ± 2.3	10.8 ± 2.1	11.2 ± 1.9	11.2 ± 2.2	11.5 ± 1.5	11.8 ± 2.0
Live Fetuses/dam	I	11.0 ± 1.3	11.2 ± 1.7	10.4 ± 2.6	11.2 ± 1.3	11.3 ± 2.4	10.7 ± 2.6
	II	11.0 ± 3.3	10.0 ± 4.1	11.0 ± 2.6	10.6 ± 3.0	10.3 ± 3.5	11.5 ± 3.1
	III	11.1 ± 2.2	10.4 ± 2.3	10.9 ± 1.9	10.6 ± 2.7	11.0 ± 1.8	11.2 ± 2.4
% Female	I	51.8	44.6	50.0	51.8	44.2	47.7
	II	49.1	54.0	50.9	59.4	43.7	48.7
	III	50.5	47.1	52.3	53.8	51.8	46.4
Total fetal wastage (number/dam; dead + resorped)	I	0.49 ± 0.72	0.30 ± 0.66	1.37* ± 1.17	0.67 ± 0.84	0.29 ± 0.56	0.60 ± 0.68
	II	0.63 ± 0.98	2.05* ± 3.35	0.59 ± 1.18	0.39 ± 0.80	0.52 ± 0.77	0.67 ± 0.86
	III	0.62 ± 0.92	0.38 ± 0.58	0.32 ± 0.56	0.63 ± 1.10	0.57 ± 0.79	0.61 ± 0.89
Resorptions (number/dam)	I	0.46 ± 0.64	0.25 ± 0.55	1.32* ± 1.20	0.67 ± 0.84	0.29 ± 0.56	0.60 ± 0.68
	II	0.63 ± 0.98	2.05* ± 3.35	0.59 ± 1.18	0.39 ± 0.80	0.48 ± 0.77	0.67 ± 0.86
	III	0.56 ± 0.86	0.29 ± 0.55	0.24 ± 0.44	0.58 ± 1.10	0.57 ± 0.79	0.61 ± 0.89

Halo = halothane; Iso = isoflurane; Enf = enflurane; Period I = exposure days 14–16; Period II = exposure days 11–13; Period III = exposure days 8–10.

* $P < 0.05$ *versus* control group (same exposure period).

Data were analyzed by analysis of variance (ANOVA) and by Student's *t* test using the Bonferroni correction for multiple analyses when differences were found with ANOVA. $P < 0.05$ was considered significant.

Results

Exposure to the volatile anesthetics resulted in light general anesthesia; when exposure was terminated, rats were awake within 10–15 min. Animals exposed to nitrous oxide remained conscious throughout the experiment. Of the 537 rats studied, 476 (88.6%) were pregnant (table 2). They were delivered of 5178 offspring, all of which were examined. Compared with control rats, pregnant rats exposed on days 14–16 (period I) to nitrous oxide, isoflurane, and enflurane had smaller weight gains. A similar finding was noted in pregnant dams exposed on days 8–10 (period III) to halothane and isoflurane (table 2). The apparent difference in weight gain among dams exposed during each of the exposure periods was due to the differences in their gestational age when they were received from the breeder and first weighed.

There were two major findings in the study. The first was the absence of cleft palate or other major abnormalities in rats treated with isoflurane or the other inhaled anesthetics (tables 3a–3c). The second was a three-fold increase compared with control ($P < 0.001$) in the mean number of fetal resorptions in rats treated with nitrous oxide on days 14–16 (1.32 *vs.* 0.46; table 2). This was also manifested as a decreased percentage of live fetuses/implantations for the nitrous oxide group (87.8%) compared with controls (96.7%). There were no other abnormal values in reproductive indices except in the positive control group (table 2). Minor but statistically significant reductions in fetal body weight were seen in period I in all anesthetic-treated groups and in period III

TABLE 3A. Fetal Morphology (mean per cent abnormal fetuses per litter ± SD)

	Period	Control	Positive Control	75% N_2O	0.8% Halo	1.05% Iso	1.65% Enf
External Examination							
Number of fetuses	I	429	224	198	200	238	214
	II	529	211	319	276	257	241
	III	557	249	272	254	252	258
Any external abnormality	I	0.7 ± 2.5	0.4 ± 1.8	0.5 ± 2.1	2.0 ± 4.8	0.4 ± 1.7	0.0 ± 0.0
	II	0.4 ± 1.8	1.7 ± 7.4	0.0 ± 0.0	0.3 ± 1.6	0.0 ± 0.0	1.6 ± 7.2
	III	0.0 ± 0.0	3.8 ± 13.9	3.6 ± 16.0	1.3 ± 4.5	1.0 ± 3.6	0.0 ± 0.0
Major malformation	I	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	II	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	III	0.0 ± 0.0	0.0 ± 0.0	3.2 ± 16.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Minor anomaly	I	0.7 ± 2.5	0.4 ± 1.8	0.5 ± 2.1	2.0 ± 4.8	0.4 ± 1.7	0.0 ± 0.0
	II	0.1 ± 1.0	1.7 ± 7.4	0.0 ± 0.0	0.3 ± 1.6	0.0 ± 0.0	0.0 ± 0.0
	III	0.0 ± 0.0	3.3 ± 13.8	0.0 ± 0.0	1.3 ± 4.5	0.7 ± 3.1	0.0 ± 0.0

Halo = halothane; Iso = isoflurane; Enf = enflurane; Period I = exposure days 14–16; Period II = exposure days 11–13; Period III = exposure days 8–10.

* $P < 0.05$ *versus* control group (same exposure period).

in all treated groups except 75% nitrous oxide. Several developmental variants were observed in both control and treated groups, the most frequent being rudimentary ribs (tables 3*B* and 3*C*).

Discussion

The present study failed to demonstrate any significant teratogenic effect in Sprague-Dawley rats treated with isoflurane or the other inhaled anesthetics. This is in contrast to the results of our previous study[6] in which Swiss Webster mice exposed to isoflurane had a 12% incidence of cleft palate but no other defects. Mice are generally suitable for teratology studies, but an isolated finding of cleft palate in this species is of questionable significance for humans. Thus, it is likely that the high incidence of cleft palate in our previous isoflurane study (isoflurane, 12.1%; control, 0.6%),[6] typifies the tendency of mice to develop this lesion following a large variety of treatments and manipulations.[15] For example, although the spontaneous incidence of cleft palate in mice is usually less than 1%, values as great as 100% have been observed in A-strain mice after cortisone treatment.[15] The lack of confirmation of cleft palate in the present study, and the apparent absence of this malformation in the offspring of pregnant women exposed to isoflurane or any other inhaled anesthetic,[16,17] suggest that the original finding was species-specific.

The other significant finding in the present study was the confirmation of increased reproductive loss associated with nitrous oxide administration. This has been demonstrated by many laboratories,[9–12] including our own,[11] but usually in association with more stressful exposure to nitrous oxide. That is, pregnant dams have been exposed for a single 24-h period, during which time they were not given food or water,[9–11] or they have been exposed to low concentrations of nitrous oxide continuously throughout pregnancy.[12] We employed three 6-h exposures separated by 18-h recovery periods to permit usual rodent nocturnal activities. Nevertheless, we still observed a three-fold increase in resorptions, the equivalent of spontaneous abortion in humans, in dams treated with nitrous oxide on days 14–16 of pregnancy. No such findings occurred with the volatile agents, although the relative concentration of these drugs, 0.75 MAC, was 35–40% greater than that of nitrous oxide, which was 0.55 MAC. Thus, the present study emphasizes the potential for nitrous oxide to cause reproductive loss in rodents. Rats treated with retinoic acid on days 11–13 of pregnancy also had an increased incidence of resorptions. Finding a difference in the time periods when resorptions occurred following treatment with retinoic acid and with nitrous oxide is not surprising in light of the probable differences in their mechanisms of action. Although these have not been definitively established, it is most likely that retinoic acid acts by abnormally inducing or suppressing genomic expression through alterations in protein production.[18] In contrast, nitrous oxide probably acts by directly inhibiting vitamin B_{12} formation and subsequently inhibiting DNA synthesis.[19]

Can the results of these animal toxicity studies be extrapolated to pregnant patients who must undergo operation? Some believe that animal toxicity studies are of little value. However, the need to avoid potentially hazardous experiments in humans, the proven usefulness of such studies in many areas and, indeed, the rules established by government regulatory agencies dictate that they be done. Thus, it seems only reasonable to be cautious in extrapolating the results of these studies to human populations. Of particular interest in the series of teratology studies we have done is that the results of the present

TABLE 3B. Fetal Morphology (mean per cent abnormal fetuses per litter ± SD)

	Period	Control	Positive Control	75% N_2O	0.8% Halo	1.05% Iso	1.65% Enf
Skeletal Examination							
Number of fetuses	I	213	111	98	101	119	108
	II	268	105	159	141	129	126
	III	285	127	135	128	132	130
Any skeletal abnormality	I	30.5 ± 34.7	94.8* ± 14.1	38.9 ± 30.7	38.9 ± 20.4	32.5 ± 25.7	34.1 ± 24.8
	II	20.7 ± 24.9	35.8 ± 33.3	25.4 ± 27.2	22.3 ± 20.6	31.8 ± 24.3	51.9* ± 49.3
	III	24.1 ± 20.5	45.2* ± 25.7	21.3 ± 20.8	26.0 ± 18.6	26.1 ± 26.9	26.0 ± 31.0
Major malformation	I	0.0 ± 0.0	0.0 ± 0.0	1.1 ± 4.6	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 3.1
	II	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	6.0 ± 27.3
	III	0.0 ± 0.0	4.2 ± 20.4	0.0 ± 0.0	1.7 ± 8.2	0.0 ± 0.0	0.0 ± 0.0
Minor anomaly	I	0.5 ± 3.2	0.0 ± 0.0	1.9 ± 5.9	3.8 ± 11.3	0.0 ± 0.0	0.7 ± 1.3
	II	0.0 ± 0.0	5.7 ± 22.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	7.1 ± 32.7
	III	0.0 ± 0.0	7.7* ± 21.3	1.6 ± 5.5	0.0 ± 0.0	0.6 ± 2.9	0.9 ± 4.2
Developmental variant	I	30.5 ± 34.7	94.8* ± 14.1	35.4 ± 27.4	35.1 ± 23.0	32.5 ± 25.7	34.1 ± 24.8
	II	18.6 ± 22.0	35.1 ± 32.6	25.4 ± 27.2	22.3 ± 20.6	31.8 ± 24.3	50.7* ± 45.0
	III	24.1 ± 20.5	43.0 ± 27.4	21.3 ± 20.8	24.3 ± 19.1	24.8 ± 27.4	23.1 ± 26.6
Rudimentary lumbar rib	I	24.3 ± 33.1	94.0* ± 14.2	22.4 ± 28.8	26.3 ± 22.7	26.9 ± 26.3	21.9 ± 24.9
	II	15.4 ± 21.0	12.5 ± 23.6	20.0 ± 21.6	20.0 ± 19.8	21.7 ± 19.4	40.2* ± 36.1
	III	16.3 ± 20.0	12.2 ± 15.7	8.5 ± 15.5	6.7 ± 13.4	17.3 ± 24.2	11.3 ± 19.8
Cervical rib	I	0.0 ± 0.0	1.0 ± 4.5	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 2.8	0.0 ± 0.0
	II	0.4 ± 2.9	1.7 ± 5.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	III	0.3 ± 2.4	19.2* ± 24.0	2.8 ± 6.7	3.3 ± 9.6	0.0 ± 0.0	6.4 ± 19.4
Generalized decreased ossification	I	0.0 ± 0.0	0.0 ± 0.0	2.6 ± 11.5	1.1 ± 4.7	0.0 ± 0.0	0.0 ± 0.0
	II	0.3 ± 2.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	III	0.0 ± 0.0	1.5 ± 5.2	0.7 ± 3.4	0.0 ± 0.0	1.2 ± 4.0	5.1 ± 21.0

Halo = halothane; Iso = isoflurane; Enf = enflurane; Period I = exposure days 14–16; Period II = exposure days 11–13; Period III = exposure days 8–10.

* $P < 0.05$ *versus* control group (same exposure period).

study with rats appear to contradict those of our previous isoflurane study with mice.[6] Such contradictions are not uncommon in animal toxicity studies, causing the question to be asked, "Which results are correct?". In fact, the results of both studies are valid. What must be emphasized is that no single protocol and no single species are ideal for all toxicity studies. Interpretation of the results of toxicity studies must take into account many factors, such as

TABLE 3C. Fetal Morphology (mean per cent abnormal fetuses per litter ± SD)

	Period	Control	Positive Control	75% N_2O	0.8% Halo	1.05% Iso	1.65% Enf
Internal Examination							
Number of fetuses	I	216	113	100	99	119	106
	II	261	106	160	135	128	115
	III	272	122	137	126	120	128
Any internal abnormality	I	6.1 ± 11.5	8.1 ± 9.3	4.3 ± 9.2	14.6 ± 18.2	6.9 ± 9.8	7.5 ± 11.0
	II	8.5 ± 12.9	10.4 ± 14.0	6.3 ± 10.5	8.0 ± 14.0	10.8 ± 18.4	14.7 ± 17.0
	III	2.6 ± 7.6	5.0 ± 10.6	3.5 ± 10.4	3.5 ± 8.5	7.2 ± 14.9	4.5 ± 9.3
Major malformation	I	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	II	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	III	4.0 ± 28.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Minor anomaly	I	6.1 ± 11.6	8.1 ± 9.3	4.3 ± 9.2	10.9 ± 14.3	6.9 ± 9.8	7.5 ± 10.8
	II	7.5 ± 12.2	9.1 ± 13.7	5.2 ± 9.3	7.2 ± 13.9	10.8 ± 18.4	13.7 ± 17.1
	III	2.6 ± 7.6	3.3 ± 7.6	2.2 ± 8.4	3.5 ± 8.5	6.1 ± 14.5	4.5 ± 9.3
Developmental variant	I	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	47.2* ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	II	10.2 ± 39.7	12.5 ± 55.9	11.4 ± 61.4	8.0 ± 40.0	0.0 ± 0.0	20.0 ± 64.4
	III	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.9 ± 52.1	0.0 ± 0.2

Halo = halothane; Iso = isoflurane; Enf = enflurane; Period I = exposure days 14–16; Period II = exposure days 11–13; Period III = exposure days 8–10.

* $P < 0.05$ *versus* control group (same exposure period).

the prevalence of a particular abnormal finding in a study, the occurrence of other abnormalities in that study, the numbers of different species that develop the same abnormality, and, perhaps most important, the tendency of the test species to develop a particular lesion in comparison with the tendency in humans. Because cleft palate was the only significant teratogenic finding in our isoflurane study in mice,[6] this lesion was not seen in our rat study, and the incidence of cleft palate does not appear to be increased in pregnant women having surgery,[16,17] we believe that neither isoflurane nor any of the other inhaled anesthetics, when administered under the usual clinical conditions, will cause teratogenic effects in humans.

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