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# The Role of Cytochrome P450 2E1 in the Species-Dependent Biotransformation of 1,2-Dichloro-1,1,2-trifluoroethane in Rats and Mice

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**1,2-Dichloro-1,1,2-trifluoroethane (HCFC-123a) is a potential alternative to replace ozone-depleting chlorofluorocarbons. The metabolism of HCFC-123a was studied in microsomes of rats, mice, and humans as well as in rats and mice *in vivo*. Rat, mouse, and human liver microsomes metabolized HCFC-123a to inorganic fluoride and chlorodifluoroacetic acid. Fluoride formation was dependent on time and NADPH, HCFC-123a, and protein concentration. Microsomes from untreated rats oxidized HCFC-123a at low rates (0.49 nmol fluoride/20 min × mg protein). Pretreatment of rats with pyridine and ethanol, inducers of P450 2E1, increased the rates of fluoride release. In mouse liver microsomes, the rates of HCFC-123a oxidation to release fluoride were significantly higher (1.68 nmol fluoride/20 min × mg) than in rat liver microsomes. Incubation of HCFC-123a with microsomes and diethyldithiocarbamate (100 μM), an inhibitor of P450 2E1, reduced fluoride formation by more than 60%. In different samples of human liver microsomes, rates of fluoride formation were between two- and fourfold higher than those observed in liver microsomes from untreated rats. In rats and mice exposed to concentrations of HCFC-123a up to 5000 ppm in a closed recirculating exposure system, chlorodifluoroacetic acid, and inorganic fluoride were identified as urinary metabolites. The biotransformation of HCFC-123a in rats was saturated after exposure to more than 2000 ppm HCFC-123a for 6 hr, whereas no saturation was evident in mice exposed to concentrations of up to 5000 ppm. The obtained results suggest a major role of P450 2E1 in the oxidation of HCFC-123a and in the different capacities for oxidative biotransformation of HCFC-123a in rodents. Mice may thus be more sensitive to toxic effects of HCFC-123a depending on biotransformation after administration of high doses.** © 1995 Academic Press, Inc.

Chlorofluorocarbons are very stable in the atmosphere. Their degradation in the stratosphere has been implicated in the depletion of stratospheric ozone (Fisher *et al.*, 1990; Hofmann *et al.*, 1992; Ko *et al.*, 1994; McFarland and Kaye, 1992; Molina and Rowland, 1994). Since depletion of stratospheric ozone may cause adverse effects in humans such as skin cancer, international agreements have forced a reduction of the use of chlorofluorocarbons. For many technical applications of chlorofluorocarbons, replacements such as hydrochlorofluorocarbons and hydrofluorocarbons, having many of the beneficial properties of chlorofluorocarbons but little ozone-depleting potential, are needed (Deger, 1992a,b). The atmospheric half-lives of hydrochlorofluorocarbons and hydrofluorocarbons are decreased due to the presence of C—H bonds. The C—H bonds make chlorofluorocarbons more prone to atmospheric degradation, but may also increase the possibility of biotransformation and biotransformation-dependent toxicity in humans. One of the most beneficial properties of chlorofluorocarbons was their lack of biotransformation in mammals.

Biotransformation of hydrochlorofluorocarbons likely proceeds through a cytochrome P450 (P450)-mediated oxidation of C—H bonds. This reaction has been demonstrated to occur in the metabolism of 1,1-dichloro-1-fluoroethane and 1,1,1,2-tetrafluoroethane (Harris and Anders, 1991a,b). 1,2-Dichloro-1,1,2-trifluoroethane (HCFC-123a) is a hydrochlorofluorocarbon presently under development. It is a structural analog to the widely used anesthetic halothane (1-bromo-1-chloro-2,2,2-trifluoroethane) and an isomer of 1,1-dichloro-2,2,2-trifluoroethane (HCFC-123). Halothane and HCFC-123 have been demonstrated to be metabolized to trifluoroacetic acid via a reactive acyl halide as a major metabolite (Gandolfi *et al.*, 1980; Harris *et al.*, 1991, 1992; Satoh *et al.*, 1985; Urban *et al.*, 1994). These reactions could initiate toxic effects due to binding of the formed reactive intermediates to cellular macromolecules. Indeed, chlorofluorohydrocarbons have a significantly higher potential for chronic toxicity than chlorofluorocarbons (Rusch *et al.*, 1994). The P450 enzyme P450 2E1 has been implicated in the metabolism of halothane and several hydrochlorofluorocarbons and hydrofluorocarbons (Gruenke *et al.*, 1988; Loizou *et al.*, 1994; Olson *et al.*, 1990, 1991a,b; Olson and Surbrook, 1991; Surbrook and Olson, 1992; Urban *et al.*, 1994). Constitutive expression of P450 2E1 in rat liver is low, but enzyme activity in rats is induced by ethanol, pyri-

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dine, and many other xenobiotics (Kim and Novak, 1993; Kim *et al.*, 1988). Diseases and lifestyle factors influence the concentration of P450 2E1 in humans (Brady *et al.*, 1991; Koop, 1992; Koop and Tierney, 1990; Yang *et al.*, 1992; Yoo *et al.*, 1991).

The objective of this work was to compare the metabolism of HCFC-123a in rat, mouse, and human liver subcellular fractions and in rats and mice *in vivo*. The obtained results suggest that species differences in the hepatic concentrations of P450 2E1 are responsible for a higher extent of HCFC-123a biotransformation in mice compared to rats.

## MATERIALS AND METHODS

***Chemicals.*** HCFC-123a, 99.8% pure (containing 0.2% HCFC-123 as impurity, percentage based on flame ionization detector response) was supplied from Hoechst (Frankfurt, FRG). Chlorodifluoroacetic acid, *p*-nitrophenol, chlorzoxazone, and all other chemicals were purchased from Aldrich Chemical Co. (Steinheim, FRG) in the highest purity available. Enzymes and cofactors were obtained from Sigma Chemical Co. (Deisenhofen, FRG).

***Animals and treatment.*** Male Sprague–Dawley rats (Zentralinstitut für Versuchstierzucht, Hannover, FRG, 220–250 g body weight) and Swiss–Webster mice were used for all studies. To induce P450 2E1, rats were pretreated with ethanol or pyridine. For ethanol pretreatment, animals were administered 10% (w/v) ethanol in drinking water over 10 days. For pyridine pretreatment, animals received an ip injection of 100 mg/kg pyridine (dissolved in isotonic sodium chloride solution) once daily for 4 consecutive days. Pretreatment with phenobarbital and 3-methylcholanthrene was performed according to standard protocols (Rodriguez and Prough, 1991). All animals were fasted 18 hr before euthanization and preparation of microsomes.

***Metabolism of HCFC-123a in rodents.*** To study the biotransformation of HCFC-123a *in vivo*, rats and mice were exposed to varying concentrations of HCFC-123a in a closed, recirculating exposure chamber for 6 hr. Two rats exposed to 1000 ppm HCFC-123a were pretreated with diallyl sulfide to inhibit P450 2E1 *in vivo* (Loizou *et al.*, 1994). After the end of the exposure, the animals were transferred to an all-glass metabolic cage and urine was collected at 4°C for 24 hr. On collection, the urine was quickly frozen at −78°C and stored at −20°C. To record NMR spectra, the urine was filtered through Millipore HV filters (45 $\mu$m) and 400 $\mu$l of urine was mixed with 50 $\mu$l of $D_2O$.

***Microsome preparation.*** Human liver specimens were obtained from the Keystone Skin Bank (Exton, PA) and the liver bank in Kiel (FRG). Human liver samples, medically unsuited for liver transplantation, were also acquired under the auspices of the Washington Regional Transplant Consortiums (Washington, DC). All procedures were performed at 4°C. To minced livers (rats) and tissue specimens (human), a threefold volume (w/v) of 0.154 M KCl was added and tissues were homogenized. The homogenate was then centrifuged at 10,000 rpm for 10 min and the resulting supernatant was subjected to a centrifugation at 40,000 rpm for 60 min to sediment microsomes. The microsomal pellet was resuspended in 0.01 M phosphate buffer (pH 7.4) and centrifuged again for 60 min at 40,000 rpm. The resulting pellet was suspended in 0.01 M phosphate buffer (pH 7.4) and aliquots of the microsomal preparations were quickly frozen in liquid nitrogen and stored at −70°C. Protein concentrations were determined with a Bio-Rad protein assay kit with bovine serum albumin as standard (Bradford, 1976).

***Instrumental analyses.*** A double-beam spectrophotometer (Hitachi U-2000, Tokyo, Japan) with an accuracy of ±0.002 mAU and a reproducibility of ±0.001 mAU was used for *p*-nitrocatechol quantification in 1-cm plastic cuvettes. $^{19}$F NMR spectra were recorded with a Bruker AMX 500 NMR spectrometer with a 5-mm fluorine probe operating at 470 MHz. $^{19}$F chemical shifts are referenced to $C_6F_6$ ($\delta$ = 0 ppm) in a coaxial tube. $^{19}$F NMR spectra were recorded with a pulse length of 6 $\mu$sec and an interpulse time of 1.5 sec. Fifteen thousand datapoints with 5000 to 20,000 scans were recorded for Fourier transformation.

***Incubation conditions.*** Hydroxylation of *p*-nitrophenol was determined as previously described (Harris *et al.*, 1991; Koop, 1986). The absorbance of 4-nitrocatechol was measured spectrophotometrically at 510 nm. Final protein concentrations were 0.5 and 1 mg/ml for pretreated Sprague–Dawley rats and human liver microsomes, respectively, and 2 mg/ml for naive Sprague–Dawley rat microsomes. Formation rates of *p*-nitrocatechol are based on a molar extinction coefficient $\epsilon$ = 15.4 mmol$^{-1}$ cm$^{-1}$. Diethyldithiocarbamate, a selective inhibitor for P450 2E1 was used in final concentrations of 100 and 300 $\mu$M (Guengerich *et al.*, 1991). For all these incubations, microsomes, the NADPH-generating system and diethyldithiocarbamate were preincubated for 5 min at 25°C in absence of *p*-nitrophenol. Incubations with HCFC-123a were performed in a total volume of 6 ml and contained microsomes, substrates, or inhibitors as noted, a NADPH-generating system (final concentrations: 9 mM glucose 6-phosphate, 1 mM $NADP^+$, 0.2 unit/ml glucose-6-phosphate dehydrogenase) in 0.1 M phosphate buffer with 1 mM EDTA (pH 7.4). Microsomes (0.5, 1, and 2 mg/ml protein) and the corresponding amount of buffer were placed in 38.7 ml Wheaton serum bottle. HCFC-123a was added (at 4°C) with a syringe into the sealed vials (Teflon septa) and preincubated for 10 min at 37°C to equilibrate the gas with the liquid phase. After addition of the NADPH-generating system, the mixtures were incubated for 10–20 min at 37°C in a shaking water bath. The vials were submerged in water to ensure constant temperatures in the vials. The reactions were stopped by putting the vials on ice. Every incubation was repeated four times. For the quantitation of inorganic fluoride and the NMR analyses, the microsomal protein was removed by centrifugation at 40,000 rpm for 20 min at 4°C. The 6-hydroxylation of chlorzoxazone was determined in a incubation mixture containing 0.5 mM chlorzoxazone, microsomal protein and an NADPH-generating system in 100 mM phosphate buffer (pH 7.4) in a final volume of 1.0 ml (Urban *et al.*, 1994).

***Western blot analysis for P450 2E1.*** Anti-rat P450 2E1 IgG (Amersham Buchler, Braunschweig, FRG) was used to quantitate P450 2E1 content in microsomes. Quantitation of antibody binding to P450 2E1 in immunoblots in microsomal proteins was accomplished by scanning blots with a laser densitometer (Urban *et al.*, 1994).

***Quantitation of inorganic fluoride.*** To quantitate microsomal metabolism of HCFC-123a to inorganic fluoride, 5 ml of supernatant from the microsomal incubation or 5 ml of urine was combined with an equal volume of total ionic strength-adjustment buffer (TISAB: 1 M acetic acid, 1 M sodium chloride, 0.012 M (±)-*trans*-1,2-diaminocyclohexane-*N,N,N',N'*-tetraacetic acid monohydrate in deionized water, pH 5.5) and analyzed with a fluoride-selective electrode and a reference electrode (Metrohm, Herisau, CH). Samples were constantly stirred, and values for the samples were taken after a 10-min equilibration period. Fluoride contents were quantitated by comparing the values obtained with those of standard curves. The fluoride-selective electrode was calibrated daily with freshly prepared solutions of known $F^-$ content. For calibration, solutions containing 0.1, 0.2, 0.5, 0.75, 1, and 10 ppm sodium fluoride were prepared in distilled water. Samples were constantly stirred and values for response (mV) were read after 10 min, and calibration curves were established by plotting the response against the fluoride concentration. The response of the fluoride selective electrode was not influenced by the protein and other contents of the microsomal incubation system.

## RESULTS

*Endproducts of HCFC-123a metabolism in liver microsomes and rodents in vivo.* $^{19}$F NMR analysis of incubation

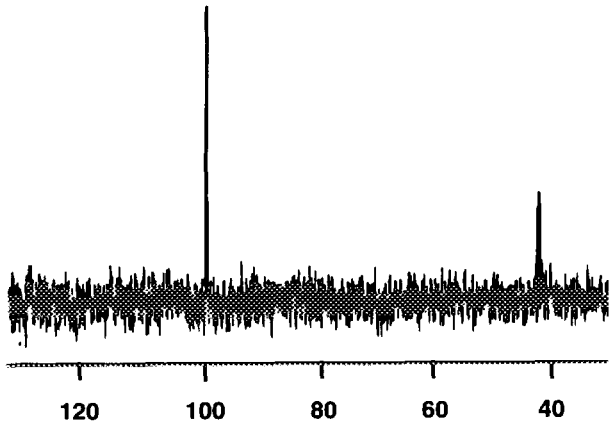


FIG. 1. $^{19}F$ NMR of microsomal incubations with HCFC-123a in the presence of NADPH-generating system. The signals at $\delta$ = 100.1 and $\delta$ = 42.1 ppm represent chlorodifluoroacetic acid and inorganic fluoride, respectively. Spectra were recorded with a Bruker AMX-500 Spectrometer operating at 470 MHz. Data points (2048) were collected for Fourier transformation. External $CFCl_3$ was used as chemical shift reference $\delta$ = 0 ppm.

mixtures of rat and human liver microsomes with HCFC-123a without an NADPH-generation system showed no signals indicating formation of metabolites. Incubations with HCFC-123a in the presence of NADPH contained a resonance identical in chemical shift to chlorodifluoroacetic acid ($\delta$ = 100.1) and a singlet at $\delta$ = 43.1 assigned to inorganic fluoride (Fig. 1). The resonance at $\delta$ = 87 ppm was identical in chemical shift to that of trifluoroacetic acid (Urban *et al.*, 1994). Trifluoroacetic acid is likely formed by oxidative biotransformation of HCFC-123 present as an impurity in the sample of HCFC-123a used. These resonances were not formed when using heat-inactivated microsomes. Mass spectrometry after chemical derivatization definitively identified chlorodifluoroacetic acid (Urban *et al.*, 1994) as a HCFC-123a metabolite (data not shown). $^{19}F$ NMR spectra of rat urine collected for 24 hr after exposure of rats to 2000 ppm HCFC-123a (6 hr) also showed the presence of chlorodifluoroacetic acid and increased concentrations of fluoride (Fig. 2).

*The role of P450 2E1 in the oxidation of HCFC-123a in rats and mice.* HCFC-123a was incubated with microsomes obtained from rats and mice after different pretreatments and metabolism was assessed by quantitation of inorganic fluoride. Since *p*-nitrophenol oxidation was not observed in the microsomes of kidney and lung of untreated and ethanol-treated rats (data not shown), all experiments on the metabolism of HCFC-123a were performed in liver microsomes only. Microsomes from naive rats formed only low amounts of fluoride from HCFC-123a. The formation of fluoride was dependent on time, microsomal protein, and NADPH. No significant differences were seen in the extent of HCFC-123a and *p*-nitrophenol oxidation in microsomes from male and female rats (data not shown). The rates of fluoride formation, *p*-nitrophenol oxidation, and chlorzoxazone oxidation were increased approximately 2-fold by pretreatment of rats with the P450 2E1 inducer ethanol and 10-fold with pyridine (Table 1). The increased rates of fluoride formation from HCFC-123a in microsomes from induced animals correlated with the increased content of P450 2E1 protein as determined by Western blotting (for data, see Urban *et al.*, 1994). Inhibition of P450 2E1 with diethyldithiocarbamate resulted in an almost 70% decrease in the rates of fluoride formation from HCFC-123a (Fig. 3). Fluoride formation was also inhibited competitively by *p*-nitrophenol (data not shown). The rates of HCFC-123a oxidation to fluoride in liver microsomes obtained from rats pretreated with inducers of P450 1A1/2 (3-methylcholanthrene) and P450 3A1/2 (dexamethasone) were not significantly higher than those observed in liver microsomes from untreated rats (data not shown). A sixfold increase in the rates of HCFC-123a oxidation was observed in liver microsomes from rats pre-

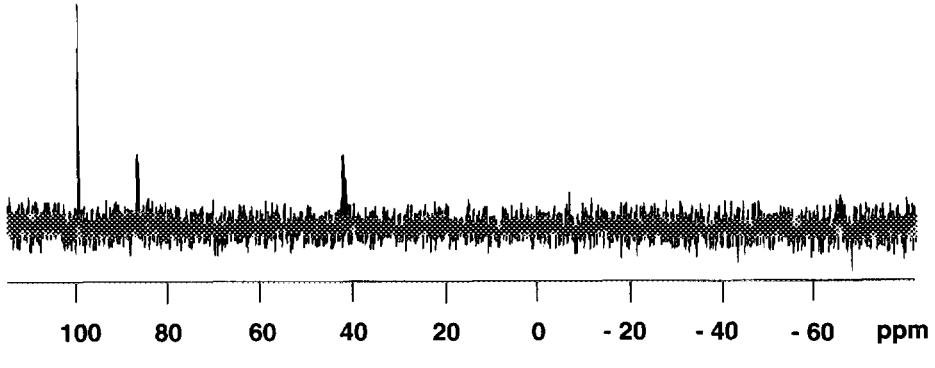


FIG. 2. $^{19}F$ NMR of rat urine collected for 24 hr after inhalation of 5000 ppm HCFC-123a for 6 hr. Spectra were recorded with a Bruker AMX-500 Spectrometer operating at 470 MHz. The signal at $\delta$ = 101.1 represents chlorodifluoroacetic acid and the signal at $\delta$ = 42.1 inorganic fluoride. The signal at $\delta$ = 87 ppm represents trifluoroacetic acid and results probably from oxidative metabolism of the impurity HCFC-123. Data points (2048) were collected for Fourier transformation. External $C_6F_6$ was used as chemical shift reference $\delta$ = 0 ppm.

TABLE 1
**Rates of HCFC-123a, *p*-Nitrophenol, and Chlorzoxazone Oxidation in Liver Microsomes from Male Rats and Mice**

| Species | Formation of fluoride from HCFC-123a (nmol/mg/20 min) | *p*-Nitrocatechol from p-nitrophenol (nmol/mg/20 min) | 6-Hydroxychlorzoxazone from chlorzoxazone (nmol/mg/20 min) |
|---|---|---|---|
| Sprague–Dawley rat | 0.5 ± 0.01 | 1.9 ± 0.3 | 31.9 ± 1.4 |
| Sprague–Dawley rat, ethanol pretreated | 1.4 ± 0.09 | 5.8 ± 1.2 | 61.2 ± 1.8 |
| Sprague–Dawley rat, ethanol pretreated + DDTC | 0.4 ± 0.10 | nd | 20.2 ± 3.4 |
| Sprague–Dawley rat, pyridine pretreated | 6.3 ± 0.20 | 18.0 ± 1.0 | 325.0 ± 4.3 |
| Swiss–Webster mice | 1.1 ± 0.30 | 6.1 ± 0.3 | 116.8 ± 6.4 |
| Swiss–Webster mice + DDTC | 0.3 ± 0.22 | 0.3 ± 0.1 | 36.1 ± 1.2 |

*Note.* nd, not detected; DDTC, diethyldithiocarbamate.

treated with phenobarbital, an inducer of P450 2B (Fig. 4). However, the 6-fold increase in the rates of oxidation compared to uninduced rats was contrasted by a more than 25-fold increase in the capacity of the liver microsomes to oxidize pentoxyresorufin, a specific P450 2B substrate, and a 50-fold increase in the amount of P450 2B protein as quantified by immunoblotting with a rat liver P450 2B-specific antibody (Köster and Dekant, unpublished observations).

*HCFC-123a metabolism in human liver microsomes.* Fluoride formation from HCFC-123a in the presence of a NADPH-generating system was observed in all six samples of human liver microsomes available (Table 2). Immunoblot analysis of the human microsomes available revealed considerable heterogeneity in the microsomal P450 2E1 protein content in the human liver specimen examined. The extent of HCFC-123 oxidation to release fluoride observed in the different samples of human liver microsomes correlated with the ability of the microsomes to oxidize chlorzoxazone and *p*-nitrophenol (Table 2) and with the amount of P450 2E1 protein as indicated by immunoblotting ($r^2 > 0.85$ for all correlations, for immunoblots of human liver microsomal samples, see Urban *et al.*, 1994). Low concentrations of diethyldithiocarbamate again inhibited HCFC-123a oxidation by more than 80% in two samples tested (Table 2).

*Differences in the biotransformation of HCFC-123a in rats and mice.* Rats and mice were exposed to HCFC-123a in a closed recirculating exposure chamber and the urinary excretion of the HCFC-123a metabolite inorganic fluoride was determined in the urine collected for 24 hr after the end of the exposure. NMR spectra of pooled urines (24-hr collection periods) showed the presence of chlorodifluoroacetic acid in the 24-, 48-, and 72-hr urines. However, in-

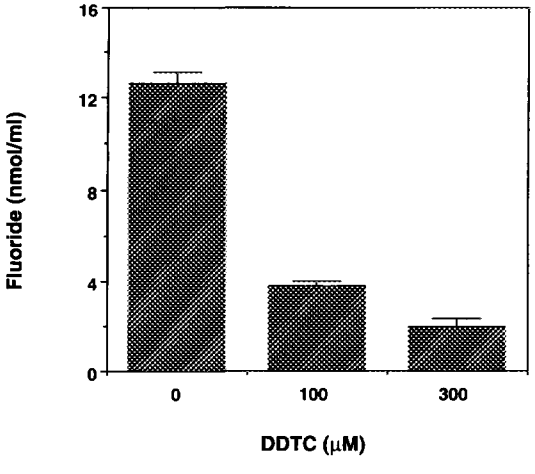


FIG. 3. Concentration-dependent inhibition by diethyldithiocarbamate on the rate of oxidative fluoride release from HCFC-123a in liver microsomes from ethanol-induced rats (mean ± SD, $n = 4$).

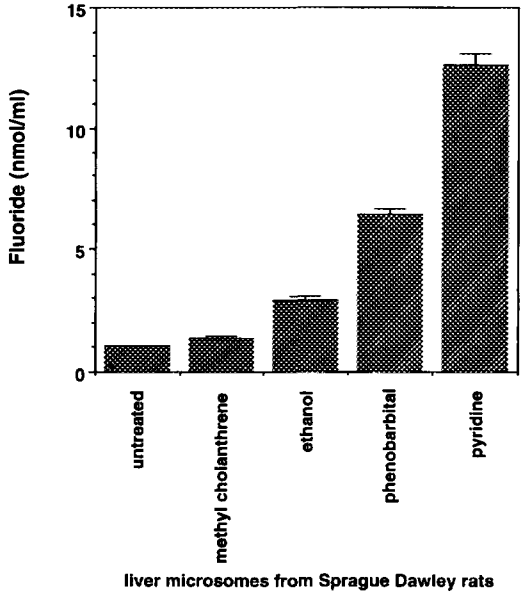


FIG. 4. Rates of oxidative fluoride release from HCFC-123a in microsomes obtained from rats after pretreatment with inducers of different P450 enzymes.

TABLE 2
**Rates of HCFC-123a, *p*-Nitrophenol, and Chlorzoxazone Oxidation in Human Liver Microsomes**

| Human liver sample/year of birth | Formation of fluoride from HCF-123a (nmol/mg/20 min) | *p*-Nitrocatechol from *p*-nitrophenol (nmol/mg/20 min) | 6-Hydroxychlorzoxazone from chlorzoxazone (nmol/mg/20 min) | Immunoblot density (20 μg protein per lane) |
|---|---|---|---|---|
| L4-1923 | 1.3 ± 0.1 | 10.7 ± 0.5 | 68.0 ± 1.9 | 2.6 |
| H1-1968 | 0.9 ± 0.1 | 6.0 ± 0.5 | 27.1 ± 0.6 | 1.0 |
| H3-1948 | 1.1 ± 0.2 | 6.7 ± 0.7 | 45.3 ± 5.3 | 1.1 |
| H5-1937 | 1.6 ± 0.2 | 15.4 ± 0.7 | 99.1 ± 0.8 | 2.4 |
| H4-1953 | 3.4 ± 0.4 | 28.0 ± 2.0 | 141.3 ± 26.3 | 6.7 |
| H4-1953 + DDTC | 0.5 ± 0.3 | 7.4 ± 2.1 | 37.8 ± 10.5 | na |
| H2-1941 | 1.9 ± 0.4 | 16.6 ± 0.4 | 98.0 ± 7.4 | 4.8 |
| H2-1941 + DDTC | 0.4 ± 0.2 | 5.3 ± 1.2 | 28.0 ± 6.3 | na |

*Note.* na, not applicable; DDTC, diethyldithiocarbamate (100 μM). The rates of fluoride formation from HCFC-123a were correlated to *p*-nitrophenol oxidation ($r^2$ = 0.96), chlorzoxazone 6-hydroxylation ($r^2$ = 0.85), and relative P450 2E1 protein content ($r^2$ = 0.90).

creased contents of inorganic fluoride were only observed in the urine collected within 24 hr after the end of the exposure (data not shown). Due to the rapid elimination of inorganic fluoride (Whitford, 1989, 1990), fluoride excretion is a better method for quantifying HCFC-123a biotransformation than chlorodifluoroacetic acid, which is likely eliminated slowly as are other trihaloacetates. The urinary excretion of inorganic fluoride was dependent on the HCFC-123a concentration in the exposure chamber in both mice and rats. A plot of the initial exposure concentrations against metabolite excretion indicated that the metabolism of HCFC-123a in the rat was a saturable process, saturation being reached at exposure concentrations below 2000 ppm HCFC-123a. In the mouse, the biotransformation of HCFC-123a was linear up to exposure concentrations of 5000 ppm (Fig. 5). In two rats exposed to 1000 ppm HCFC-123a and pretreated with the P450 2E1 inhibitor diallyl sulfide, the excretion of inorganic fluoride was reduced by more than 75% compared to control rats exposed to identical concentrations (data not shown).

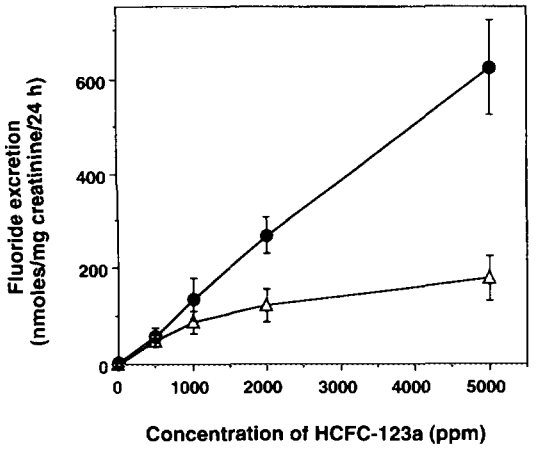


FIG. 5. Dose-dependent excretion of inorganic fluoride with urine after exposure of mice (●) and rats (△) to HCFC-123a (initial concentrations) in a closed recirculating exposure system for 6 hr. Fluoride concentrations in the pooled urine collected for 24 hr were quantified by a fluoride-specific electrode. Data represent the mean ± SD of three exposures with one rat each and one exposure with three mice each. For urine collection, mice were housed individually in the metabolic cages.

## DISCUSSION

Our results demonstrate that HCFC-123a is metabolized by P450 to yield inorganic fluoride and chlorodifluoroacetic acid. Chlorodifluoroacetate is formed by the oxidation of the carbon hydrogen bond in HCFC-123a followed by the loss of hydrochloric or hydrofluoric acid to give a chlorodifluoroacyl halide. This reactive acylating agent is further hydrolyzed to give chlorodifluoroacetate (Fig. 6). As observed with other HCFCs, the intermediate acylating agent may also react with lysine residues in proteins (Harris *et al.*, 1991, 1992). When compared to the rates of oxidation of HCFC-123 in liver microsomes, oxidation of HCFC-123a by P450

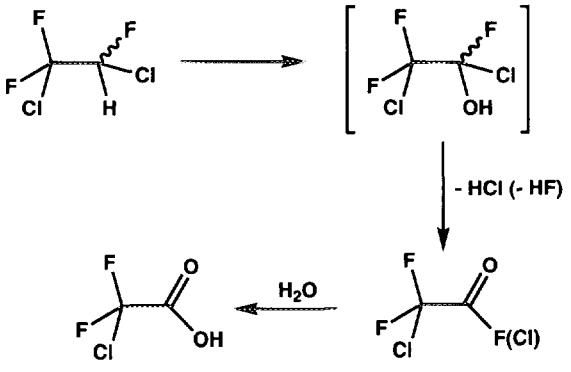


FIG. 6. Biotransformation of HCFC-123a in rodents.

was less efficient. This observation is in agreement with predictions of the effects of different substituents at the carbon atom on the rates of hydrogen abstractions from dihaloalkyl moieties catalyzed by P450 (Harris *et al.*, 1992; Korzekawa *et al.*, 1990).

The cytochrome P450 enzyme 2E1 has been implicated in the biotransformation of many low-molecular-weight xenobiotics (Guengerich *et al.*, 1991). Our results demonstrate that this enzyme also plays a major role in the oxidation of HCFC-123a. Liver microsomes from rats treated with the P450 2E1 inducers ethanol and pyridine showed significantly higher rates of fluoride formation from HCFC-123a. Moreover, microsomes from untreated mice exhibited higher rates of fluoride formation than rats. The increases in chlorofluoroalkane oxidation paralleled with the increased rates of oxidation of *p*-nitrophenol and chlorzoxazone, specific P450 2E1 substrates in rats and mice. Further evidence for a major role of P450 2E1 in the oxidation of HCFC-123a in human scan be derived from the correlation of the rates of fluoride formation from HCFC-123a in human liver microsomes with markers of P450 2E1 activity ($r^2 > 0.85$ were obtained for all correlations). In the different samples used, fluoride formation correlated well with *p*-nitrophenol oxidation, chlorzoxazone 6-hydroxylation and the relative P450 2E1 protein content. A major role of P450 2E1 is also indicated by significant reduction of fluoride formation from HCFC-123a afforded by the P450 2E1 inhibitor diethyldithiocarbamate in all liver microsome samples investigated. Diethyldithiocarbamate, at low concentrations, is a specific inhibitor of P450 2E1 in humans. Moreover, in rats, the amounts of fluoride excreted within 24 hr after exposure to HCFC-123a were significantly reduced by the mechanism-based P450 2E1 inhibitor diallyl sulfide *in vivo*.

As observed with other P450 2E1 substrates, at higher substrate concentrations, P450 2B could also catalyze the oxidation (Gruenke *et al.*, 1988; Nakajima *et al.*, 1990, 1992). The participation of P450 2B1/2 in the oxidation of HCFC-123a, however, seems to be only minor. Even at high substrate concentration of HCFC-123a, fluoride formation was still inhibited by almost 70% by diethyldithiocarbamate. Moreover, in microsomes from phenobarbital treated rats, whose P450 2B content was increased between 50- and 100-fold as measured by the dealkylation of 7-pentoxyresorufin, a similar increase in the rates of fluoride formation from HCFC-123a was not observed. The higher rates of HCFC-123 biotransformation in mice compared to rats are most likely due to the higher basal expression of P450 2E1 in mouse liver (Nakajima *et al.*, 1993). When comparing the capacity for biotransformation of other P450 2E1 substrates such as trichloroethene and perchloroethene in rats and mice, higher rates of biotransformation were frequently observed in mice (Odum *et al.*, 1988; Prout *et al.*, 1985; Schumann *et al.*, 1980). Moreover, saturation of metabolism under the applied experimental conditions was also often observed in rats but not in mice (Buben and O'Flaherty, 1985; Schumann *et al.*, 1980; Stott *et al.*, 1982). For example, rats eliminated the majority of the administered dose (2000 mg/kg) of trichloroethene as unchanged parent compound, whereas mice transformed most of an identical dose of trichloroethene to urinary metabolites. The higher capacity of mice for the oxidative biotransformation of HCFC-123a may cause differences in the toxic responses to HCFC-123a in these species. Since the acute toxicity of HCFC-123a in both species is low, differences in toxic responses will likely be observed in subchronic or chronic toxicity studies. Similarly, the role of reactive intermediates and protein acylation in the rodent toxicity will play only a minor role in the induction of chronic effects in rodents since, at least with the HCFC-123a analog HCFC-123, toxic effects observed after long-term exposures seem to be mediated by high blood levels of the stable trihaloacetates formed as metabolites. Trifluoroacetic acid, trichloroacetic acid (Bentley *et al.*, 1993; Bruschi and Bull, 1993; DeAngelo *et al.*, 1989; Elcombe *et al.*, 1985), and, likely, chlorodifluoroacetic acid are peroxisome proliferators; the higher rates of formation of the potential peroxisome proliferator chlorodifluoroacetic acid may render mice more susceptible to chronic toxicity (liver adenoma and carcinoma) associated with peroxisome proliferation (Parnell *et al.*, 1986; Reddy and Rao, 1986; Styles *et al.*, 1991).

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