Title: HALOTHANE AND FLUROXENE ARE MUTAGENIC IN DROSOPHILA

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Introduction: Epidemiologic surveys have led to the suggestion that exposure to waste anesthetic gases is associated with a higher than normal incidence of cancer and adverse reproductive effects. Thus, many investigators have examined toxicity of inhaled anesthetics and, in particular, have studied their mutagenic potential in a variety of test systems. Most tests have shown that nitrous oxide, halothane, enflurane and isoflurane are not mutagenic, whereas fluroxene which contains a vinyl moiety, is mutagenic. However, studies of halothane and nitrous oxide with Drosophila have been positive.[1,2] Because of this inconsistency, we undertook to examine all five anesthetics for their ability to induce sex-linked recessive lethals in Drosophila.

Methods: Male wild-type flies of Drosophila melanogaster, 2-3 days old, were exposed to an acute dose of halothane, enflurane, isoflurane or fluroxene at vapor concentration of 1 or 2%. For nitrous oxide, the concentrations used were 40 and 80%. These dosages were found in previous studies not to cause an increased rate of lethality. Control flies were exposed to room air alone. All exposures were for 1 h at 25 $\pm$ 1°C and 50 $\pm$ 5% relative humidity. During exposure the concentration of oxygen was maintained at 21 $\pm$ 1%. Immediately after exposure, 30 male flies from each treatment group were selected at random and each male was individually mated with three untreated virgin female flies of the Basc strain. The brooding technique was applied; that is, treated males were mated at regular intervals of 3 days to 3 successive waves (I,II,III) of untreated virgin females. The numbers of sex-linked recessive lethals were determined in the F_2 generation of flies. The Kastenbaum-Bowman test was used to compare the mutation rate in each brood of each anesthetic with that of the untreated controls.

Results: Control flies exposed to room air alone produced only one or two lethals per brood. The total lethality rate was 4 out of 1,788 gametes tested, or 0.244% (table). Halothane and fluroxene produced a dose-dependent increase in the rate of lethal mutation above control. At 2% vapor concentration, halothane produced about a 7-fold increase ($P < 0.01$) and fluroxene produced about a 3.5-fold increase ($P < 0.05$). Brooding pattern analysis showed that all stages of germ cell development appeared to be equally sensitive to the mutagenic effects of halothane and fluroxene. Enflurane, isoflurane and nitrous oxide were not mutagenic at the concentrations tested (table).

Discussion: Although our results and those of Kramers and Burm[1] provide strong evidence that halothane is mutagenic in Drosophila at clinical concentrations, halothane has not been found to be mutagenic in other test systems. The most likely explanation for the different results among the tests is differences in pathways and degrees of metabolism. Fluroxene, an anesthetic which is now seldom used, also showed a dose-dependent increase in mutation frequency. At equipotent concentrations, fluroxene would be more mutagenic than halothane. In contrast to halothane, all other systems which have been used to test fluroxene for mutagenicity have given positive results. Nitrous oxide was not mutagenic in our study despite the findings of Garrett and Fuerst[2] that nitrous oxide marginally increased the number of recessive sex-linked lethal mutation in Drosophila. However, a hypoxic gas mixture was used in their studies which raises an uncertainty about their results. The lack of mutagenicity of nitrous oxide seen in our study and in many other studies suggests that nitrous oxide has no mutagenic potential. Our negative findings with enflurane and isoflurane are in agreement with many mutagenicity studies using other test models. Factors such as species variation, dose, exposure time, hypoxia must be considered before assessing the risk of these agents to humans. Nonetheless, the possibility that fluroxene and perhaps halothane have contributed to the increase in malignancy and adverse reproductive effects reported to occur in operating room personnel cannot be ignored.

Table: Induction of sex-linked recessive lethals

Anesthetic	Dose (%v/v)	Brood	No.Chrom. Tested	% Lethals
Control	0	I-III	1,788	0.224
Halothane	1	I-III	1,760	1.136*
	2	I-III	1,818	1.539*
Fluroxene	1	I-III	1,792	0.670**
	2	I-III	1,843	0.760**
Enflurane	1	I-III	1,815	0.275
	2	I-III	1,808	0.166
Isoflurane	1	I-III	1,797	0.278
	2	I-III	1,801	0.321
Nitrous oxide	40	I-III	1,820	0.274
	80	I-III	1,795	0.279

*$P < 0.01$; **$P < 0.05$

References:
1. Kramers PG, Burm GL: Mutagenicity studies with halothane in Drosophila melanogaster, Anesthesiology 50: 510-513, 1979.
2. Garrett S, Fuerst R: Sex-linked mutations in Drosophila after exposure to various mixtures of gas atmospheres, Environ. Res. 7:286-293, 1974.