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Metabolism and Toxicity of 2,2,2-Trifluoroethyl Vinyl Ether

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A review on metabolism and toxicity of the fluorinated anesthetic agent, fluroxene, is presented. Fluroxene anesthesia is nontoxic to man but fatal to many experimental animals. The fluroxene molecule (2,2,2-trifluoroethyl vinyl ether) is composed of two moieties; both are toxic as a result of their metabolism: the vinyl moiety destroys heme of cytochrome P-450 while being metabolized to the final product, CO_2. The trifluoroethyl moiety is oxidized to trifluoroethanol (TFE) and trifluoroacetic acid (TFAA), and the acute toxicity of fluroxene is related to this pathway. The ratio of metabolites (TFAA to TFE) excreted by different species exposed to fluroxene varies; whenever highly toxic TFE is the major metabolite, fluroxene toxicity is high (rodents, dogs, phenobarbital pretreated monkeys), whenever TFAA is the major metabolite (man, monkey) fluroxene is not toxic. Toxicity in different species also correlates with the extent of glutathione depletion following fluroxene exposure.

Fluroxene metabolism and toxicity are modified by drugs metabolized by or affecting the activity of the microsomal cytochrome P-450-system or enzymes involved in ethanol metabolism. The susceptibility of fluroxene to two enzymatic systems which are modified by environmental and genetic factors may explain the large differences in fluroxene toxicity to various species.

The fate of one-third of fluroxene administered to man remains unknown.

Trifluoroethyl vinyl ether, fluroxene, was introduced in 1953 as the first fluorinated inhalation anesthetic (*1*), under the trade name Fluoromar. It is slightly flammable, but was considered to be an anesthetic agent of high clinical safety. In 1975, after enflurane had appeared on the market, the manufacture of fluroxene was discontinued. The 22-year history of fluroxene (Table 1) is an interesting one. During this period, fluroxene was found to be toxic to surgical patients in only four cases, and in these four cases other medications were involved (*9, 10, 13, 14*). As good as this record is, fluroxene would never have been approved for use in humans if it had to undergo present-day testing standards, because it happens to be highly toxic to experimental animals (recognized in 1972) (*7*) and is extensively metabolized to highly toxic metabolites (discovered in 1967) (*5*).

Metabolites of Fluroxene

When labeled fluroxene was injected into mice and dogs, trifluoroacetic acid (TFAA) and trifluoroethanol—free (TFE) and conjugated with glucuronide (TFEG)—were found in urine of injected animals, and if the vinyl moiety was labeled, $^{14}CO_2$ was found in exhaled air. According to urinary excretion, TFE and TFEG are the major metabolites of fluroxene in mice and dog, respectively. The TFEE/TFAA excretion ratio is approximately 2:1. TFAA, TFE, and TFEG were also identified in urine of anesthetized rhesus monkeys (*16*) and man (*15*). However, TFAA was the major metabolite, and TFE and TFEG were excreted only in traces.

In our laboratory, we administered fluroxene to nine informed surgical patients (*15*). The anesthesia was administered in a closed-circuit absorption system by injection of liquid fluroxene in the circuit by a Harvard infusion pump, the speed controlled by a Data-Trak curve follower programmed to maintain a constant alveolar concentration around 3.3%. This system allowed us to measure uptake by the amount of fluroxene injected into the circuit, and/or by measurement of the uptake rate from difference of fluroxene concentrations in inhaled and exhaled air and minute ventilation. The anesthesia lasted 77 to 210 min. The administered dose was 32 g (SD = 10), corresponding to an average dose of 0.43 g/kg (SD = 0.14). Fluroxene concentrations in exhaled

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Table 1. History of fluroxene.

Year	Observation	Reference
1953	Beginning of clinical anesthesia	(*1*)
1960	Hepatotoxicity in fasting rats	(*2*)
1965	*In vitro* metabolism	(*3*)
1966	Toxicity in dog	(*4*)
1967	First report on biotransformation in mice and dog metabolites: trifluoroethanol glucuronide, free trifluoroethanol, trifluoroacetic acid, CO_2	(*5*)
1970	Biotransformation of labeled fluroxene in two volunteers	(*6*)
1972	Postanesthetic death in experimental animals	(*7, 8*)
	Postanesthetic toxicity in man (2 cases, one fatal)	(*9, 10*)
1973	Modification of fluroxene toxicity by other drugs	(*11, 12*)
	Postanesthetic toxicity in man (2 cases, one fatal)	(*13, 14*)
1974	Biotransformation of fluroxene in surgical patients	(*15*)
1975	Withdrawn from the market	
	Exhalation of trifluoroethanol in monkeys	(*16*)
	Destruction of cytochrome P-450	(*17*)
1976	Sex differences in anesthetic toxicity	(*18*)
1977	Glutathione depletion	(*19, 20*)

air and minute ventilation were measured for 5 days, and it was calculated that 58% of fluroxene was exhaled unaltered. Total amount of TFAA excreted during and following anesthesia was equivalent to 10% of the administered dose of fluroxene, and the quantity of TFE and TFEG excreted was equivalent to less than 1% of the administered dose. The fate of 31% of fluroxene is unknown.

In another study (*16*), we anesthetized rhesus monkeys for 4 hr with fluroxene. During anesthesia the concentrations of TFAA in urine and in blood were 27.1 mg-% and 2.57 mg-%, respectively, and TFE concentrations in urine were 4.11 mg-%, and in blood less than 3 mg-%. Traces of TFE were found in exhaled air. If the animals were pretreated with phenobarbital, the concentrations in urine as well as in blood increased (TFAA, 49.7 mg-% and 4.22 mg-% respectively; TFEE, 10.9 mg-% and 17 mg-%, respectively). A significant amount of TFE was exhaled during anesthesia. The TFE concentration in exhaled air increased with exposure duration. Enhanced fluroxene metabolism was previously observed in dogs and mice pretreated with phenobarbital, 3-methylcholanthrene, or 3,4-benzyprene (*5*).

When the monkeys were sacrificed at the end of exposure, a nonvolatile fluorinated compound was found in all tissues. In pretreated animals the fluorine concentrations were significantly greater than in nonpretreated animals. The same observation was made in similar experiments in mice (*19, 20*). After exposure, the amount of fluorinated compound in all tissues of pretreated mice (except in the liver) kept increasing up to 10 hr, the maximal survival time (Fig. 1).

No fluoride ion was released by fluroxene

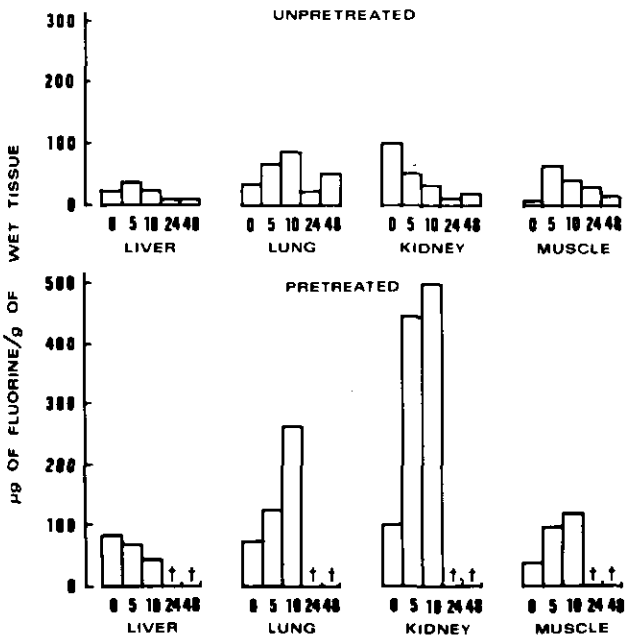

FIGURE 1. Concentrations of nonvolatile fluorine in mouse tissues following 2 hr exposure to 0.8% fluroxene. The bar indicates the fluorine concentration in a pool of organs of two animals. The numbers under the bars indicate the time intervals (in hours) between end of exposure and sacrifice of animals. The dagger (†) indicates that all animals were dead.

metabolism since none was found in tissues or excreta of any studied species. There is also indirect evidence that the trifluoroethyl moiety is not defluorinated: metabolites of fluroxene were administered to mice, and no changes in citric acid concentrations in tissues were observed, which means that no monofluoro derivatives were formed, since otherwise the Krebs cycle would be blocked (*21*).

Toxicity of Fluroxene

Fluroxene has been found to be highly toxic to mice (*7, 12, 18–20, 22*), dogs, cats, and rabbits (*8, 23*), but not to man (*1, 8, 15*). Terminal events in dogs, cats and rabbits include diarrhea, convulsion, and reduction in clotting factors. Autopsy revealed hemorrhagic gastrointestinal mucose, lung congestion, and haemorrhages. Histologic examination of tissues has shown inflammatory and vascular involvement. Mice anesthetized with fluroxene may recover from light anesthesia, mortality being related to concentration used, duration of anesthesia (*7*), and sex (*18*). In mice fluroxene is more toxic in males than in females. In our experiments, mice anesthetized for 2 hr with 3% fluroxene recovered rapidly from anesthesia, within 2 hr became unconscious again, and died within 5 hr in convulsions. In gross examination, no apparent changes in organs were observed. Histological examination showed hepatocellular changes, predominantly centrolobular vacuolization, moderate fatty metamorphosis, passive hyperemia, and sinusoidal congestion (*19*). Similar hepatotoxicity was observed in *rats*, but the fluroxene anesthesia was not fatal (*11*). There were no toxic side effects of fluroxene in man (*1, 8, 15*) or rhesus monkey (*16*), with the exception of the four surgical patients mentioned before. Postoperatively these latter patients suffered from nausea, vomiting, fever, jaundice, and hemorrhagic disorder, and histologic examination showed centrolobular necrosis and fatty degeneration of the liver. In all four cases, previous extensive drug exposure (such as phenobarbital, diphenylhydantoin) was involved (*9, 10, 13, 14*).

Modification of Fluroxene Toxicity by Other Drugs

Fluroxene anesthesia was fatal in all experimental animals pretreated with phenobarbital (*7, 11, 16*). The most remarkable observation is that monkeys (*16*) and rats (*11*) tolerated fluroxene anesthesia without any toxic side effect, but none of the animals pretreated with phenobarbital survived a four hour anesthesia. The target organ in rats is the liver (histological examination revealed massive liver necrosis), and in monkeys the lung (pink-staining edema fluid). Hepatotoxicity in pretreated monkey was indicated only by increasing SGOT and SGPT in blood.

In contrast, post anesthetic death was reduced in mice by pretreatment with microsomal enzyme inhibitors, such as carbon tetrachloride (*12*) or 2-allyl-2-isopropylacetamide (*24*).

Administration of sex hormones reverses the fluroxene toxicity in male and female mice (*18*): Estradiol administration reduces toxicity in males, while testosterone enhances toxicity in females.

Studies in mice (*12*) and dogs (*23*) demonstrated that postanesthetic mortality may be reduced or abolished by ethanol, and by drugs which affect ethanol metabolism such as pyrazole, aminotriazol and disulfiram.

Death resulting from fluroxene anesthesia may be delayed for 6 hr if the mice are simultaneously treated with L-cysteine (*19*).

Toxicity of Fluroxene Metabolites

The toxicity of fluroxene metabolites is much greater than toxicity of fluroxene itself, and is comparable to toxicity of chlorinated derivatives which are metabolites of trichloroethylene (Table 2). TFE toxicity is reduced if administered IV (*25*). The lethal effect is usually delayed 18-32 hr after administration. We administered fluroxene intraperitoneally in olive oil to white Wistar mice. The LD_{50} was 6.9 g/kg. This means that TFE is 16 times more toxic than fluroxene and 6 times more toxic than TFAA. Because of its high toxicity, TFE was studied intensively, mainly in mice (*21, 22, 25, 26*) and dogs (*23*). It was concluded that TFE produces death in a manner indistinguishable from death resulting from fluroxene anesthesia, and that like fluroxene, TFE toxicity is depressed by ethanol administration (*23, 25*) and accompanied by an increase of TFEG and decrease of TFAA in urine (*25*).

Table 2. LD_{50} of metabolites administered IP in mice.

	LD_{50}, g/kg	
	Fluroxene X = F	Trichloroethylene X = Cl
CX_3CH_2OH	0.42	0.2
$CX_3CH(OH)_2$	0.65	—
CX_3COOH	2.6	2.4
CH_2XCOOH	0.0005	0.1

It was demonstrated in mice that, contrary to fluroxene, TFE toxicity is reduced or not affected at all by phenobarbital pretreatment, but like fluroxene, the sex differences in toxicity favor females (*18*). Also, toxicity is diminished by administration of ethanol and drugs interferring with ethanol metabolism (*25*) (carbon tetrachloride, pyrazol, hydrazide, disulfiram), by administration of L-cysteine (*27*), and by halothane pretreatment (*27*).

Modification of trifluoroacetaldehyde toxicity by drugs was qualitatively similar to TFE, but usually more profound (*21*). The effects of drugs on toxicity

of fluroxene and its metabolites are summarized in Table 3.

Table 3. Modification of toxicity of fluroxene and its metabolites by other drugs.

Drug	Species	Toxicity		
		Fluroxene	TFE	TFAald
Phenobarbital	Mice	↑	0	0
	Cats, monkeys, dogs	↑		
CCl_4	Mice	↓	↓	↓
2-Allyl-2-isopropyl-acetamide	Rats	↓		
Ethanol	Mice, dogs	↓	↓	↓
Pyrozole	Mice	↓	↓	↓
Aminotriazol	Mice	↓	↓	
Disulfiram	Mice	↓	↓	↓
Allopurinol	Mice		↑	↑
L-Cysteine	Mice	↓	↓	
Hydrazide	Mice		↓	↓
Estradiol	Mice (male)	↓	↓	
Testosterone	Mice (female)	↑	↑	
Halothane	Mice		↓	

Effect of Fluroxene and its Metabolites on Cellular Constituents

Massive platelet and fibrinogen depletion was observed in dogs (*8*) and in phenobarbital-pretreated monkeys (*16*) during and following fluroxene anesthesia. This has not been observed in man or in nonpretreated monkeys.

During and following fluroxene exposure, glutathione was profoundly depleted in blood and tissues of mice and rats (the depletion in mice was more extensive) and slightly depleted in blood of anesthetized monkeys (*19*). No depletion in human blood was observed (Our unpublished observation; tissues of monkeys and man were not examined). Phenobarbital pretreatment enhanced glutathione depletion in rodents anesthetized with fluroxene (*19*). Glutathione depletion was also observed in liver and erythrocytes of mice exposed to TFE and trifluoroacetaldehyde, but no depletion was observed in experiments *in vitro* (*27*).

In liver of mice anesthetized with fluroxene, ATP was depleted, depletion being inversely related to the amount of TFE in urine (*22*). TFE or TFAA administered to guinea pigs also lowered ATP in liver but did not affect ATP in mouse livers (*26*). Fluroxene metabolites also decrease NADPH concentration in mouse livers and increase concentration of NADP, without changing total concentration of nucleotides (*27*). TFE and trifluoroacetaldehyde also inhibit D-glucose-6-phosphate dehydrogenase and significantly decrease the lactate-pyruvate pool in mouse livers. This data leads to the conclusion that fluroxene metabolites cause inhibition of anaerobic glycolysis (*27*).

Mechanisms of Toxicity and Metabolic Pathways of Fluroxene

The metabolic pathway of fluroxene appears to be very simple. After splitting of the ether linkage by microsomal enzymes, the vinyl moiety is oxidized to carbon dioxide and the trifluoroethyl moiety is oxidized to TFE. TFE either undergoes detoxification by glucuronization or is oxidized to TFAA by enzymes involved in ethanol metabolism, the unstable trifluoroacetaldehyde being postulated intermediate. Only a few observations remain to be added in order to complete the picture of metabolic pathways as they are illustrated in Figure 2.

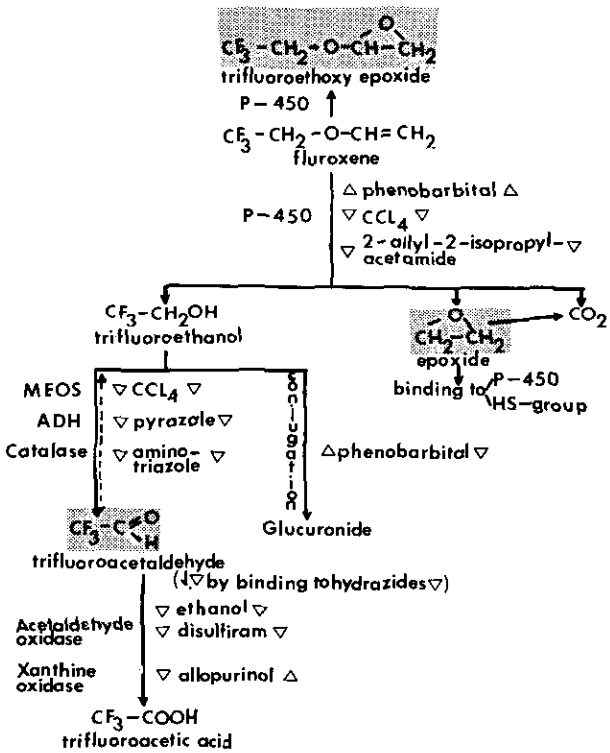

FIGURE 2. Metabolic pathways of fluroxene. The metabolites in shaded areas are hypothetical. The lines represent the individual steps in metabolism, to the left are the enzymes involved, to the right are modifiers of enzyme activity. Triangles in front of modifiers mean stimulation (Δ) or inhibition (∇) of enzymatic activity, triangles behind the modifiers indicate increase (Δ) or decrease (∇) of fluroxene toxicity in mice. P-450 is microsomal cytochrome P-450 system, MEOS is microsomal ethanol oxidizing system, ADH is alcohol dehydrogenase.

Fluroxene metabolites were administered to rodents and male volunteers. It was observed that TFAA is not further metabolized, and is quantitatively excreted in urine (D. A. Holaday, and R. Cunnah, personal communication, experiments in humans). Furthermore, trifluoroacetaldehyde is oxidized to TFAA and reduced to TFE (*21*) (experiments in rats), and TFE is oxidized to TFAA (*25*) (experiments in mice).

The fate of the vinyl moiety of fluroxene was recently studied in rats (*17, 24, 28*). It was shown in microsomal enzyme preparations that fluroxene and its saturated homolog, 2,2,2-trifluoroethyl ethyl ether, bind to cytochrome P-450 but not to cytochrome P-448. In contrast to 2,2,2-trifluoroethyl ethyl ether, fluroxene destroys cytochrome P-450 by degradation of its heme moiety. Since the destruction was prevented by preincubation of microsomes with carbon monoxide, it was postulated that cytochrome P-450 is destroyed during the metabolism of the vinyl moiety. We speculate that this destruction could be caused by reactive epoxide formed from the vinyl group, either after splitting of the ether linkage or by epoxidation of the fluroxene molecule. If trifluoroethoxy epoxide is formed and bound to cellular constituents, this could also explain the large fraction of fluorine unrecovered in patients anesthetized with fluroxene. (Another explanation could be that trifluorometabolites are bound to poly-L-lysine or proteins, as suggested for halothane) (*29, 30*).

The toxic effect of fluroxene and 2,2,2-trifluoroethyl ethyl ether was attributed to the trifluoroethyl moiety, because the toxicity of both ethers is comparable (*24*). Since toxicity of fluroxene is reduced by all inhibitors of enzymes catalyzing alcohol oxidation or by drugs binding trifluoroacetaldehyde, and since TFAA is not toxic, the toxicity-limiting factor in fluroxene metabolism is trifluoroacetaldehyde, or its oxidation to TFAA. Trifluoroacetaldehyde is also presumably formed from halothane, which is not fatal to experimental animals. It is believed that trifluoroacetaldehyde was not proved as a halothane metabolite, for the reason that it is instantly oxidized to TFAA. However, it is unlikely that the trifluoroacetaldehyde generated from halothane and fluroxene would be detoxified differently.

The genetic and environmental effects of enzyme synthesis and activity are today recognized and differences in toxicity and metabolism of a variety of drugs in different species have been reported (*31, 32*). In most cases, Old World monkeys showed the closest resemblance to humans (*32*). Fluroxene is no exception in this respect. [Surprising was the dramatic effect of phenobarbital pretreatment on toxicity and metabolism of fluroxene in rhesus monkey (*16*). However, similar information on other drugs is not available]. Species differences and the variety of drugs modifying fluroxene toxicity and metabolism are unusually large. This may be related to the involvement in fluroxene metabolism of numerous enzymes (Fig. 2) which are enhanced or inhibited differently by genetic and environmental factors. For example, it might be expected that in man, who is exposed to alcoholic beverages, the alcohol-metabolizing enzymes will react differently than in experimental animals. Also, induction of microsomal enzymes which catalyze the initial step of fluroxene metabolism is not necessarily accompanied by increase of activity of the other enzymes. This could upset the balance in normal metabolic pathways of fluroxene and might increase the toxicity.

Finally, with respect to mechanisms of fluroxene toxicity following anesthesia, the large dose administered has to be kept in mind. For example, following clinical anesthesia, approximately 15 g fluroxene undergoes metabolism. This requires a large amount of oxygen and energy, the transfer of which employs numerous enzymes and coenzymes, participating in normal metabolism. Thus sharing of these resources might result in slowing important vital processes in the body. The inhibition of anaerobic glycolysis (*27*) and increase of fluroxene toxicity under hypoxic conditions (*2*) already have been suggested.

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REFERENCES

1. Krantz, J. C., et al. Anaesthesia. XL. The anesthetic action of trifluoroethyl vinyl ether. J. Pharmacol. Exptl. Therap. 108: 488 (1953).
2. Morris, E. L. Comparison studies of hepatic function following anesthesia with the halogenated agents. Anesthesiology 21: 109 (1960).
3. Van Dyke, R. A., and Chenoweth, M. B. Metabolism of volatile anesthetics. Anesthesiology 26: 348 (1965).
4. Bagwell, E. E., et al. Blood levels and cardiovascular dynamics during fluroxene anesthesia in dogs. Can. Anaesth. Soc. J. 13: 378 (1966).
5. Blake, D. A., et al. Anesthesia. LXXIV. Biotransformation of fluroxene-I. Metabolism in mice and dogs *in vivo*. Biochem. Pharmacol. 16: 1237 (1967).
6. Blake, D. A., and Cascorbi, H. F. A note on the biotransformation of fluroxene in two volunteers. Anesthesiology 32: 560 (1970).
7. Cascorbi, H. F., and Singh-Amaranath, A. V. Fluroxene toxicity in mice. Anesthesiology 37: 480 (1972).
8. Johnston, R. R., et al. The toxicity of fluroxene in animals and man. Anesthesiology 38: 313 (1973).
9. Reynolds, E. S., Brown, B. R., and Vandam, L. D. Massive hepatic necrosis after fluroxene anesthesia—a case of drug

interaction? New Engl. J. Med. 286: 530 (1972).
10. Harris, J. A., and Cromwell, T. H. Jaundice following fluroxene anesthesia. Anesthesiology 37: 462 (1972).
11. Harrison, G. G., and Smith, J. S. Massive lethal hepatic necrosis in rats anesthetized with fluroxene, after microsomal enzyme induction. Anesthesiology 39: 619 (1973).
12. Cascorbi, H. F., and Singh-Amaranath, A. V. Modification of fluroxene toxicity. Anesthesiology 38: 454 (1973).
13. Wollman, S. B., and Surks, S. N. Case history number 75: hepatic damage after fluroxene anesthesia. Anesth. Analg. 52: 942 (1973).
14. Tucker, W. K., et al. Hepatorenal toxicity following fluroxene anesthesia. Anesthesiology 39: 104 (1973).
15. Gion, H., et al. Biotransformation of fluroxene in man. Anesthesiology 40: 553 (1974).
16. Munson, E. S., et al. Fluroxene toxicity induced by phenobarbital. Clin. Pharmacol. Therap. 18: 687 (1975).
17. Ivanetich, K. M., et al. Fluroxene (2,2,2-trifluoroethyl vinyl ether) mediated destruction of cytochrome P-450 *in vitro*. Biochem. Pharmacol. 24: 1933 (1975).
18. Cascorbi, H. F., Gonsky, B. H., and Redford, J. F. Sex differences in anaesthetic toxicity: fluoroxene and trifluoroethanol in mice. Brit. J. Anesth. 48: 399 (1976).
19. Zumbiel, M. A., et al. Glutathione depletion following inhalation anesthesia. Anesthesiology, in press.
20. Fiserova-Bergerova, V. Species differences in metabolism and toxicity of fluroxene. Xenobiotica 7: 113 (1977).
21. Airaksinen, M. M., Rosenberg, P. H. and Tammisto, T. A possible mechanism of toxicity of trifluoroethanol and other halothane metabolites. Acta Pharmacol. Toxicol. 28: 299 (1970).
22. Yoshimura, N., Fiserova-Bergerova, V., and Holaday, D. A. Relationship between trifluoroethanol and fluroxene toxicity in mice. Anesthesiology 23: 835 (1974).
23. Johnston, R. R., et al. Fluroxene toxicity in dogs: possible mechanisms. Anesth. Analg. 53: 998 (1974).
24. Ivantich, K. M., et al. The role of cytochrome P-450 in the toxicity of fluroxene (2,2,2,-trifluoroethyl vinyl ether) anaesthesia *in vivo*. Biochem. Pharmacol. 25: 773 (1976).
25. Blake, D. A., et al. Animal toxicity of 2,2,2-trifluoroethanol. Toxicol. Appl. Pharmacol. 15: 83 (1969).
26. Airaksinen, M. M., and Tammisto, T. Toxic actions of the metabolites of halothane: LD_{50} and some metabolic effects of trifluoroethanol and trifluoroacetic acid in mice and guinea pigs. Ann. Med. Exp. Fenn. 46: 242 (1968).
27. Rosenberg, P. H. Decrease in reduced glutathione and NADPH and inhibition of glucose-6-phosphate dehydrogenase activity caused by metabolites of fluroxene and halothane. Ann. Med. Exp. Fenn. 49: 84 (1971).
28. Ivanetich, K. M., et al. The interaction of hepatic microsomal cytochrome P-450 with fluroxene (2,2,2-trifluroethyl vinyl ether) *in vitro*. Biochem. Pharmacol. 25: 779 (1976).
29. Rosenberg, P. H., and Wahlstrom, T. Trifluoroacetic acid and some possible intermediate metabolites of halothane as haptens. Anesthesiology 38: 224 (1973).
30. Van Dyke, R. A., and Wood, C. L. *In vitro* studies on irreversible binding of halothane metabolite to microsomes. Drug Metab. Dispos. 3: 51 (1975).
31. Williams, R. T. Species variation in drug biotransformation. In: Fundamentals of Drugs Metabolism and Drug Disposition. B. N. LaDu, H. G. Mandel, and E. L. Way, Eds., Williams & Wilkins, Baltimore, Md., 1972, pp. 187-205.
32. Smith, R. L., and Caldwell, J. Drug metabolism in nonhuman primates. In: Drug Metabolism from Microbe to Man. D. V. Parke and R. L. Smith, Eds., Taylor & Francis, London, 1977, pp. 331-356.