Environmental Health Perspectives
Vol. 15, pp. 111-119, 1976

Methoxyflurane Nephropathy

by Richard I. Mazze*

Investigations of methoxyflurane-induced nephrotoxicity in man have been extensively aided by the use of an animal model. To be of value the animal model must share similar metabolic pathways with man and have the same clinical manifestations of the disease process. The Fischer 344 rat appears to meet these criteria.

The predominant factors in the production of methoxyflurane nephrotoxicity appear to be high methoxyflurane dosage and serum inorganic fluoride concentration. It is likely that secondary factors include: (1) a high rate of methoxyflurane metabolism and sepsitivity of the kidney to inorganic fluoride toxicity; (2) concurrent treatment with other nephrotoxic drugs; (3) preexisting renal disease; (4) surgery of the urogenital tract, aorta, or renal vasculative; (5) repeat administration of methoxyflurane due to accumulation of inorganic fluoride and, perhaps, methoxyflurane induction of its own metabolism: and (6) concurrent treatment with enzyme-inducing drugs such as phenobarbital.

Specific anesthetic-induced nephrotoxicity was first reported by Crandell et al. (*1*) in 13 of 41 patients anesthetized with methoxyflurane (MOF) for abdominal surgery. They noted polyuria with a negative fluid balance, elevation of serum sodium, serum osmolality, and blood urea nitrogen, and fixed urine osmolality close to that of serum. Patients were unable to concentrate urine despite fluid deprivation and vasopressin administration, suggesting that the difficulty was of renal origin and not due to antidiuretic hormone (ADH) deficiency. Impairment lasted from 10 to 20 days in most patients, but in three, abnormalities persisted for longer than one year. In 1971, Mazze et al. (*2,3*) reported a controlled, randomized, prospective clinical evaluation of renal function following methoxyflurane anesthesia. Abnormalities were found in all cases; patients exhibited polyuria unresponsive to ADH administration, marked weight loss, delayed return of normal urine concentrating ability, hypernatraemia, serum hyperosmolality, elevated BUN and serum creatinine, increased serum uric acid and a decrease in uric acid clearance. Nephrotoxicity was not permanent in any of these patients.

* Department of Anesthesia, Stanford University School of Medicine, Stanford, California 94305 and Veterans Administration Hospital, Palo Alto, California 94304.

Initial Evidence Relating Methoxyflurane Metabolism to Nephrotoxicity

It had been suggested by Taves et al. (*4*) that a metabolite of methoxyflurane, inorganic fluoride, was related to its nephrotoxicity. They reported increased concentrations of inorganic fluoride in the serum and urine of a patient who had renal dysfunction following methoxyflurane anesthesia. About the same time, Frascino et al (*5*) noted oxalic acid crystals in renal biopsy specimens and increased urinary oxalic acid excretion in several patients with renal insufficiency following methoxyflurane anesthesia, Mazze et al. (*3*) subsequently reported increased concentrations of inorganic fluoride in all patients anesthetized with methoxyflurane with the highest levels found in those patients with the greatest impairment of renal function (Fig. 1). Similarly, patients with clinically evident nephrotoxicity had mean peak oxalic acid excretion significantly greater than patients with laboratory abnormalities only. To explain the increased excretion of inorganic fluoride and oxalic acid following methoxyflurane anesthesia, two complementary metabolic routes for biotransformation of methoxyflurane were proposed (Fig. 2). Both inorganic fluoride and oxalic acid have nephrotoxic potential with the former the most likely cause of polyuria.

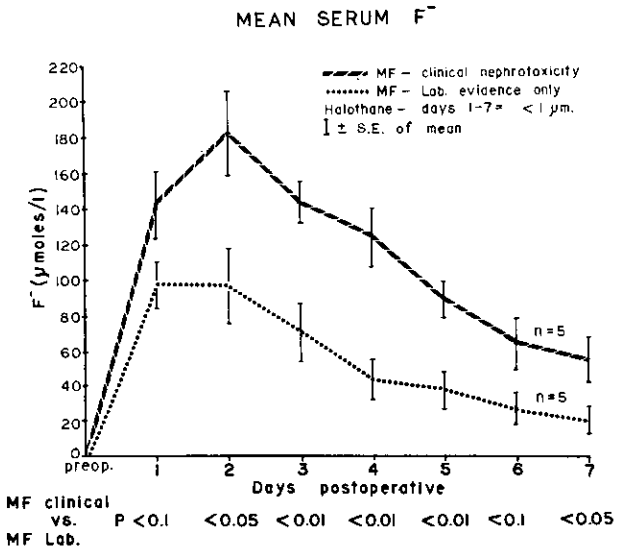

FIGURE 1. Mean daily serum inorganic fluoride concentrations. Patients anesthetized with methoxyflurane were divided into two subgroups, those with laboratory abnormalities in renal function and those with laboratory abnormalities as well as clinically evident renal dysfunction (see text). Preoperative inorganic fluoride concentration was approximately 1 μm/1. in all patients, with no change noted following halothane anesthesia. F^- (μmoles) = inorganic fluoride, μmoles/l. From Mazze et al. (*3*), reproduced with permission of Anesthesiology.

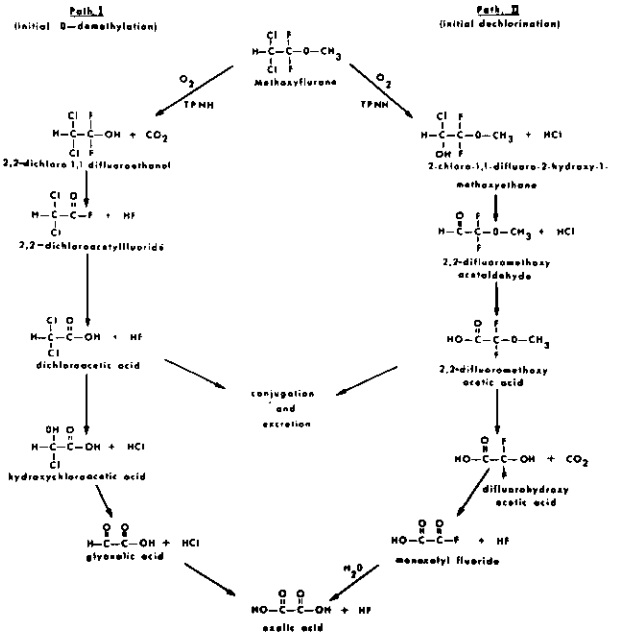

FIGURE 2. Metabolic pathways for the metabolism of methoxyflurane. In pathway I, methoxyflurane is O-demethylated in the liver, producing 2,2-dichloroacetyl fluoride and a molecule of hydrogen fluoride. Acetyl fluoride is further hydrolyzed to dichloracetic acid, liberating another molecule of hydrogen fluoride. A portion of the dichloroacetyl acid is excreted in the urine while a second part is oxidatively dechlorinated to glyoxalic acid. The latter is enzymatically oxidized to oxalic acid. In pathway II, Methoxyflurane is enzymatically dechlorinated to 2,2-difluoromethoxyacetic acid. A portion is excreted in the urine and a second part O-demethylated in the liver. Subsequent dehydrofluorination and hydrolysis result in oxalic acid formation. From Mazze et al. (*3*), reproduced with persmission of Anesthesiology.

Evidence of Methoxyflurane Nephrotoxicity in an Animal Model

A direct cause-effect relationship of methoxyflurane administration and renal dysfunction was still not conclusively established, in part due to the absence of nephrotoxicity in animal experiments (*6*). After studying the renal effects of methoxyflurane in five rat strains, Mazze et al. (*7*) found that Fischer 344 and Buffalo rats metabolized methoxyflurane to a greater extent that the other three strains as evidenced by higher serum and urine inorganic fluoride activity (Fig. 3). However, only Fischer 344 rats developed functional (Fig. 4) and pathological evidence of a renal lesion (*7*). Injection of inorganic fluoride (NaF) produced a much greater degree of renal insufficiency in Fischer 344 rats than in Buffalo rats. It was concluded that there were strain differences among rats in the rate of metabolism of methoxyflurane to inorganic fluoride and in its nephrotoxic effects. A high rate of methoxyflurane metabolism and increased susceptibility to the nephrotoxic effects of inorganic fluoride resulted in polyuric renal insufficiency in Fischer 344 rats. Other studies in Fischer 344 rats showed that renal functional and morphological changes (Fig. 5) were proportional to the dose of methoxyflurane; polyuria, whether resulting from methoxyflurane anesthesia or from direct injection of inorganic fluoride, was ADH-resistant (Figs. 6 and 7). (*7-9*). Subsequently, it was conclusively established (*10*) that metabolism of methoxyflurane was related to nephrotoxicity; induction of the mixed function oxidase system in Fischer 344 rats by phenobarbital treatment increased defluorination and nephrotoxicity (Fig. 8), whereas enzyme inhibition with SKF 525A resulted in decreased defluorination and an attenuated renal lesion (Fig. 9).

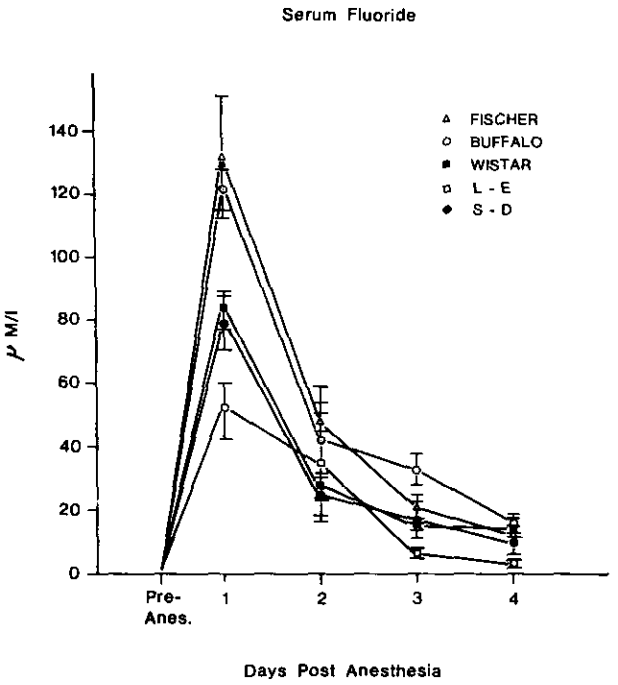

FIGURE 3. Daily serum inorganic fluoride activity (mean ± S.E.) before anesthesia and after 3 hr of 0.5% methoxyflurane anesthesia. Fischer 344 and Bufaalo strain rats had significantly greater increases than rats of the other three strains; $n = 6$: (L-E) Long-Evans rats; (S-D) Sprague-Dawley rats. From Mazze et al. (7), reproduced with permission from the Journal of Pharmacology and Experimental Therapeutics.

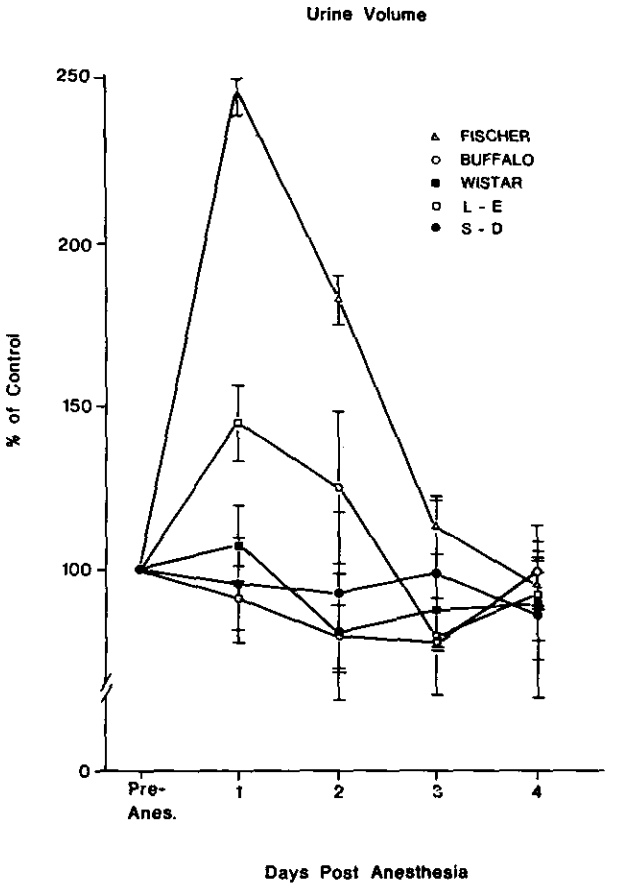

FIGURE 4. Daily 24-hr urine volume (mean ± S.E.) before and after 3 hr of 0.5% methoxyflurane anesthesia. Significant postanesthetic increases occurred only in Fischer 344 rats. From Mazze et al. (7), reproduced with permission from the Journal of Pharmacology and Experimental Therapeutics.

The Fischer 344 rat is an excellent model for studying methoxyflurane nephrotoxicity in man because (1) both metabolize methoxyflurane to F^- and oxalic acid; (2) both develop polyuria after MOF which is proportional to serum inorganic F^- level and is pitressin-resistant; (3) both develop renal function abnormalities (hypernatremia, elevated BUN, polyuria) after methoxyflurane; (4) the degree of nephrotoxicity is proportional to the dose of methoxyflurane.

Etiology of Nephrotoxicity

Studies in both man (*2,11*) and animals (*7-9*) have shown that the degree of nephrotoxicity following methoxyflurane anesthesia is proportional to the amount of metabolite resulting from biotransformation of the drug. The following evidence points to inorganic fluoride rather than oxalic acid as the primary nephrotoxic metabolite. Sodium fluoride injection in Fischer 344 rats resulted in dose-related polyuric renal insufficiency and pathological abnormalities similar to those found after methoxyflurane anesthesia (*8*). Injection of oxalic acid did not cause these changes (Fig. 10) (*10*). In man, acute oxalic acid intoxication results in classical anuric renal failure; chronic elevation of oxalic acid excretion is associated with kidney stone formation (*12,13*). Also there is a known potent inhibitory effect of inorganic fluoride on many enzyme systems, including those thought to be involved in sodium transport (Na^+ + K^+ ATPase) and ADH action (*14*).

Thus, it is unlikely that oxalic acid is of importance in the production of the acute lesion of methoxyflurane nephrotoxicity. However, it is possible that oxalic acid is a factor in the production of the chronic lesion seen in patients with

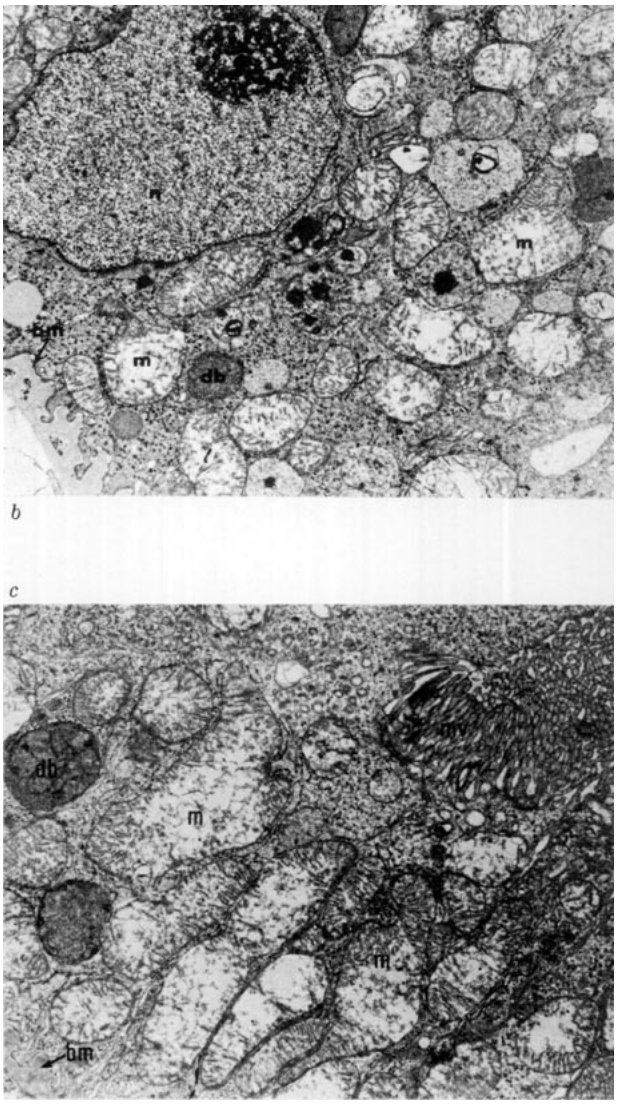

FIGURE 5. *Electron micrographs of proximal convoluted* tubular cells from the rat. (*a*) In the unanesthetized control rat, note the predominance of normally long and thin mitochondria (m). Also identified are nucleus (n), basement membrane (bm), and a few scattered dense bodies (db). (*b*) In a cell obtained 24 hr following 0.5% methoxyflurane anesthesia for 180 min, mitochondria are swollen and there is some indentation of the nucleus. (*c*) In the proximal convoluted tubular cell obtained 24 hr following 0.75% methoxyflurane anesthesia for 360 min, there is pronounced mitochondrial swelling, degeneration and rupture. (mv denotes luminal *microvilli). Electron photomicrographs by courtesy of Dr.* Jon C. Kosek; magnification: (*a*) 3200×; (*b*) 2580×; (*c*) 2900×.

permanent renal impairment. Although the number of oxalate crystals observed in kidney sections obtained at renal biopsy or autopsy from patients with methoxyflurane induced renal failure have been insufficient to cause significant obstruction to urine flow (*4,15,16*), they could act as a nidus for imflammation resulting in scarring of the renal tubules and interstitium.

Site and Mechanism of the Acute Renal Lesion

Because polyuria following methoxyflurane anesthesia is ADH-resistant, it was originally postulated (*3*) that inorganic fluoride caused nephrotoxicity by interfering with the action of ADH in the distal convoluted tubule and collecting duct. However, subsequent animal studies (*7-9*) showed that the major pathological lesions were in the proximal convoluted tubule with damage ranging from mitochondrial swelling to necrosis of epithelial cells. Few abnormalities were noted in the distal nephron. In biopsy and autopsy specimens, proximal tubular dilatation, focal necrosis and oxalate crystal deposition also have been described (*4,15,16*). Thus it is likely that inorganic fluoride acts by a number of mechanisms. (1) Interference with iso-osmotic reabsorption of proximal tubular fluid would place an excessive load on the more distal nephron, particularly the ascending limb of the

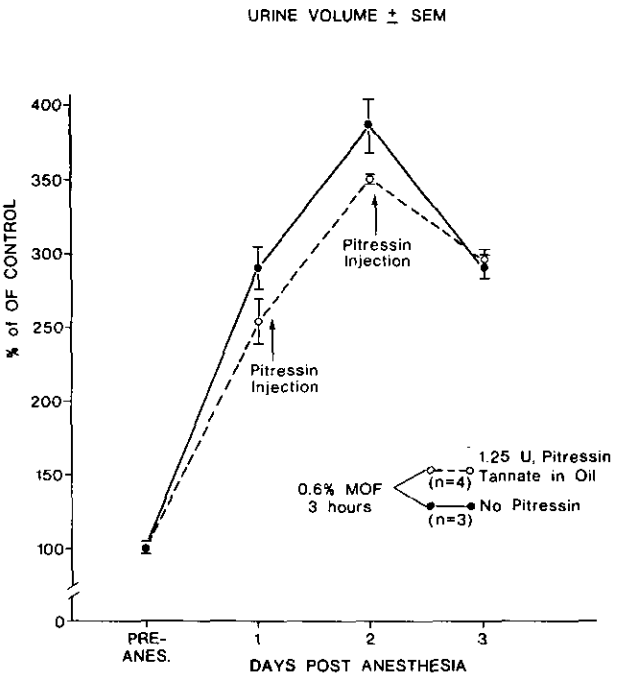

FIGURE 6. Pitressin (ADH) administration had no effect on the increase in 24 hr urine volume following administration of 0.6% methoxyflurane for 3 hr. SEM=standard error of mean. From Mazze et al. (*8*), reproduced with permission of Anesthesiology.

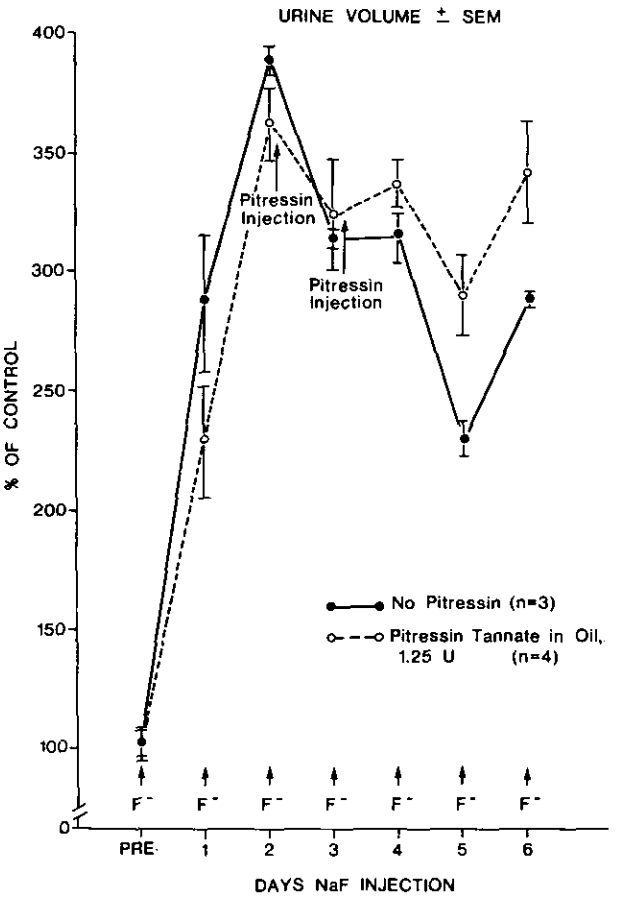

FIGURE 7. Daily injection of 1.0 ml of 0.1*M* NaF produced a sustained increase in 24 hr urine volume which was not affected by pitressin (ADH) administration. F^- denotes inorganic fluoride injection. From Mazze et al. (*8*), reproduced with permission of Anesthesiology.

loop of Henle, leading to reduced renal medullary hyperosmolality and decreased reabsorption of water from the collecting ducts. Large volumes of dilute urine could then be formed in spite of the presence of ADH. (2) Inhibition of enzyme systems necessary for sodium (or chloride) pumping in the ascending limb of the loop of Henle would result in decreased renal medullary hyperosmolality and polyuric renal insufficiency. (3) Damage to the collecting ducts would make them insensitive to ADH. The morphological components of the lesions suggested in (2) and (3) may be to subtle to be detected by available histological techniques.

Evidence of Dose-Related Nephrotoxicity in Man

Although animal studies appeared conclusive, there was still doubt as to the nephrotoxicity of methoxyflurane in man (*17*). In part this was due to the fact that in the initial study of surgical patients by Mazze et al. (*2,3*), methoxyflurane was not administered in the usual clinical manner, i.e., with anesthetic adjuvant drugs. Also, methoxyflurane dosage was not precisely defined in that study. This left unanswered the question of the relationship of methoxyflurane dosage and renal dysfunction. Additionally, it was possible that anesthetic adjuvants such as thiopental and nitrous oxide might have protected the kidney against nephrotoxicity. Therefore a randomized, prospective study was undertaken in which methoxyflurane was administered with anesthetic adjuvant drugs; methoxyflurane dosage in each patient was precisely documented and the renal functional changes that occurred at each dosage were carefully determined (*10*). The results of that study conclusively established that methoxyflurane was a nephrotoxin in man. A positive correlation was observed among the

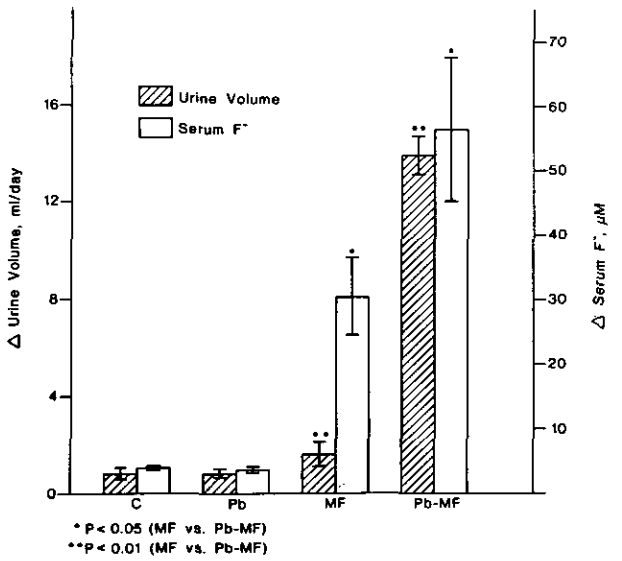

FIGURE 8. Changes in serum inorganic fluoride (F−) concentration and urine volume for the first 2 days after anesthesia (day 15): (C) control group I; (Pb) phenobarbital, 25 mg/kg, b.i.d., days 11 to 14, group II; (MF) methoxyflurane, 0.25% for 1.5 hr, day 15, group III; (Pb-MF) phenobarbital preceding methoxyflurane, group IV. Δ = days 7 to 10 minus days 16 to 17, mean ± S.E. From Cousins et al. (*10*), reproduced with permission of Journal of Pharmacology and Experimental Therapeutics.

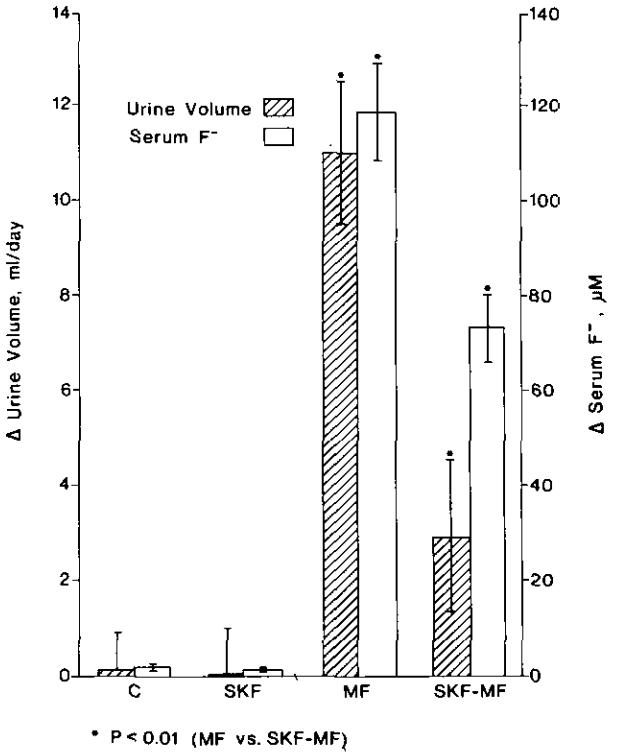

FIGURE 9. Changes in serum inorganic fluoride (F^-) concentration and urine volume for the first 2 days after anesthesia (day 11): (C) control, group I; (SKF) SKF 525-A, 50mg/kg, group II; (MF) methoxyflurane, 0.5% for 3 hr, group III; (SKF-MF) SKF 525-A followed by methoxyflurane, group IV. Δ = days 7 to 10 minus days 12 to 13, mean ± S.E. From Cousins et al. (*10*), reproduced with permission of the Journal of Phamacology and Experimental Therapeutics.

variables which included degree of nephrotoxicity, methoxyflurane dosage and serum inorganic fluoride concentration. Patients anesthetized with a methoxyflurane dose of 2.0 MAC-hr or less* had peak serum inorganic fluoride concentrations below 40 μM; this was not associated with nephrotoxicity (Fig. 11). The threshold of subclinical toxicity occurred at a dosage of 2.5–3 MAC-hr, corresponding to peak serum inorganic fluoride levels of 50–80 μM. These patients had delayed return to maximum preoperative urine osmolality, unresponsiveness to ADH administration and elevated serum uric acid concentration.

Mild clinical toxicity occurred at a dosage of 5 MAC-hr (serum inorganic fluoride concentration 90–120 μM). In addition to the abnormalities noted above, serum hyperosmolality, hypernatremia, polyuria, and low urine osmolality were present. Finally, clinical toxicity occurred in all three patients with methoxyflurane dosage greater than 7 MAC-hr (peak serum inorganic fluoride levels 80–175 μM). Abnormalities in serum and urine variables were even more pronounced than at lower methoxyflurane dosages. In addition, all three patients were resistant to ADH (Fig. 12) and had thirst and polyuria which added difficulty to their clinical management.

A significant permanent reduction in creatinine clearance was seen only in one patient who received 9 MAC-hr of methoxyflurane and required a course of gentamicin for a postoperative Psuedomonas wound infection. His creatinine clearance was reduced from 107 ml/min prior to gentamicin treatment to 40 ml/min after 4 days of antibiotic therapy (Fig. 13) (*18*). It was subsequently shown in Fischer 344 rats that the adverse renal functional and pathological effects of gentamicin and methoxyflurane are additive (*19*). Other studies

*MAC is the minimum alveolar concentration of anesthetic necessary to prevent movement in response to surgical incision in 50% of patients. MAC hours is determined by multiplying alveolar anesthetic concentration by the duration of administration of anesthetic.

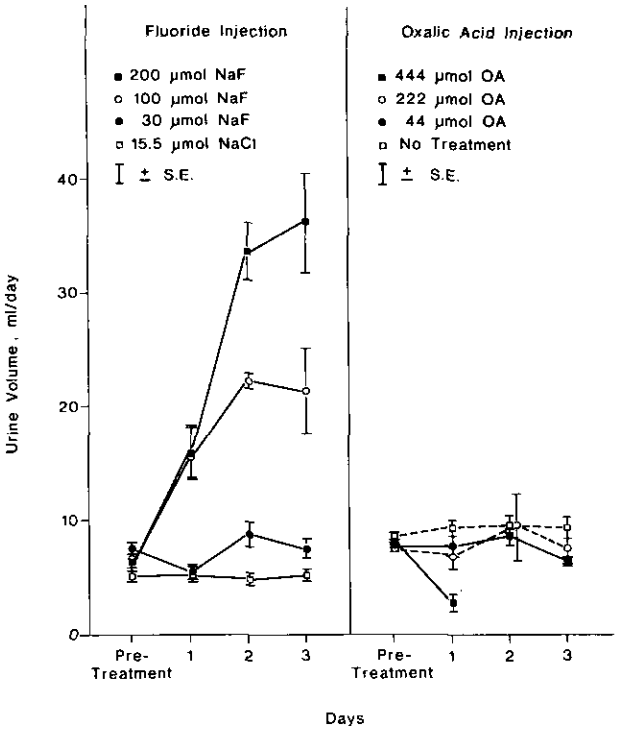

FIGURE 10. Daily urine volume (mean ± S.E.) before and after treatment with oxalic acid (OA) and sodium fluoride (NaF). Dose-related polyuria occurred after NaF injection but not after OA. Treatment with 444 µmole of oxalic acid resulted in oliguria. From Mazze et al. (*10*), reproduced with permission of the Journal of Pharmacology and Experimental Therapeutics.

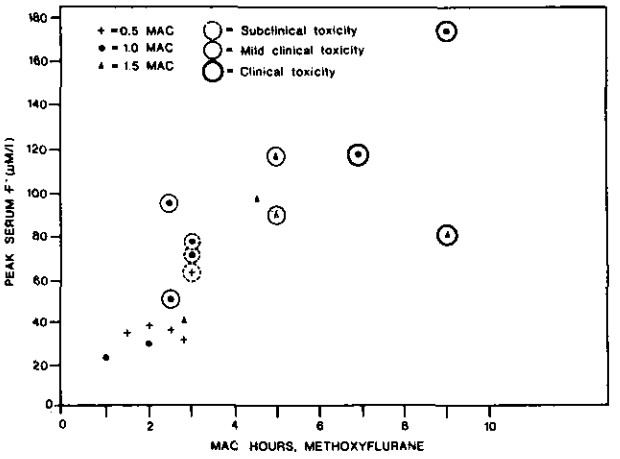

FIGURE 11. Peak serum inorganic fluoride concentration (F^-) and degree of nephrotoxicity are shown at increasing doses of methoxyflurane. Methoxyflurane dose correlated with both peak serum inorganic fluoride concentration and degree of nephrotoxicity. From Cousins and Mazze (*11*), reproduced with permission of the Journal of the American Medical Association.

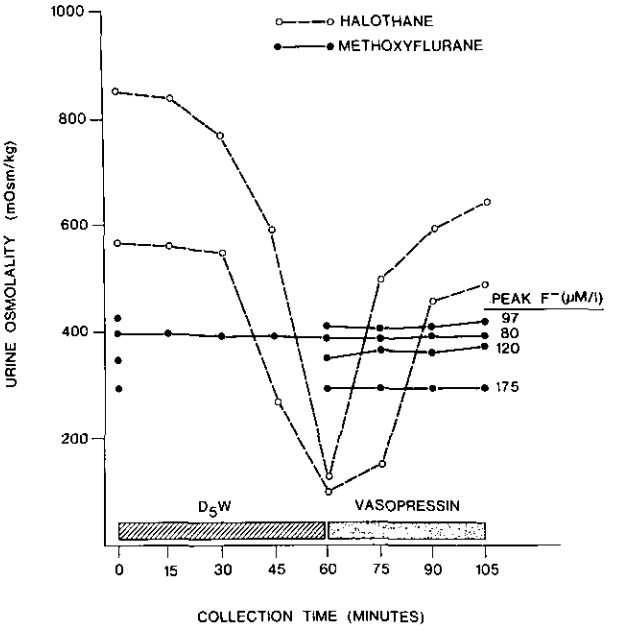

FIGURE 12. Vasopressin (ADH) infusion tests in four patients with polyuric renal dysfunction following methoxyflurane anesthesia. Control infusions are shown for two patients following halothane anesthesia. Patients anesthetized with methoxyflurane were unable to decrease urine osmolality in response to a 1-liter fluid load of 5% dextrose in water or increase urine osmolality after ADH infusion. From Cousins and Mazze (*11*), reproduced with permission of the Journal of the American Medical Association.

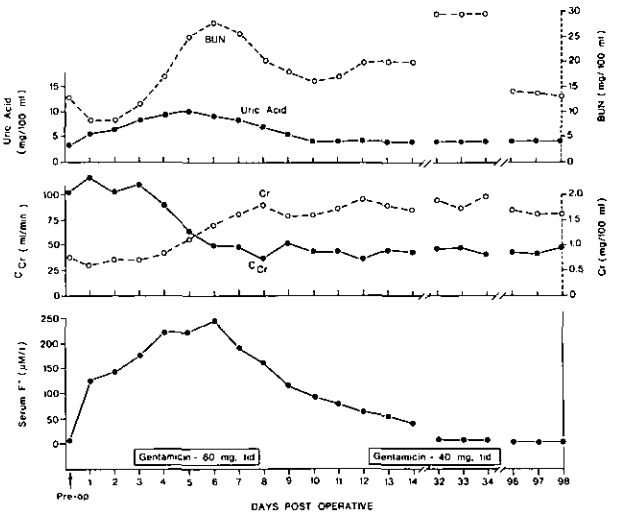

FIGURE 13. Preoperative and postoperative uric acid, blood urea nitrogen (BUN), creatinine clearance (C_{Cr}), creatinine (Cr), and serum inorganic fluoride (F^-). Preoperative values are means for the three days preceding operation. From Mazze and Cousins (*18*), reproduced with permission of the British Journal of Anaesthesia.

indicated that the toxicity of methoxyflurane is also increased by concurrent treatment with tetracycline (*20*) and other potentially nephrotoxic drugs.

Finally, a comment about age as a factor in methoxyflurane nephrotoxicity. Nephrotoxicity has not been reported in children. Studies in the Fischer 344 rat animal model suggest this may be due to greater deposition of inorganic fluoride in developing bone, thus lowering serum inorganic fluoride levels (Fig. 14) (*21*).

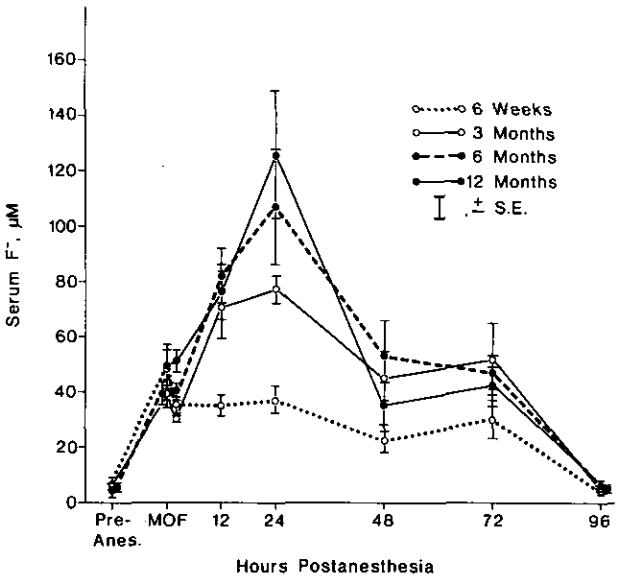

FIGURE 14. Serum inorganic fluoride in rats of various ages after 2 hr exposure to 0.5% methoxyflurane. Peak values were directly related to the age of rats. From Bell et al. (*21*), reproduced with permission of the Journal of Pharmacology and Experimental Therapeutics.

Summary of Factors in the Production of Methoxyflurane Nephrotoxicity

The predominant factors in the production of methoxyflurane nephrotoxicity appear to be mehtoxyflurane dosage and serum inorganic fluoride concentration. It is likely that secondary factors include: (1) a high rate of methoxyflurane metabolism and sensitivity of the kidney to inorganic fluoride toxicity; (2) concurrent treatment with other nephrotoxic drugs; (3) preexisting renal disease; (4) surgery of the urogenital tract, aorta, or renal vasculative; (5) repeat administration of methoxyflurane due to accumulation of inorganic fluoride and, perhaps, methoxyflurane induction of its own metabolism; and (6) concurrent treatment with enzyme inducing drugs such as phenobarbital.

In summary, investigations of methoxyflurane-induced nephrotoxicity in man have been extensively aided by the use of an animal model. To be of value the animal model must share similar metabolic pathways with man and have the same clinical manifestations of the disease process. The Fischer 344 rat appears to meet these criteria.

This work was supported in part by the Veterans Administration Hospital, Palo Alto, California, U.S. Public Health Service Grants GM 18514 and GM 22746 and Abbott Laboratories, North Chicago, Ill.

The author wishes to thank Michael J. Cousins, Ben A. Hitt, and Jon T. Sosek for their collaborative efforts in the studies referred to in this publication.

REFERENCES

1. Crandell, W. B., Pappas, S. G., and Macdonald, A. Nephrotoxicity associated with methoxyflurane anesthesia. Anesthesiology 27: 591 (1966).
2. Mazze, R. I., Shue, G. L., and Jackson, S. H. Renal dysfunction associated with methoxyflurane anesthesia. A randomized prospective clinical evaluation. J. Amer. Med. Assoc. 216: 278 (1971).
3. Mazze, R. I, Trudell, J. R., and Cousins, M. J. Methoxyflurane metabolism and renal dysfunction: Clinical correlation in man. Anesthesiology 35: 247 (1971).
4. Taves, D. R., et al. Toxicity following methoxyflurane anesthesia. II. Fluoride concentration in nephrotoxicity. J. Amer. Med. Assoc. 214:91 (1970).
5. Frascino, J. A., Vanamee, P., and Rosen, P. P. Renal oxalosis and azotemia after methoxyflurane anesthesia. N. Engl. J. Med. 283: 676 (1970).
6. Cale, J. O., Orarjs, C. R., and Jenkins, M. T. Hepatic and renal effects of methoxyflurane in dogs. Anesthesiology 23: 248 (1967).
7. Mazze, R. I., Cousins, M. J., and Kosek, J. C. Strain differences in metabolism and susceptibility to the nephrotoxic effects of methoxyflurane in rats. J. Pharmacol. Exptl. Therap. 184: 481 (1973).
8. Mazze, R. I., Cousins, M. J., Kosek, J. C. Dose-related methoxyflurane nephrotoxicity in rats: A biochemical and pathologic correlation. Anesthesiology 36: 571 (1972).
9. Kosek, J. C., Mazze, R. I., and Cousins, M. J. The morphology and pathogenesis of nephrotoxicity following methoxyflurane (Penthrane) anesthesia: An experimental model in rats. Lab. Invest. 27: 575 (1972).
10. Cousins, M. J., et al. The etiology of methoxyflurane nephrotoxicity. J. Pharmacol. Exptl. Therap. 190: 530 (1974).
11. Cousins, M. J., and Mazze, R. I. Methoxyflurane nephrotoxicity: A study of dose-response in man. J. Amer. Med. Assoc. 225: 1611 (1973).
12. Hockaday T. D. R., et al. Primary hyperoxaluria. Medicine 43: 315 (1964).
13. Jegher, H., and Murphy, R. Medical progress, practical aspects of oxalate metabolism. N. Engl. J. Med. 233: 208 (1945).

14. Wiseman, A. Effect of inorganic fluoride on enzymes. In: Handbook of Experimental Pharmacology, Pharmacology of Fluorides, F. A. Smith, Ed., Springer-Verlag, New York, 1970, pp. 48-93.
15. Aufderheide, A. C. Renal tubular calcium oxalate crystal deposition. Arch. Pathol. 92: 162 (1971).
16. Halpren, B. A., Kempson, P. L., and Coplon, N. S. Interstitial fibrosis and chronic renal failure following methoxyflurane anesthesia. J. Amer. Med. Assoc. 223: 1239 (1973).
17. Methoxyflurane nephrotoxicity. Committee on Anesthesia, National Academy of Science, National Research Council. J. Amer. Med. Assoc. 217: 958 (1971).
18. Mazze, R. I., and Cousins, M. J. Combined nephrotoxicity of gentamicin and methoxyflurane anaesthesia in man. A case report. Brit. J. Anaesth. 45: 394 (1973).
19. Barr, G. A., et al. An animal model for combined methoxyflurane and gentamicin nephrotoxicity. Brit. J. Anaesth. 45: 306 (1973).
20. Kuzucu, K. Y. Methoxyflurane, tetracycline, and renal failure. J. Amer. Med. Assoc. 211: 1162 (1970).
21. Bell, L. E., Hitt, B. A., and Mazze, R. I. The influence of age on the distribution, metabolism and excretion of methoxyflurane in Fischer 344 rats: A possible relationship to nephrotoxicity. J. Pharmacol. Exptl. Therap. 195: 34 (1975).