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Fluoroacetate-Mediated Toxicity of Fluorinated Ethanes

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A series of 1-(di)halo-2-fluoroethanes reported in the literature to be nontoxic or of low toxicity were found to be highly toxic by the inhalation route. Experiments were performed that showed the compounds, 1,2-difluoroethane, 1-chloro-2-fluoroethane, 1-chloro-1,2-difluoroethane, and 1-bromo-2-fluoroethane to be highly toxic to rats upon inhalation for 4 hr. All four compounds had 4-hr approximate lethal concentrations of ≤100 ppm in rats. In contrast, 1,1-difluoroethane (commonly referred to as HFC-152a) has very low acute toxicity with a 4-hr LC50 of >400,000 ppm in rats. Rats exposed to the selected toxic fluoroethanes showed clinical signs of fluoroacetate toxicity (lethargy, hunched posture, convulsions). 1,2-Difluoroethane, 1-chloro-2-fluoroethane, 1-chloro-1,2-difluoroethane, and 1-bromo-2-fluoroethane were shown to increase concentrations of citrate in serum and heart tissue, a hallmark of fluoroacetate intoxication. ^{19}F NMR analysis confirmed that fluoroacetate was present in the urine of rats exposed to each toxic compound. Fluorocitrate, a condensation product of fluoroacetate and oxaloacetate, was identified in the kidney of rats exposed to 1,2-difluoroethane. There was a concentration-related elevation of serum and heart citrate in rats exposed to 0–1000 ppm 1,2-difluoroethane. Serum citrate was increased up to 5-fold and heart citrate was increased up to 11-fold over control citrate levels. Metabolism of 1,2-difluoroethane by cytochrome P450 (most likely CYP2E1) is suspected because pretreatment of rats or mice with SKF-525A, disulfiram, or dimethyl sulfoxide prevented or delayed the toxicity observed in rats not pretreated. Experimental evidence indicates that the metabolism of the toxic fluoroethanes is initiated at the carbon–hydrogen bond, with metabolism to fluoroacetate via an aldehyde or an acyl fluoride. The results of these studies show that 1-(di)halo-2-fluoroethanes are highly toxic to rats and should be considered a hazard to humans unless demonstrated otherwise.

Fluorinated ethanes are among the potential replacements for the chlorofluorocarbons (CFCs) as refrigerants, blowing agents, and solvents (Manzer, 1990). Humans could therefore be exposed to fluorinated ethanes in the workplace and in the environment. CFCs were distinguished by their low acute toxicity, resulting in relatively high workplace exposure limits. The low acute toxicity of the CFCs is attributable to the relative biological inertness of the molecules. Methane- and ethane-based CFCs with relatively high F/Cl ratios are relatively resistant to metabolic processes, and the products of metabolism are often of little toxicological consequence. CFCs also have relatively poor solubility in blood, leading to low blood concentrations when inhaled.

New chemistries being investigated for CFC replacements include ethane-based molecules in which one or more hydrogens have replaced halogens (HCFCs, HFCs). The introduction of hydrogen not only makes the molecule more prone to atmospheric destruction, resulting in a reduced ozone depletion potential, but also makes the molecule more susceptible to biological oxidation reactions. The increased biological reactivity of the CFC replacements makes it necessary to investigate the potential toxicity of the compounds through metabolism-based studies.

The high acute toxicity of alkanes with an even number of carbon atoms greater than 2, and with a single terminal fluorine, has been known for more than 30 years (Pattison, 1959). These compounds were thought to produce their toxicity through oxidation or hydrolysis of a carbon–halogen bond, followed by metabolism to an acid and β-oxidation resulting in the production of fluoroacetate. Some other compounds, such as fluoroethylnitrosoureas, were shown to be metabolized to fluoroacetate (Tisdale and Brennan, 1985).

Fluoroacetate (FA) has been used as a rodenticide for over 40 years. The mechanism of action of FA toxicity is generally accepted to be metabolism of FA to fluorocitrate (FC), and the resultant blockage of the citric acid cycle enzyme aconitase (Peters, 1963). Inhibition of aconitase leads to a buildup of citrate in the animal, and the production of ATP is severely limited. Toxicity and death are thought to be caused by severe impairment of energy production (Bosakowski and Levin, 1986).

Long-standing dogma has been that, for metabolism of a fluoroalkane to FA to occur, the number of carbons must be 4

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or greater in order to accommodate a β-oxidation mechanism (Pattison, 1959). We present in this paper a series of fluorinated ethanes that are metabolized to fluoroacetate, resulting in high acute toxicity in the rat.

MATERIALS AND METHODS

Animals. Male CD rats, ages 50–60 days, were obtained from Charles River Breeding Laboratories (Raleigh, NC). All animals were cared for according to the NIH Guide for Care and Use of Laboratory Animals. Rats were used at a weight range of 250–350 g. Animals were given certified rodent chow and tap water *ad libitum,* except for during inhalation exposures, when food and water were withheld (maximum of 5 hr).

Chemicals. 1,2-Difluoroethane and 1-chloro-1,2-difluoroethane were obtained from DuPont Chemicals (Deepwater, NJ). 1-Chloro-2-fluoroethane, 1-bromo-2-fluoroethane, and 1,1-difluoroethane were obtained from PCR, Inc. (Gainesville, FL). SKF-525A was a gift of SmithKline Beechum (Philadelphia, PA). All other chemicals and reagents were obtained from commercial sources and were of the highest quality available.

Inhalation exposures. Rats (3-4 per exposure) were exposed to the test compound in a closed, recirculating chamber as described in Gargas *et al.* (1986). Exposures were designed to be 4 hr in length, but were terminated early if at least half of the rats died. O_2 was monitored constantly and added as needed to maintain a 19–22% atmosphere. Carbon dioxide was removed with a soda lime trap. Exposures performed with no rats in the chamber showed that each compound was stable with soda lime. Atmospheres for all compounds except 1,1-difluoroethane were generated by adding the liquid compound into the system through a septum. All compounds were sufficiently volatile to achieve the desired chamber concentration within 10 min. 1,1-Difluoroethane is a gas at room temperature and was added as a specific volume with a gas-tight syringe.

The chamber concentration of the test chemical was monitored continuously throughout the exposure by gas chromatography with automatic sampling of the chamber every 10 min. Chromatography was performed with a Hewlett–Packard 5880 GC equipped with a 1/8-in. by 5-ft stainless-steel column of 5% Krytox on Carbopak B and a flame ionization detector. Atmospheres were quantitated by comparing peak areas to standard curves prepared with authentic standards.

For determination of approximate lethal concentration (ALC), rats were exposed to 1,2-difluoroethane at mean concentrations of 567, 116, 100, and 24 ppm; to 1-chloro-2-fluoroethane at mean concentrations of 136, 45, 13, and 7 ppm; 1-bromo-2-fluoroethane at mean concentrations of 40, 11, and 5 ppm; and to 1-chloro-1,2-difluoroethane at mean concentrations of 238, 40, 15, and 8 ppm for 4 hr. The ALC is defined as the lowest concentration that causes at least one death during or postexposure.

For citrate measurements, rats were exposed to room air, 25, 100, 1000, or 10,000 ppm 1,2-difluoroethane, 700 ppm 1-chloro-2-fluoroethane, 330 ppm 1-bromo-2-fluoroethane, or 700 ppm 1-chloro-1,2-difluoroethane for 4 hr, or less if half of the rats died early. Heart and kidney tissues were removed from rats immediately after the end of the exposure for citrate analysis. Euthanization was by chloroform overdose and exsanguination. Serum was prepared from blood collected via cardiac puncture. Samples were only taken from rats alive when the exposure was stopped.

In some experiments rats were pretreated with 300 mg/kg metyrapone, 50 mg/kg SKF-525A, 75 mg/kg disulfiram, or 500 mg/kg dimethyl sulfoxide (DMSO) ip before exposure to 500 ppm 1,2-difluoroethane for 4 hr.

Positive controls. Sets of three male rats were dosed orally by gavage with 3 mg/kg fluoroacetate or 80 mg/kg fluorocitrate. Two hours after dosing, the rats were euthanized by chloroform overdose and serum and heart tissue were removed for citrate analysis.

Citrate analyses. Citrate analyses were performed with a modification of the method of Bosakowski and Levin (1986). Tissue samples were homogenized in 25% trichloroacetic acid. Serum samples were brought to 25% TCA by adding a 50% TCA solution. Samples were centrifuged and the supernatant was extracted with 3 vol of diethyl ether. The ether layer was discarded, and the aqueous layer was analyzed for citrate by the method of Moellering and Gruber (1966).

Metabolite identification. Urine from rats exposed to 6600 ppm 1,2-difluoroethane, 500 ppm 1-chloro-2-fluoroethane, 500 ppm 1-bromo-2-fluoroethane, 1000 ppm 1-chloro-1,2-difluoroethane, and 3000 ppm 1,1-difluoroethane, and a homogenate of kidney from a rat exposed to 6600 ppm 1,2-difluoroethane for 4 hr were analyzed by ^{19}F NMR. Fluoroacetate and fluorocitrate were identified by NMR spectroscopy with a General Electric OMEGA NMR spectrometer equipped with a 5-mm probe. The field strength for ^{19}F was 470.5 MHz. A 4-μsec pulse was used with a 1.33-sec relaxation time. The chemical shift was referenced to $CFCl_3$ at 0 ppm. Authentic standards of fluoroacetate and fluorocitrate (Sigma Chemical Co., St. Louis, MO) were used for purposes of identification.

Statistical analyses. Group means of citrate concentrations were compared with a one-way analysis of variance at a significance level of 0.05.

RESULTS

Acute Toxicity

In rats, 4-hr ALCs for 1,2-difluoroethane, 1-chloro-2-fluoroethane, 1-bromo-2-fluoroethane, and 1-chloro-1,2-difluoroethane were 100, 13, 11, and 15 ppm, respectively (Table 1). Rats exposed to each of these compounds showed classical symptoms of fluoroacetate intoxication (Pelfrene, 1991) after approximately 30 min of exposure. These symptoms were lethargy, hunched posture, and convulsions. When rats exposed to lethal levels of these chemicals were necropsied after exposure, blood flowed freely and was not clotted in the lung or body cavities, a sign of citrate accumulation. Rats exposed to 1,2-difluoroethane were overtly hypothermic, a sign of reduced energy production.

Lethal concentration experiments were not performed with 1,1-difluoroethane, since the lethal concentration is reported in the literature as 500,000 ppm for 30 min in rats (Lester and Greenberg, 1950) and a 2-yr inhalation study was run with rats at concentrations up to 50,000 ppm (DuPont, unpublished data). In the current study, rats were exposed to approximately 3000 ppm for 4 hr with no effects. Urine was collected for NMR analysis from the rats exposed to 3000 ppm.

Citrate Analyses

Exposure of rats to 1,2-difluoroethane, 1-chloro-1,2-difluoroethane, 1-chloro-2-fluoroethane, and 1-bromo-2-fluoroethane all resulted in statistically significant elevations of serum and heart citrate concentrations (Fig. 1). Lethal concentrations of 1,2-difluoroethane resulted in serum citrate levels two- to fourfold higher than control, while the chlorinated and brominated analogs caused slightly higher citrate levels in the serum. Exposure of rats to increasing concentrations of 1,2-difluoroethane for 4 hr resulted in a dose-related increase in serum citrate at concentrations up to 1000 ppm

TABLE 1
Approximate Lethal Concentration (ALC) of Fluoroethanes

Compound	Exposure concentration (ppm)	No. dead/no. alive	ALC (ppm)
1,2-Difluoroethane	567 ± 153	4/0	
	116 ± 24	4/0	
	100 ± 35	2/2[a]	100
	24 ± 4	0/4	
1-Chloro-2-fluoroethane	136 ± 40	3/0	
	45 ± 8	2/1[a]	
	13 ± 5	3/0	13
	7 ± 4	0/3	
1-Bromo-2-fluoroethane	40 ± 10	3/0	
	11 ± 5	3/0	11
	5 ± 3	0/3	
1-Chloro-1,2-difluoroethane	238 ± 57	3/0	
	40 ± 4	3/0	
	15 ± 3	3/0	15
	8 ± 2	0/3	

[a] Alive rats were anesthetized 4 hr after the exposure and kept for 2 days following the exposure to monitor for delayed effects.

(Fig. 2a). Citrate levels in serum did not increase between 1000 and 10,000 ppm, perhaps due to the extreme toxicity of 1,2-difluoroethane at concentrations above 1000 ppm or saturation of metabolism.

Heart citrate levels were elevated to a greater extent in fluoroethane-exposed rats than were serum citrate levels (Fig. 1b). 1-Chloro-1,2-difluoroethane caused an 11-fold increase in heart citrate, compared to control, while the other fluoroethanes caused citrate to be elevated 5- to 8-fold over control. Increasing concentrations of 1,2-difluoroethane also caused a dose-related increase in heart citrate at concentrations up to 1000 ppm (Fig. 2b).

Rats exposed to fluoroacetate or fluorocitrate at lethal doses had serum citrate levels significantly elevated above control, but slightly lower than the concentrations in fluoroethane-exposed rats (Fig. 1). Heart citrate was significantly elevated in rats exposed to FA, but not to FC (Fig. 1).

Serum from rats exposed to 1,1-difluoroethane was not analyzed for citrate, since the lethal concentration of the compound is very high and no FA was found in urine samples. It is not expected, however, that 1,1-difluoroethane exposure would lead to citrate accumulations, and there is no evidence that acetyl fluoride would cause inhibition of aconitase.

Metabolism of Fluoroethanes

NMR spectroscopy demonstrated that fluoroacetate was present in the urine (Fig. 3), and fluoroacetate and fluorocitrate were present in the kidney of rats exposed to 1,2-difluoroethane. FA was identified in the urine of rats exposed to 1-chloro-2-fluoroethane, 1-bromo-2-fluoroethane, and 1-chloro-1,2-difluoroethane, but tissues from rats exposed to these compounds were not analyzed. The only other metabolite identified in kidney or serum of rats exposed to 1,2-difluoroethane and 1-chloro-1,2-difluoroethane was fluoride ion.

The only metabolites identified in the urine of rats exposed to 1,1-difluoroethane were fluoride ion and a trace of acetyl fluoride. No fluoroacetate was detected.

Rats pretreated with 500 mg/kg dimethyl sulfoxide ip $\frac{1}{2}$ hr before exposure to 1,2-difluoroethane at a normally lethal level (500 ppm for 4 hr) had no clinical signs of toxicity during or after a 4-hr exposure. SKF-525A (50 mg/kg) and 75 mg/kg disulfiram attenuated the toxicity of a 500 ppm exposure such that no signs of toxicity appeared during the exposure and deaths were delayed by 12 hr or more. Metyrapone (300 mg/kg) did not appear to attenuate the toxicity of 1,2-difluoroethane.

DISCUSSION

Replacements for CFCs must not only show economic and engineering efficiencies and minimal ozone-depleting potential, but must also be toxicologically acceptable. The introduction of hydrogen into the CFC structure raises the potential for increased biological reactivity. In some cases, increased biological reactivity could result in increased toxicity. Since most commercial applications of HCFCs and HFCs require exposure limits of 25 ppm or greater for 8 hr, relatively low toxicity of the CFC replacements is a significant factor in determining their use.

Metabolism of xenobiotics to FA is documented in the literature. Production of FA through biological reactions is suspected to be the cause of the high acute toxicity of fluor-

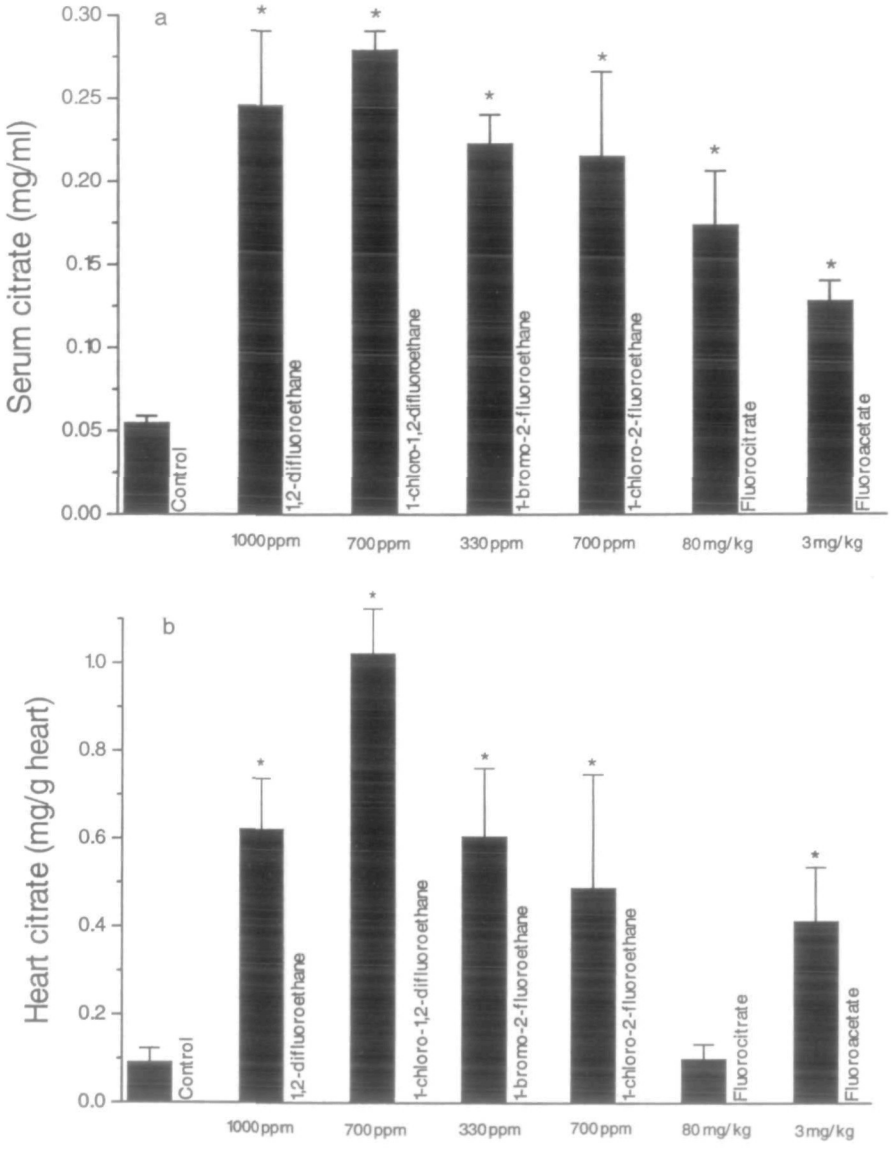

FIG. 1. (a) Serum citrate levels in rats treated with fluoroethanes, fluorocitrate, or fluoroacetate. (b) Heart citrate levels in rats treated with fluoroethanes, fluorocitrate, or fluoroacetate. Values represent the mean ± SD for three rats. Inhalation exposures were for 4 hr, rats dosed with FA or FC were euthanized after 2 hr because of toxicity. *Significantly different from control ($p < 0.05$).

oalkanes with the structure $F(CH_2)_nCH_3$, where n is an odd number greater than 2 (Pattison, 1959). Biological synthesis of FA was also shown with several fluorinated nitrosoureas (Tisdale and Brennan, 1985).

The results presented in this paper clearly show that FA is produced in rats exposed to 1,2-difluoroethane, 1-chloro-2-fluoroethane, 1-bromo-2-fluoroethane, and 1-chloro-1,2-difluoroethane, and that 1-(di)halo-2-fluoroethanes are highly toxic to rats and should be considered a hazard to humans unless demonstrated otherwise. The amount of FA produced is sufficient to cause citrate accumulation in serum and heart as high or higher than in rats exposed to lethal doses of FA or FC. All four fluoroethanes tested increased citrate in serum and heart tissue to levels previously reported to be caused by lethal doses of FA and FC (Bosakowski and Levin, 1986). The levels of heart citrate in rats exposed to fluoroethanes are comparable to the citrate levels in hearts of mice exposed to lethal doses of bis-fluoroethylnitrosourea (Tisdale and Brennan, 1985). The inability of FC itself to cause changes in heart citrate concentrations agrees with the data from Bosakowski and Levin (1986). It is possible that orally administered FC is metabolized by a first-pass effect in the liver and is not available in high enough concentrations to cause a significant inhibition of the citric acid cycle in the heart. The bioavailability of FC is unknown.

Evidence for the critical involvement of FA in the toxicity

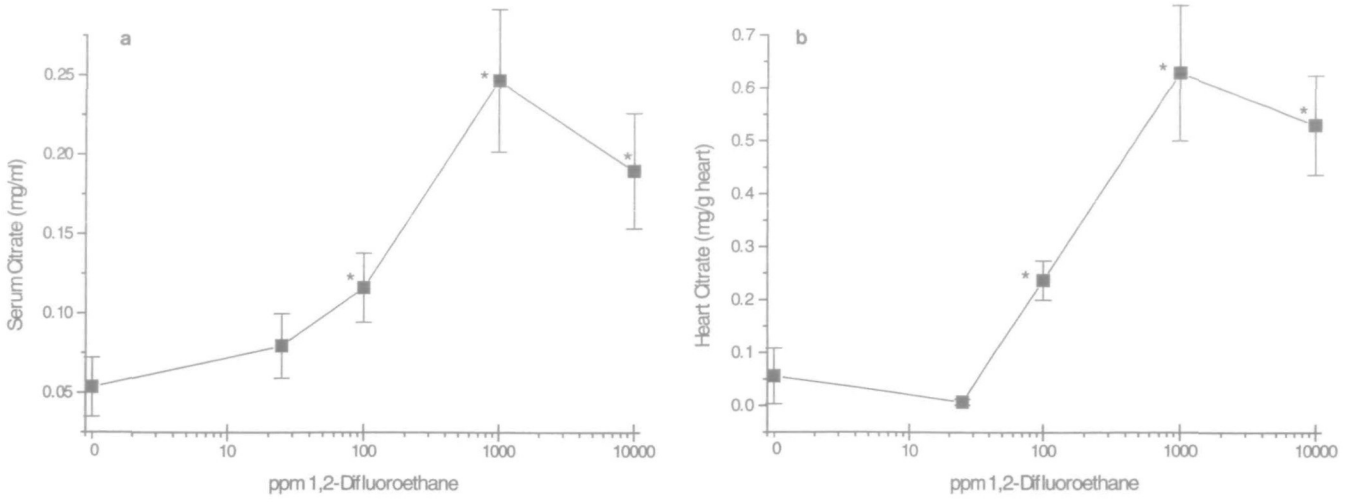

FIG. 2. (a) Serum citrate levels in rats exposed to 1,2-difluoroethane. Groups of three rats were exposed to 1,2-difluoroethane for 4 hr. Values represent means ± SD. *Significantly different from control ($p < 0.05$). (b) Heart citrate levels in rats exposed to 1,2-difluoroethane. Groups of three rats were exposed to 1,2-difluoroethane for 4 hr. Values represent mean ± SD. *Significantly different from control ($p < 0.05$).

of 1,2-difluoroethane is strengthened by the ability of DMSO, SKF-525A, and disulfiram to inhibit toxicity of 1,2-difluoroethane. DMSO is known to be a substrate of cytochrome P450, with a high affinity for the CYP2E1 enzyme (Feierman and Cederbaum, 1989). Additionally, CYP2E1 is known to contribute significantly to the metabolism of chlorocarbons, chlorofluorocarbons, and fluorocarbons (Harris and Anders, 1990; Olson *et al.*, 1991). SKF-525A is a broad-spectrum CYP inhibitor, and disulfiram is an excellent inhibitor of CYP2E1 (Feierman and Cederbaum, 1989). Thus, it appears that metabolism of 1,2-difluoroethane (and perhaps 1-chloro-2-fluoroethane, 1-bromo-2-fluoroethane, and 1-chloro-1,2-difluoroethane) may be mediated primarily through CYP2E1. The reason for the inability of metyrapone to attenuate the toxicity of 1,2-difluoroethane is unknown.

In contrast to the striking toxicity of 1,2-difluoroethane, 1-chloro-2-fluoroethane, 1-bromo-2-fluoroethane, and 1-chloro-1,2-difluoroethane, 1,1-difluoroethane has very low acute toxicity and is not metabolized to fluoroacetate. The positions of halogens on the ethane backbone is obviously critical to the metabolic fate and eventual toxicity of the compound.

The postulated pathways for metabolism of 1,2-difluoroethane, 1-chloro-2-fluoroethane, 1-bromo-2-fluoroethane, 1-

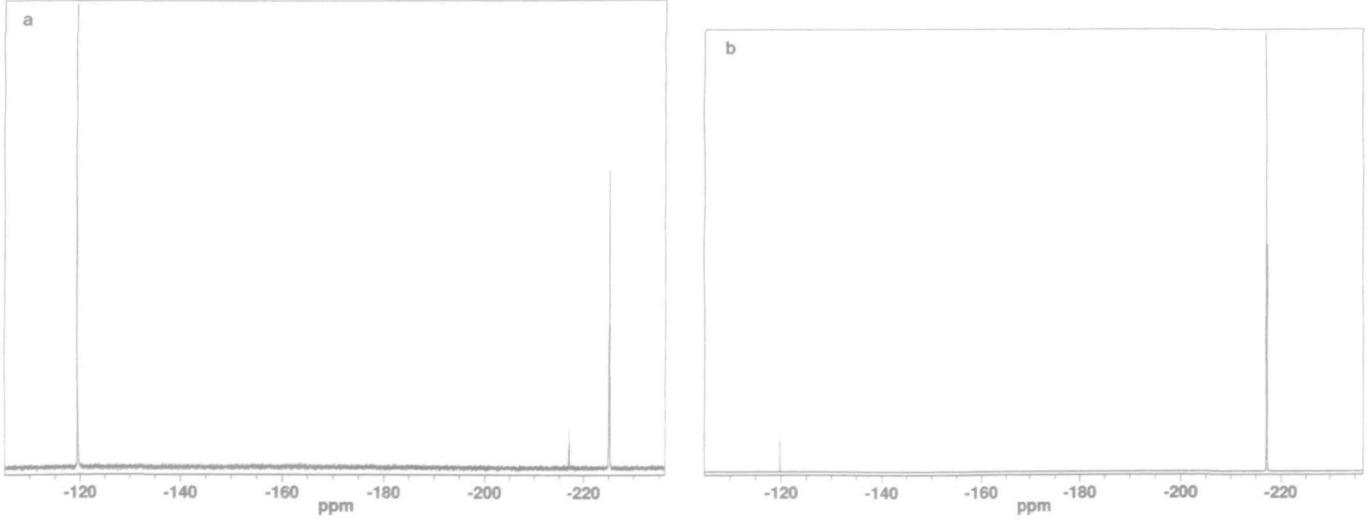

FIG. 3. ^{19}F NMR spectra (a) of urine from rats exposed to 10,000 ppm 1,2-difluoroethane for 4 hr and (b) a reference standard of fluoroacetate. The peak at −225 ppm is 1,2-difluoroethane, the peak at −217 ppm is fluoroacetate, and the peak at −120 ppm is fluoride ion.

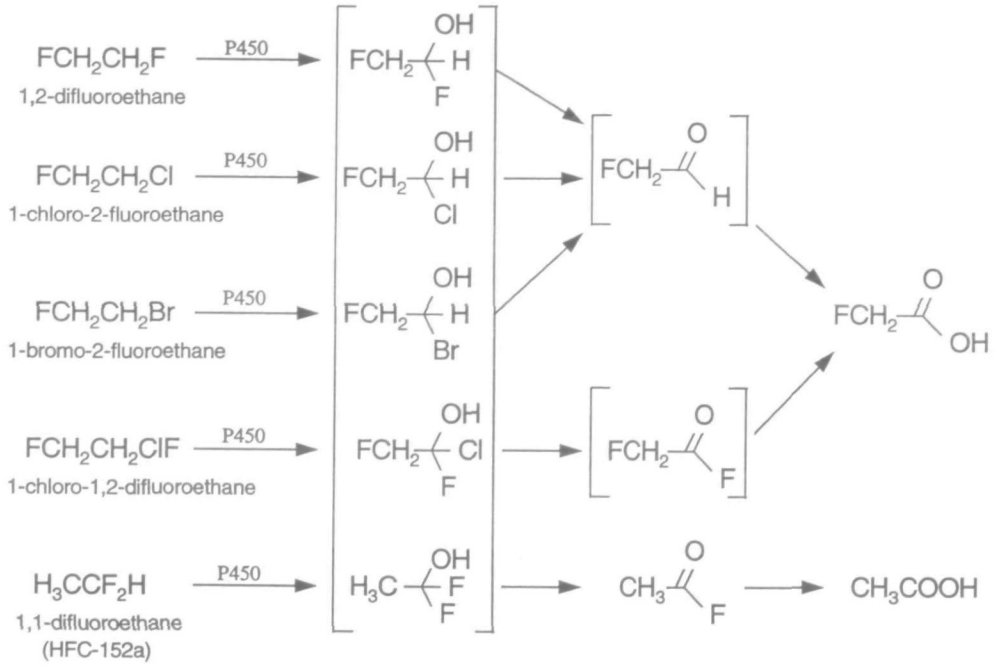

FIG. 4. Postulated pathways of metabolism for selected fluoroethanes. Brackets indicate potential metabolites not identified experimentally

chloro-1,2-difluoroethane, and 1,1-difluoroethane are shown in Fig. 4. Metabolism of each compound is initiated at the carbon–hydrogen bond by cytochrome P450. In the case of 1,2-difluoroethane, 1-chloro-2-fluoroethane, and 1-bromo-2-fluoroethane, an intermediate fluoroacetaldehyde is formed, which is oxidized to FA. A likely intermediate in 1-chloro-1,2-difluoroethane metabolism is fluoroacetyl fluoride, which is unstable and hydrolyzes to FA, releasing F^-. 1,1-Difluoroethane is likely metabolized through an acetyl fluoride, which subsequently hydrolyzes to acetate. NMR data show that higher than basal levels of F^- are present in the urine of rats exposed to 1,2-difluoroethane and 1-chloro-1,2-difluoroethane, but not in the urine of rats exposed to 1-chloro-2-fluoroethane or 1-bromo-2-fluoroethane. These data support the hypothesis of attack by cytochrome P450 on the hydrogen adjacent to the chlorine or bromine, preferential to a hydrogen adjacent to the single fluorine. The detection of F^- and acetyl fluoride, but no difluoroacetaldehyde, in the urine of rats exposed to 1,1-difluoroethane supports the hypothesis of preferential attack by cytochrome P450 on the hydrogen adjacent to the fluorines as opposed to attack on the hydrogens on the methyl carbon.

The significant toxicity of inhaled 1,2-difluoroethane, 1-chloro-2-fluoroethane, 1-bromo-2-fluoroethane, and 1-chloro-1,2-difluoroethane in rats and mice contradicts previous reports of low toxicity for some of these compounds. Saunders *et al.* report 1-chloro-2-fluoroethane and 1-bromo-2-fluoroethane to be "nontoxic" and "relatively nontoxic," respectively. However, examination of the experimental details shows that animals were exposed to 1-chloro-2-fluoroethane for 10 min at 55 ppm, while no experimental details were reported for 1-bromo-2-fluoroethane. Exposure times of 4 hr or longer are standard for evaluating toxicity of inhaled materials.

Overall, the results of this study show toxicologically significant metabolism of 1-(di)halo-2-fluoroethanes to FA, likely mediated by CYP2E1. Since CYP2E1 is present in human liver (Guengerich and Shimada, 1991), and perhaps other tissues, metabolism of 1-(di)halo-2-fluoroethanes to FA could occur in humans. The effects of FA in humans are similar to those in rats (Pelfrene, 1991). Therefore, compounds metabolized to FA in significant amounts are not suitable CFC replacements.

ACKNOWLEDGMENT

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