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INTERACTION OF FLUOROETHANE CHLOROFLUOROCARBON (CFC) SUBSTITUTES WITH MICROSOMAL CYTOCHROME P450

STIMULATION OF P450 ACTIVITY AND CHLORODIFLUOROETHENE METABOLISM

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Abstract—The abilities of halothane and the fluoroethane chlorofluorocarbon (CFC) substitutes, FC-123, FC-133a, FC-124, FC-134a and FC-125, to stimulate cytochrome P450 activities and 2-chloro-1,1-difluoroethene (CDE) defluorination in hepatic microsomes from phenobarbital-treated rabbits were compared. At 1% (v/v) each, halothane and FC-123 similarly increased the consumption of NADPH and O_2 by 300 and 100%, respectively, over that in microsomes without substrate. FC-133a and FC-124 were less effective, increasing NADPH and O_2 consumption by 150–200 and 70%. FC-134a and FC-125 were the least effective, increasing NADPH and O_2 consumption by only 70 and 50%, respectively. No metabolism of any fluoroethane could be detected under the incubation conditions used. Halothane and FC-123 were most effective in stimulating CDE metabolism with increases of CDE defluorination ranging from 1.5- to 2-fold. FC-133a and FC-124 enhanced CDE oxidation 89 and 74%, respectively, and FC-134a and FC-125 had no effect. While CDE metabolism was enhanced in the presence of the fluoroethanes, no additional NADPH or O_2 was consumed when halothane or FC-124 was incubated with CDE compared with incubations containing only halothane or FC-124. Log-log plots of NADPH consumption and CDE metabolism with the olive oil/gas partition coefficients of each fluoroethane showed linear relationships. These data demonstrate that the activity of the fluoroethanes in stimulating P450 activity and CDE metabolism is a function of their lipid solubility, and fluoroethane-enhanced CDE metabolism is related to the ability of these compounds to increase uncoupled P450 activity.

Highly fluorinated hydrocarbons are largely resistant to metabolism by cytochrome P450 enzymes [1, 2]. However, fluorocarbon binding to P450 can nevertheless stimulate P450 activity that is uncoupled from substrate oxidation [3–6]. Uncoupled activity entails NADPH and O_2 consumption greater than that utilized for substrate metabolism where the excess activity involves the reduction of molecular O_2 to H_2O_2, a two-electron reduction, or H_2O, a four-electron reduction [4–7].

In addition to greater hepatic O_2 consumption, an increase in uncoupled P450 activity caused by short-chained fluoro- and chlorofluorocarbons may be of toxicological or pharmacological significance since there is evidence that this activity can support the oxidation of gaseous haloethenes at rates greater than expected. For example, 1,1,1-trichloroethane, an uncoupler of P450 [8], has been shown to stimulate vinyl chloride metabolism and activation in liver microsomes from phenobarbital-treated rats [9]. Isoflurane and halothane, which are highly fluorinated anesthetics metabolized at low rates [10], stimulate 2-chloro-1,1-difluoroethene (CDE§) and trifluoroethene metabolism in microsomes from rats or rabbits [11–13]. Evidence that this effect is related to excess P450 activity is derived from studies of the stoichiometry of NADPH and O_2 utilization during isoflurane-stimulated CDE metabolism. In microsomes from phenobarbital-treated rabbits, NADPH oxidation and O_2 consumption were strongly increased by isoflurane, whereas CDE was much less effective. When CDE was incubated with isoflurane, CDE metabolism was increased 3-fold; however, the consumption of NADPH or O_2 was the same for CDE plus isoflurane or isoflurane alone [13].

Recently a number of 2-halo- and 2-dihalo-1,1,1-trifluoroethanes have been developed for use as alternatives to chlorofluorocarbons (CFCs) as refrigerants, solvents and in other applications. Since halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) facilitates CDE metabolism [11], it seems likely that these CFC substitutes may also increase haloethene metabolism via mechanisms involving excess P450 activity. While these compounds are expected to stimulate uncoupled P450 activity, it is also likely that members of this class of compounds will stimulate this activity to different degrees even

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§ Abbreviations: CDE, 2-chloro-1,1-difluoroethene; CFC, chlorofluorocarbon; TFA, trifluoroacetic acid; FC-123B1, 2-bromo-2-chloro-1,1,1-trifluoroethane (halothane); FC-123, 2,2-dichloro-1,1,1-trifluoroethane; FC-133a, 2-chloro-1,1,1-trifluoroethane; FC-124, 2-chloro-1,1,1,2-tetrafluoroethane; FC-134a, 1,1,1,2-tetrafluoroethane; and FC-125, pentafluoroethane.

though they are structurally similar. This is due to the fact that halosubstitution influences the inherent susceptibility of these compounds to metabolism and affects their solubility in biological media. Chlorine substitution, and to a greater degree bromine substitution, increases the rate of fluoroethane metabolism, a factor that decreases excess uncoupled activity. On the other hand chlorine or bromine substitution increases the solubilities of these compounds in lipophilic media, which enhances their ability to bind to and stimulate P450 activity [1–3]. The balance of fluoroethane metabolism and lipophilicity will thus determine the activity of individual haloethane CFC substitutes to stimulate P450 activity toward a second substrate.

The objectives of the present study were to determine the abilities of several commercially useful 2-halosubstituted-1,1,1-trifluoroethanes to stimulate P450 activity and, based on their effects, to evaluate the relationship between the generation of excess P450 activity and increased haloethene metabolism. This was done by comparing the effects of these compounds on P450 activities, including CDE metabolism, in hepatic microsomes from phenobarbital-treated rabbits. Rabbit liver microsomes have been shown previously to be suitable preparations for measurement of P450 cofactor utilization and product formation [4].

MATERIALS AND METHODS

Chemicals. Halothane (>99% purity) was purchased from Ayerst Laboratories, Inc. (New York, NY). CDE (>97%), FC-123 (>99%), FC-133a (>99%), FC-124 (>99.4%), FC-134a (>99%) and FC-125 (>98%) were obtained from PCR Chemicals, Inc. (Gainesville, FL). Trifluoroacetic acid (TFA) and dimethyl sulfate were purchased from EM Science (Cherry Hill, NJ).

Animals. Male New Zealand rabbits, 3.0 to 3.5 kg, were obtained from Bakkom Rabbit Try Co. (Viroqua, WI). The animals were housed individually in metal cages at the University of Iowa Animal Care Facility and maintained in accordance with the NIH guidelines for animal care. The rabbits were allowed to drink water containing 0.2% sodium phenobarbital for 7 days, and on day 8, the animals were killed by inhalation of 100% nitrous oxide. The livers were removed and weighed, and hepatic microsomes were prepared by differential centrifugation.

NADPH consumption. Liver microsomes (2.80 nmol P450/mg protein) were suspended in 50 mM Tris–HCl/0.5 mM NaN_3, pH 7.4, at a concentration of 1.5 mg protein/mL. Microsomes (1.95 mL) were placed in 4-mL cuvettes and sealed under air. Two micromoles of each fluoroethane and/or CDE were introduced into the cuvettes using a gas-tight micro-syringe. Halothane and FC-123 were added as saturated vapors in air, and FC-133a, FC-124, FC-134a, FC-125 and CDE were added as pure gases. The microsomal mixtures were warmed at 30° for 5 min and placed in a Cary 3E UV/Visible spectrophotometer with a thermostatically controlled cuvette holder maintained at 30°. The reaction was initiated by injection of 0.05 mL of NADPH solution giving a final concentration of 0.2 mM NADPH. The A_{340} of the incubation mixtures were recorded for the first 5 min immediately following the addition of NADPH. NADPH consumption was calculated based on the NADPH extinction coefficient of 6.22 $mM^{-1}\,cm^{-1}$ at 340 nm [4].

Oxygen uptake. O_2 consumption was evaluated under incubation conditions identical to those described above except that incubations were performed in sealed 6.3-mL plastic vials with septa adapted for the accommodation of an O_2 electrode. Aqueous O_2 concentrations were measured with a semi-micro Clark-type electrode in conjunction with an A-M polarographic amplifier model 1900 (A-M Systems, Inc., Everett, WA). The O_2 electrode was calibrated using *N*-methylphenazonium methosulfate (PMS) as described by Robinson and Cooper [14].

CDE metabolism. CDE (2 μmol, 1%) and/or each fluoroethane (2 μmol, 1%) were incubated with microsomes under an atmosphere of air in sealed 6.3-mL plastic vials. Each vial contained 2 mL of microsomes (1.5 mg protein/mL) suspended in 50 mM Tris–HCl/0.5 mM NaN_3, pH 7.4. An NADPH-generating system, containing 2.66 mM glucose-6-phosphate, 0.25 U glucose-6-phosphate dehydrogenase, and 0.47 mM NADP, in a volume of 50 μL, or for the stoichiometric studies, 0.2 mM NADPH was added. The mixtures were incubated at 30° for 5 min and then cooled in ice to stop CDE metabolism. Fluoride concentrations in the mixtures were determined as described below.

Hydrogen peroxide formation. Each fluoroethane and/or CDE (2 μmol) were incubated with microsomes (2 mL, 1.5 mg protein/mL) at 30° for 5 min in the presence of 0.2 mM NADPH as described above. Reactions were stopped by the addition of 1 mL of 3% trichloroacetic acid. Hydrogen peroxide formation in the incubations was determined by the thiocyanate method as described by Hildebrandt *et al.* [15].

Fluoride analysis. Fluoride analysis was performed essentially according to the method described by Van Dyke and Gandolfi [16]. Aliquots (1 mL) from the microsomal mixtures were mixed with 1 mL of TISAB IV solution (Fischer Scientific Co., Fair Lawn, NJ). Fluoride concentration in the mixtures was determined using a fluoride-specific electrode (Fisher Scientific Co.) and an Orion model 720A meter (Orion Research Inc., Boston, MA). Sodium fluoride (10^{-6}–10^{-4} M) was used as a standard. Since metabolism of one molecule of CDE releases two fluoride ions [17], the quantity of CDE oxidized was considered to be half the fluoride liberated.

Determination of trifluoroacetic acid. Trifluoroacetic acid (TFA) formation resulting from the incubation of each fluoroethane was determined according to the method described by Maiorino *et al.* [18]. Each fluoroethane was incubated with 2 mL of microsomes (1.5 mg protein/mL) and 0.2 mM NADPH for 5 min at 30° in the presence or absence of CDE. Following incubation, microsomal suspensions (0.2 mL) were mixed with 0.1 mL dimethyl sulfate and 0.5 mL concentrated sulfuric acid in a 1.7-mL glass vial sealed with a teflon-faced rubber liner. The mixture was incubated at 60° for 20 min and then kept at 37° until the head space was

Table 1. NADPH and O_2 consumption, and H_2O_2 and product formation during trifluoroethane-facilitated CDE metabolism in microsomes of phenobarbital-treated rabbits

	NADPH	O_2	H_2O_2	CDE metabolism*	Molar ratio
	(nmol/mg protein/min)				NADPH:O_2:Product†
Control	3.44 ± 0.53	2.91 ± 0.27	1.67 ± 0.12	NA†	1.18:1.0:0.57
CDE	5.82 ± 0.04§	5.19 ± 1.04§	1.73 ± 0.29	0.53 ± 0.12	1.12:1.0:0.44
FC-123B1	14.49 ± 0.24‖	7.88 ± 0.87‖	1.53 ± 0.09	NA	1.84:1.0:0.19
FC-123B1 + CDE	12.07 ± 0.29‖	8.45 ± 1.41‖	1.52 ± 0.11	1.94 ± 0.21¶	1.43:1.0:0.41
FC-124	7.87 ± 0.52	7.19 ± 0.99	1.52 ± 0.01	NA	1.09:1.0:0.21
FC-124 + CDE	7.25 ± 0.20‖	6.49 ± 1.06‖	1.75 ± 0.28	1.05 ± 0.10¶	1.12:1.0:0.43
FC-134a	5.64 ± 0.54§	6.36 ± 0.70‖	1.77 ± 0.19	NA	0.89:1.0:0.28
FC-134a + CDE	5.72 ± 0.40§	7.03 ± 0.35‖	1.95 ± 0.31	0.60 ± 0.07	0.81:1.0:0.36

Data are means ± SD, N = 3.
* CDE metabolism as determined by release of inorganic fluoride (F^-).
† Product = H_2O_2 + CDE metabolism.
‡ NA = not applicable.
§ $P < 0.05$, compared with control.
‖ $P < 0.05$, compared with control and CDE.
¶ $P < 0.005$, compared with CDE.

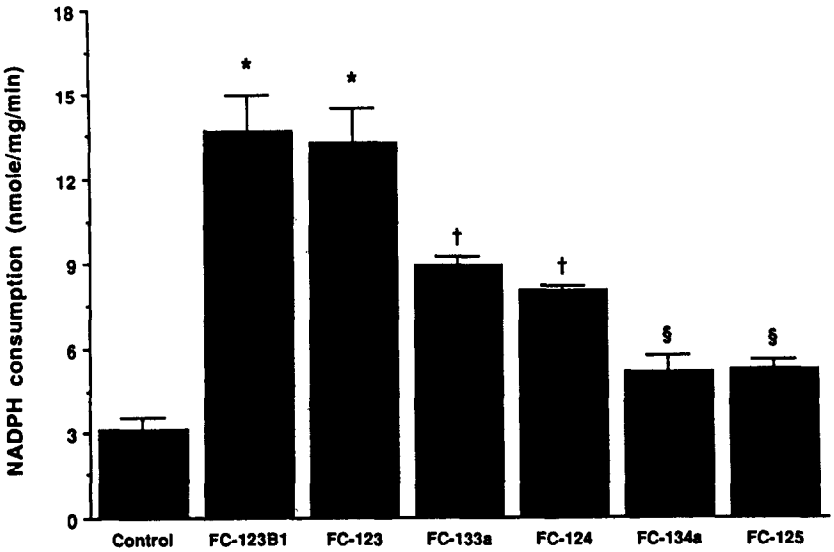

Fig. 1. Fluoroethane effects on NADPH consumption in microsomes from phenobarbital-treated rabbits. Data are means ± SD, N = 3. Key: (*) $P < 0.001$, (†) $P < 0.005$; and (§) $P < 0.01$ compared with control.

analyzed. This procedure derivatizes the water-soluble TFA to the volatile methyl trifluoroacetate. One milliliter of head-space from each vial was withdrawn with a gas-tight syringe and analyzed with a Hewlett Packard model 5830A GC equipped with a Porapak Q column (1.8 m × 2 mm, i.d.) maintained at 110° and a flame ionization detector. A standard curve was prepared by the addition of TFA (0.02 to 0.5 mM) to 0.2 mL of microsomes and derivatization as described.

Fluoroethane olive oil/gas partition coefficient determinations. The olive oil/gas partition coefficient of each fluoroethane was determined according to the method of Mushin and Jones [19]. Halothane, as a saturated vapor in air (200 μL), FC-123 in liquid form (10 μL), or FC-133a, FC-124, FC-134a, and FC-125 as gases (5 mL), were injected into 120 ± 0.1 mL sealed glass hypo-vials containing air. Initial concentrations of each fluoroethane in the vial were determined. One milliliter of pure olive oil (Bella Sales Co., Tampa, FL) was added, and the vials were maintained at 37° for 3 hr for fluoroethane equilibration between the phases. Final concentrations of each compound in the head-space were then determined. The concentrations of halothane and FC-123 were determined by capillary

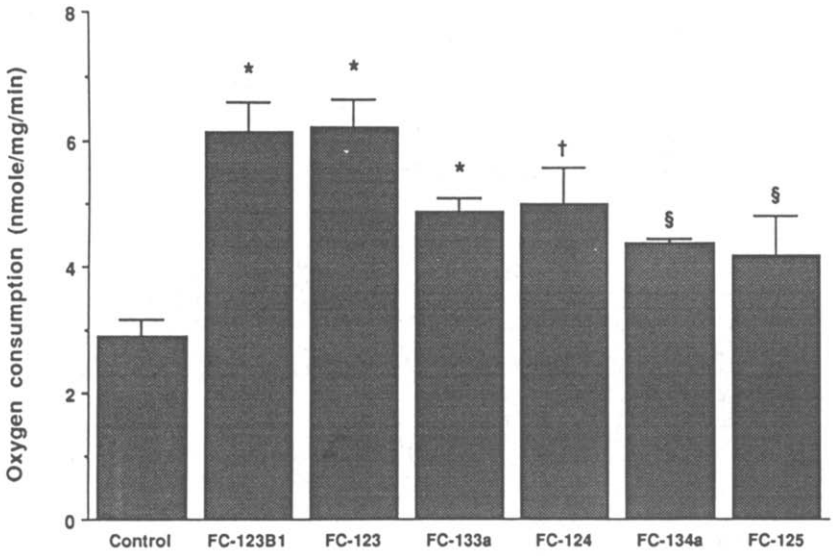

Fig. 2. Fluoroethane effects on oxygen consumption in microsomes from phenobarbital-treated rabbits. Data are means ± SD, N = 3. Key: (*) $P < 0.005$, (†) $P < 0.01$, and (§) $P < 0.05$ compared with control.

GC with an electron capture detector. An AT-624 column (30 m × 0.54 mm, i.d.) at 35° was used. The concentrations of FC-133a, FC-124, FC-134a and FC-125 were assayed with a GC equipped with Porapak Q column (1.8 mm × 2 mm, i.d., 120°) and a flame ionization detector. All values were calculated based on triplicate determinations.

Statistical analysis. Data are expressed as means ± SD, N = 3. ANOVA of multiple comparisons was utilized for analysis of significance. P values of less than 0.05 were considered statistically significant.

RESULTS

Each fluoroethane examined was found to stimulate both NADPH and O_2 consumption in liver microsomes from phenobarbital-treated rabbits (Figs. 1 and 2). However, at initial head space concentrations of 1%, the compounds showed large variations in these effects. Halothane and FC-123 were similarly effective, increasing NADPH consumption approximately 300%, and O_2 consumption 100% over that in microsomes containing no fluoroethane. The monochlorinated fluoroethanes, FC-133a and FC-124, were less effective than halothane or FC-123, increasing NADPH consumption 1.6- and 1.9-fold, and O_2 consumption 69 and 72%, respectively. The ethanes containing only fluorine substituents, FC-134a and FC-125, stimulated these activities the least, increasing NADPH consumption only 68 and 71%, and O_2 consumption, 45 and 52%, respectively.

The defluorination of each fluoroethane and the effects of these chemicals on CDE metabolism are shown in Fig. 3. No significant fluoride release could be detected following the brief aerobic incubation of the fluoroethanes with microsomes. On the other hand, halothane, FC-123, FC-133a and FC-124 were effective stimulators of fluoride production when these compounds were included in incubation mixtures containing CDE. Halothane and FC-123 increased CDE metabolism to the greatest degrees, 2.1- and 1.5-fold, respectively, compared to that resulting from CDE incubation alone. FC-133a and FC-124 enhanced CDE metabolism, but to lesser degrees, 89 and 74%. FC-134a and FC-125 at 1% neither stimulated nor inhibited CDE metabolism (Fig. 3).

To clarify the relationships of total and uncoupled P450 activity to fluoroethane-facilitated CDE metabolism, the stoichiometry of NADPH, O_2 consumption, and product formation were measured upon the incubation of halothane, FC-124 and FC-134a with microsomes in the absence and presence of CDE (Table 1). While these haloethanes alone increased microsomal NADPH and O_2 consumption as shown above, addition of CDE to the incubations had no effect on the depletion of NADPH or O_2. There were also no significant effects on the microsomal production of H_2O_2 by any of the fluoroethanes or CDE. CDE defluorination was increased in a predictable manner, where halothane was the most effective stimulator, FC-124 was less effective, and FC-134a did not increase CDE metabolism.

In an effort to more precisely assess the relationship between excess P450 activity caused by these fluoroethanes and increases in CDE metabolism, attempts were made to measure the oxidative metabolism of the fluoroethanes to TFA. Under the incubation conditions used for the stoichiometric studies (1.5 mg microsomal protein/mL and a 5-min incubation period at 30°), no TFA could be detected. Thus, although this metabolite is probably produced from the fluoroethanes, it was evolved slowly enough to fall below the lowest concentration used as a standard, 0.02 mM, which is equivalent to

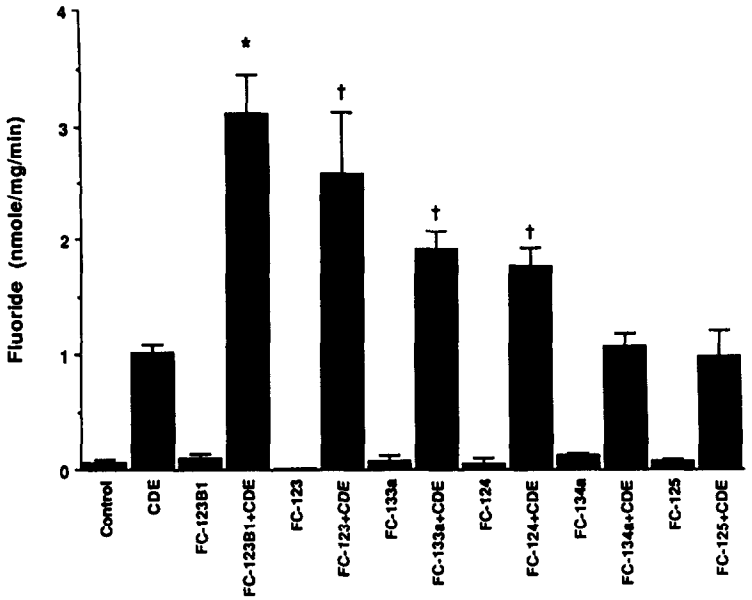

Fig. 3. Defluorination of CDE in the absence or presence of fluoroethanes in microsomes from phenobarbital-treated rabbits. Data are means ± SD, N = 3. Key: (*) $P < 0.001$, and (†) $P < 0.005$ compared with CDE.

0.0027 nmol/mg/min. TFA formation was detected when halothane, the most rapidly metabolized compound of the 1,1,1-trifluoroethanes [20], was incubated at 37° for 15 min in a suspension containing 3 mg microsomal protein/mL microsomal suspension (0.8 nmol TFA/mg/min). Based on these results, TFA formation was considered negligible in regard to the stoichiometric calculations. From the present data the quantities of NADPH consumed and not accounted for by product formation (NADPH minus H_2O_2) were: halothane, 12.96 nmol/mg/min; FC-124, 6.35; FC-134a, 3.87; control, 1.77. The addition of CDE to the incubations increased the efficiency of product formation as reflected by the molar ratios. Because NADPH consumption did not increase, excess P450 activity decreased upon the addition of CDE (Table 1).

Since uncoupled P450 activity did not inversely correlate with the relative rates of metabolism of the fluorinated ethanes, which follows the order, halothane > FC-124 > FC-134a [20], it is apparent that the lipid solubility of these compounds is more relevant to the generation of excess P450 activity. The olive oil/gas partition coefficient of each fluoroethane were determined and correlated with the other parameters observed, i.e. NADPH and O_2 consumption. The olive oil/gas partition coefficients were found to follow the order: halothane > FC-123 > FC-133a > FC-124 > FC-125 = FC-134a (Table 2). A log-log plot of the olive oil/gas partition coefficient for each compound to NADPH consumption ($r = 0.96$) or CDE defluorination ($r = 0.95$) showed similar, high degrees of correlation (Fig. 4).

DISCUSSION

The interaction of various compounds with the P450 enzymes has been shown to result in increased P450 functions including P450 reduction, and monooxygenase and oxidase activities [21–24]. The present study demonstrates that 2-halosubstituted-1,1,1-trifluoroethane CFC substitutes stimulated P450 and, at initial equimolar head space concentrations, differed in their abilities to do so. Based on measurements of O_2 consumption in rabbit liver microsomes, and, in particular, NADPH oxidation, the compounds can be divided into one of three categories: those most effective (halothane and FC-123); those moderately effective (FC-133a and FC-124); and those only minimally effective (FC-134a and FC-125).

This grouping indicates that the presence of a chlorine or bromine substituent and the total number of such substituents play a major role in the ability of the fluoroethanes to stimulate P450. Chloro- and bromo-substitution renders fluorocarbons more lipophilic as commonly demonstrated by their solubility in olive oil [25]. Correlation of the olive oil/gas partition coefficients for each fluoroethane with the respective degree of stimulation of NADPH consumption shows that the compounds fall into the same grouping as their effects on NADPH consumption: halothane ≈ FC-123 > FC-124 ≈ FC-133a > FC-134a ≈ FC-125. Since P450 possesses a lipophilic active site [21, 22, 26] and the fluoroethanes are of similar structure, the data indicate that the ability of 2-halosubstituted-1,1,1-trifluoroethanes

Table 2. Olive oil/gas partition coefficients of 2-halosubstituted-1,1,1-trifluoroethanes

	Olive oil/gas partition coefficient*
Halothane (FC-123B1)	219.8 ± 20.4
Dichlorotrifluoroethane (FC-123)	139.5 ± 3.5
Chlorotrifluoroethane (FC-133a)	22.2 ± 1.5
Chlorotetrafluoroethane (FC-124)	13.5 ± 1.9
Pentafluoroethane (FC-125)	7.3 ± 0.9
Tetrafluoroethane (FC-134a)	6.6 ± 0.9

Data are means ± SD, N = 3.
* Olive oil/gas partition coefficients were determined as described in Materials and Methods.

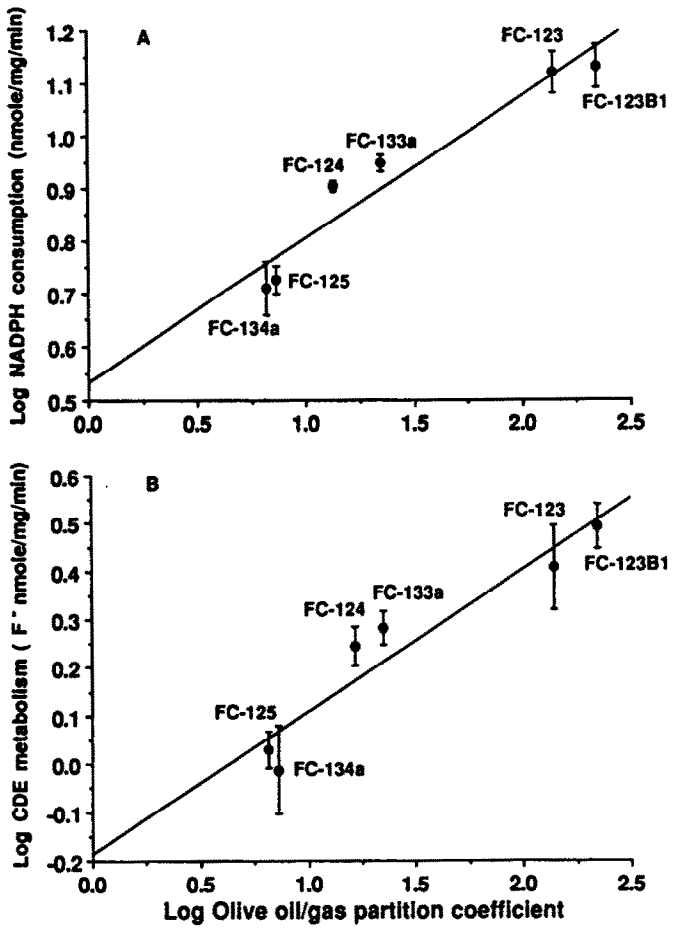

Fig. 4. Relationships of fluoroethane-stimulated NADPH consumption (A) and CDE defluorination (B) with fluoroethane olive oil/gas partition coefficients. Data are means ± SD, N = 3.

to stimulate P450 activity is primarily a function of their lipophilicity.

The same rank order for the extents to which these compounds increase NADPH consumption and stimulate CDE metabolism, and the relationship of these effects to the olive oil solubility of each, suggest that the two events are closely related. CDE oxidation, like the metabolism of the fluoroethanes [20, 27–29], is performed by the P450 isozymes [13]. Previously, it has been hypothesized that the stimulatory effect of isoflurane on CDE metabolism in rabbit liver microsomes is attributed to the ability of this anesthetic to stimulate P450 activity in excess of that used for isoflurane metabolism, and that the

excess P450 activity supports the oxidation of CDE [13]. The stoichiometry of P450 cofactor utilization and product formation for CDE metabolism in the absence and presence of three fluoroethanes, halothane, FC-124, and FC-134a, supports this hypothesis. In particular, the P450 activity generated by halothane and FC-124 is greater than the increase in CDE metabolism caused by halothane or FC-124. The addition of CDE to incubations containing these fluoroethanes did not cause additional increases in NADPH or O_2 consumption beyond that caused by each fluoroethane alone, even though CDE defluorination was enhanced. In addition, halothane produced greater excess activity than FC-124 and stimulated more CDE metabolism than FC-124. FC-124 produced more excess activity than FC-134a and stimulated CDE defluorination, whereas FC-134a did not. Even though FC-134a slightly increased NADPH consumption, it did not produce any greater NADPH consumption than CDE, which may account for its inability to facilitate CDE oxidation.

The mechanism(s) by which electron flow is enhanced by one compound to facilitate the metabolism of another P450 substrate remains to be determined. In this case, it may involve specific fluorocarbon interactions at the P450 active sites while CDE accesses the P450 heme iron without being blocked by the bound short-chain halocarbons [13]. This might come about if the haloethenes do not bind as strongly to P450 to stimulate P450 activity as the more lipid-soluble haloethanes. Second, haloethenes are more susceptible to metabolism than the fluoroethanes. The latter conclusion is reinforced by the comparative molar ratios for CDE or fluoroethane metabolism in the rabbit liver microsomes, which reflects the fact that CDE metabolism is more tightly coupled to P450 activity.

Data from this study also confirm that only a small portion of the total increase in NADPH consumption caused by fluoroethane–P450 interaction is utilized for fluoroethane metabolism. The inherent rate of metabolism of the fluoroethanes follows the order: halothane $\approx$ FC-123 > FC-124 > FC-125 $\approx$ FC-134a [20]. However, no organic or inorganic metabolites of any fluoroethane could be detected under the conditions used for the stoichiometric measurements. Although halothane is considered the fluoroethane most susceptible to metabolism, it nevertheless generated the greatest degree of excess activity when compared to FC-124 and FC-134a. The general effectiveness of halocarbons in stimulating uncoupled P450 activity is due to their resistance to attack by the reactive oxygen species generated by P450 [1, 2]. The fate of the reducing equivalents unaccounted for in fluoroethane-facilitated P450 activity is not clear. Hydrogen peroxide formation did not increase when the fluoroethanes were added to microsomal incubations. It is possible that, as previously hypothesized, the excess reducing equivalents were utilized in the reduction of molecular O_2 to H_2O [4–7].

In conclusion, this study demonstrated that the fluoroethane CFC substituents differ widely in effecting uncoupled P450 activity in rabbit liver microsomes. Of particular note is the observation that FC-123 was highly effective, whereas FC-134a, the refrigerant selected for use in automobile air-conditioning applications, was nearly inert. In addition to likely increases in hepatic O_2 consumption, stimulation of P450 by these compounds appears to be of potential toxicological importance since greater uncoupled P450 activity was associated with increases in metabolism of CDE. Given the fact that in addition to CDE, the metabolism of other gaseous haloethenes, such as vinyl chloride [9], is facilitated by short-chain halocarbons, this study suggests that inhalation of more lipophilic CFC-substitutes, e.g. FC-123, with any of a number of toxic haloethenes potentially increases the risk of individuals for metabolism-dependent haloethene toxicity. This interpretation supports the view that, if used at all, FC-123 concentrations must be controlled to very low levels in workplace, and other, environments.

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