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Metabolism and Toxicity of Hydrochlorofluorocarbons: Current Knowledge and Needs for the Future

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Hydrochlorofluorocarbons (HCFCs) are being developed as replacements for chlorofluorocarbons (CFCs) that deplete stratospheric ozone. The depletion of stratospheric ozone may increase the intensity of ultraviolet radiation at the earth's surface, which may be associated with global, adverse human health effects. The greater tropospheric lability of HCFCs, which is due to the presence of C–H bonds, reduces HCFC migration to the stratosphere; HCFCs should, therefore, cause less depletion of stratospheric ozone than CFCs. HCFCs under development include HCFC-22 (chlorodifluoromethane), HCFC-123 (2,2-dichloro-1,1,1-trifluoroethane), HCFC-132b (1,2-dichloro-1,1-difluoroethane), HCFC-134a (1,1,1,2-tetrafluoroethane), HCFC-141b (1,1-dichloro-1-fluoroethane, and HCFC-142b (1-chloro-1,1-difluoroethane). With the exception of HCFC-22, which is already in use, the metabolism and toxicity of HCFCs have not been studied in detail. By analogy to chlorinated ethanes, predictions can be made about the possible metabbolism of HCFCs, but there are insufficient data available to predict rates of metabolism. Although most HCFCs appear to show low acute toxicity, some HCFCs are mutagenic in the Ames test. Hence, future research on HCFCs should include studies on the *in vivo* and *in vitro* metabolism of HCFCs as well as on their toxicity in *in vivo* and *in vitro* systems.

Introduction

Chlorofluorocarbons (CFCs) currently in use are perhalogenated alkanes that find extensive use in four major applications: as refrigerants, as blowing agents in the manufacture of foam plastics, as cleaning fluids, and as propellants. These applications exploit the useful physical properties of CFCs, including low boiling points, specific heats and heats of vaporization, high insulating value, low surface tension and viscosity, and high vapor densities. Moreover, CFCs are nonflammable and relatively free of adverse health effects; reviews about the toxicity of CFCs have been published (*1–3*).

There is currently much concern about the potential effects of CFCs on stratospheric ozone (*4*). Because of their chemical stability, CFCs do not decompose in the troposphere but rise to the stratosphere where ultraviolet radiation catalyzes the release of chlorine atoms that destroy stratospheric ozone. Stratospheric ozone is an important barrier against ultraviolet radiation and reduces the amount of ultraviolet radiation that may otherwise reach the earth. Depletion of the ozone layer may lead to increased ultraviolet radiation at the earth's surface, and global adverse human health effects associated with increased exposure to ultraviolet radiation, such as an increase in skin cancer and cataract formation, may be seen (*5,6*). These concerns have prompted a search for CFC replacements that are less damaging to the ozone layer.

Commercially important CFCs include the perhalomethanes and ethanes fluorotrichloromethane (CFC-11),* dichlorodifluoromethane (CFC-12), 1,1,2-trichloro-1,2,2-trifluoroethane (CFC-113), 1,2-dichloro-1,1,2,2-tetrafluoroethane (CFC-114), and 2-chloro-1,1,1,2,2-pentafluoroethane (CFC-115). Some CFCs have estimated atmospheric lifetimes in the tens to hundreds of years. Bromine-containing fluorocarbons, the so-called halons, have greater ozone-depleting potential than the CFCs, but are used to a lesser extent. A cap on the production of CFC-11, 12, 113, 114, and 115 and a production freeze on halon-1211, 1301, and 2402 was mandated by the Montreal Protocol on Substances that Deplete the Ozone Layer and by Environmental Protection Agency regulations that became effective July 1, 1989 (*8,9*); hence there is a sense of urgency to develop CFC replacements.

Hydrochlorofluorocarbons (HCFCs) have been targeted as replacements for CFCs currently in use because of the greater lability imparted by the presence of C–H bonds, which makes them susceptible to oxidation in the troposphere, and because

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*The numerical codes for fluorocarbons have been established by the American Society of Refrigerating Engineers (*7*). Briefly, the first digit on the right is the number of fluorine atoms in the compound, the second digit from the right is one more than the number of hydrogen atoms in the compound, and the third digit from the right, which is omitted when the digit is zero, is one less than the number of carbon atoms in the compound; the number of chlorine atoms is found by subtracting the sum of the fluorine and hydrogen atoms from the total number of atoms that can be connected to the carbon atoms. In the case of geometric isomers, the most symmetrical isomer is indicated by the number alone; as isomers become more unsymmetrical, the letters a, b, c, etc., are appended. Symmetry is determined by summing the atomic weights of the substituents attached to each carbon and by subtracting the smaller sum from the larger sum; the smaller the difference the more symmetrical the compound.

their physical properties are similar to CFCs. CFC replacements cannot, however, be named with certainty. The toxicological, environmental, and basic thermodynamic properties of most CFC replacements have not been fully investigated, and further investigations may make some potentially useful compounds unsuitable for commercial development. Moreover, cost-effective synthetic methods that can be applied to the commercial-scale production of HCFCs are not available for all compounds. Information from several sources indicates that the HCFCs listed in Table 1, and whose chemical structures are shown in Figure 1, are candidates for development (*8–15*).

The objective of this review is to present current knowledge about the metabolism and toxicity of HCFCs, to speculate about the possible metabolic fate of HCFCs and about how metabolism may contribute to toxicity, and to indicate some future research needs.

Metabolism and Toxicity of HCFCs

Principles of Metabolism of Halogenated Hydrocarbons

Work conducted over the past two decades has defined the general metabolic pathways of halogenated hydrocarbons and their association with the toxic effects of halogenated hydrocarbons. These principles, which should be equally applicable to HCFCs, have been summarized (*16,17*).

Table 1. Hydrochlorofluorocarbon (HCFC) replacements for chlorofluorocarbons.

Chemical	HCFC
Chlorodifluoromethane	HCFC-22
2,2-Dichloro-1,1,1-trifluoroethane	HCFC-123
2-Chloro-1,1,1,2-tetrafluoroethane	HCFC-124
Pentafluoroethane	HCFC-125
1,2-Dichloro-1,1-difluoroethane	HCFC-132b
1,1,1,2-Tetrafluoroethane	HCFC-134a
1,1-Dichloro-1-fluoroethane	HCFC-141b
1-Chloro-1,1-difluoroethane	HCFC-142b

Briefly, HCFCs may undergo C–H σ-bond oxidation or oxygenation reactions catalyzed by cytochromes P-450; the expected initial metabolites would be geminal halohydrins, which may lose HX to form acyl halides or aldehydes. Such reactions may prove to be the dominant pathways of metabolism of HCFCs and may serve as detoxication or bioactivation reactions; the acyl halides may acylate nucleophilic sites in protein, as has been shown for chlorinated analogs (*18,19*). In the absence of oxygen, some HCFCs may undergo cytochrome P-450-catalyzed reduction reactions, as demonstrated for other polyhaloalkanes (*20–23*); the expected initial metabolites would be haloalkanes in which one halogen has been replaced by a hydrogen or haloethenes formed by the didehalogenation of haloalkanes. Haloalkenes formed by the didehalogenation of HCFCs may undergo bioactivation through the cysteine conjugate β-lyase pathway, which is initiated by glutathione *S*-conjugate formation and leads to the release of reactive metabolites (*24,25*). Certain HCFCs or their metabolites may be substrates for the glutathione *S*-transferases, and, depending on the compound, this may serve as a detoxication or bioactivation reaction. Indeed, recent studies indicate that fluorocarbons may be more subject to nucleophilic attack by sulfur nucleophiles than thought previously (*26,27*), indicating that the glutathione *S*-transferase-catalyzed addition of glutathione to fluorocarbons may warrant investigation. Finally, although HCFCs cannot serve as substrates for glucuronyl- or sulfotransferases, phase I functionalization reactions may yield metabolites that are conjugated by, for example, glucuronide or sulfate ester formation.

Current Knowledge and Speculations about Metabolism

Published information on the metabolism and toxicity of HCFCs that have been designated as replacements for CFCs is summarized below, and possible metabolic routes for HCFCs, based on current knowledge about halocarbon metabolism, are presented.

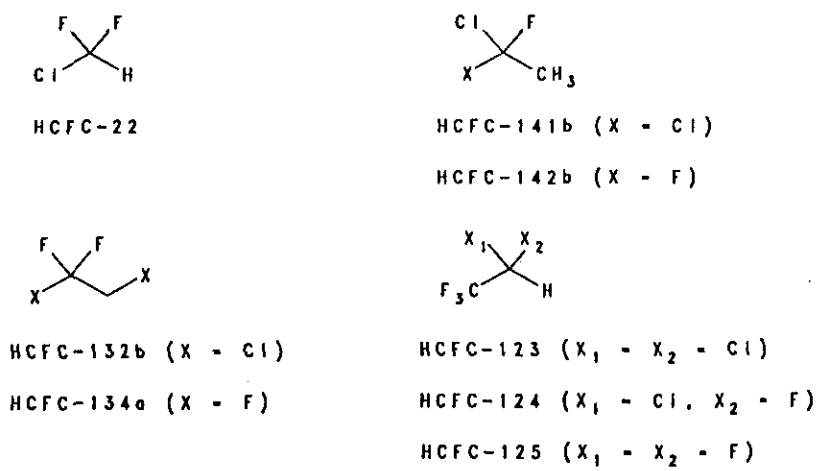

FIGURE 1. Chemical structures of hydrochlorofluorocarbons (HCFCs): HCFC-22, chlorodifluoromethane; HCFC-123, 2,2-dichloro-1,1,1-trifluoroethane; HCFC-124, 2-chloro-1,1,1,2-tetrafluoroethane; HCFC-125, pentafluoroethane; HCFC-132b, 1,2-dichloro-1,1-difluoroethane; HCFC-134a, 1,1,1,2-tetrafluoroethane; HCFC-141b, 1,1-dichloro-1-fluoroethane; HCFC-142b, 1-chloro-1,1-difluoroethane.

HCFC-22. The metabolism and toxicology of chlorodifluoromethane has been summarized (*28*). HCFC-22 shows low acute toxicity in various animal species; a concentration of 20% HCFC-22 is not lethal to any species tested (see Litchfield and Longstaff (*28*) for references to the original literature). HCFC-22 undergoes little metabolism *in vivo*; less than 0.03% of an inhaled dose of 500 ppm [^{14}C]- or [^{36}Cl]HCFC-22 was metabolized (*28*). Pharmacokinetic studies in rats demonstrated no detectable *in vivo* metabolism of HCFC-22 (*29*). *In vitro* studies with [^{36}Cl]HCFC-22 did not show the release of detectable chloride ion, indicating that little metabolism takes place.

HCFC-22 (50,000 ppm for 5 hr/day for 8 weeks) does not affect male fertility in the rat nor is there evidence of dominant lethality (*30*). Exposure of rats to 50,000 ppm HCFC-22 (6 hr/day on days 6 to 15 of pregnancy) produced a low incidence of microphthalmia and anophthalmia in rats; this effect was not observed in rabbits (*28*).

HCFC-22 is mutagenic in the Ames test in *Salmonella typhimurium* strains TA1535 and TA100 (*31*); a positive response was obtained in both the absence and presence of S-9 fractions from Aroclor 1254-treated rats. Similar results were obtained in other experiments (*28*). HCFC-22 is not mutagenic in *Schizosaccharomyces pombe* or in *Saccharomyces cerevisiae* or in a host-mediated assay with *S. pombe* or *S. cerevisiae* (*32*). Similarly, HCFC-22 was negative in an unscheduled DNA synthesis assay in the human heteroploid EUE cell line and in V-79 Chinese hamster cells (*32*) or in a CHO cell line (*28,32*). The ability of HCFC-22 to induce chromosome damage in rat bone marrow cells was studied (*28*); an apparent increase in chromosomal damage was seen at the lowest exposure level but not at two higher exposure levels. Dominant lethal assays of HCFC-22 in mice have been conducted (*28*); although significant effects were seen, the effects were not systematically related to duration of exposure or to dose, and the compound produced its effect in the premeiotic phase.

Recent studies report that no treatment-related effects were seen in Sprague-Dawley rats or Swiss mice exposed to HCFC-22 by inhalation (1,000 or 5,000 ppm for 4 hr/day, 5 days/week for 104 weeks) (*33*). HCFC-22 given by gavage (300 mg/kg, 5 days/week for 52 weeks) did not induce tumors during the 125-week observation period (*34,35*).

HCFC-123, 124, and 125. The lowest observed lethal concentration of HCFC-123 is 14 pph/4 min in the mouse (*36*); apparently data on the mammalian toxicity of HCFC-124 and 125 have not been reported. HCFC-123, 124, and 125 are not mutagenic in the Ames test with *S. typhimurium* TA1535 or TA100 as the test strains (*35*).

Because the pentahaloethanes HCFC-123, 124, and 125 are analogs of the anesthetic halothane (2-bromo-2-chloro-1,1,1-trifluoroethane), considerable insight into their possible metabolic fate is available. Recent studies on HCFC-123 and halothane metabolism have been conducted in my laboratory (*37*). Studies with ^{19}F nuclear magnetic resonance spectroscopy (^{19}F-NMR) showed that trifluoroacetylated microsomal and cytosolic proteins are present in the livers of HCFC-123- or halothane-treated rats. The adduct was identified as N^{ϵ}-trifluoroacetyllysine. Trifluoroacetic acid was identified as the only fluorine-containing metabolite in the urine of HCFC-123- or halothane-treated rats. Immunoblotting studies showed that the pattern of liver proteins immunoreactive with polyclonal, hapten-specific antitrifluoroacetyl protein antibodies was the same in the livers of HCFC-123- and halothane-treated rats. These data indicate that HCFC-123 (Fig. 2*A*, $X_1 = X_2 = Cl$) and halothane (Fig. 2*A*, $X_1 = Br$, $X_2 = Cl$) undergo a cytochrome P-450-catalyzed oxygenation to an unstable geminal halohydrin (Fig. 2*B*, $X_1 = Br$ or Cl, $X_2 = Cl$), which loses HBr or HCl to afford trifluoroacetyl chloride (Fig. 2*C*). Hydrolysis of acyl halide *C* gives trifluoroacetic acid (Fig. 2*D*), whereas reaction of acyl halide *C* with nucleophilic sites in proteins yields trifluoroacetylated proteins (Fig. 2*E*).

Although the metabolic fate of HCFC-124 and 125 has not been reported, HCFC-124 may yield the geminal halohydrin 1-chloro-1,2,2,2-tetrafluoroethanol (Fig. 2*B*, $X_1 = Cl$, $X_2 = F$) and after hydrolysis, trifluoroacetic acid (Fig. 2*D*). Finally,

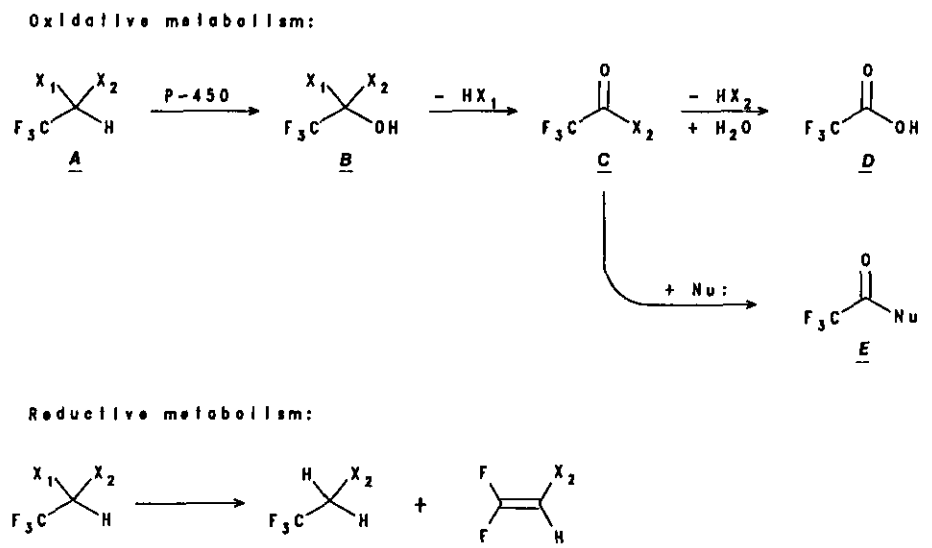

FIGURE 2. Pathways for the oxidative and reductive metabolism of HCFC-123 ($X_1 = X_2 = Cl$), HCFC-124 ($X_1 = Cl$, $X_2 = F$), and HCFC-125 ($X_1 = X_2 = F$). P-450, cytochromes P-450; Nu: tissue nucleophile.

HCFC-125 may be metabolized to pentafluoroethanol (Fig. 2*B*, $X_1 = X_2 = F$), which may lose HF to give trifluoroacetyl fluoride (Fig. 2*D*, $X_2 = F$) and, after hydrolysis, trifluoroacetic acid (Fig. 2*D*).

The oxidative metabolism of halothane is associated with the formation of hepatocyte membrane antigens, which may play a role in halothane hepatitis (*38,39*); sera from patients and animals exposed to halothane contain antibodies against trifluoroacetylated proteins. Because HCFC-123, 124, and 125 may all be metabolized to trifluoroacetylating agents, it will be important to determine whether there is cross-sensitization between HCFCs and halothane, as has been observed between halothane and enflurane (*40*).

Halothane also undergoes cytochrome P-450-catalyzed reductive metabolism to 2-chloro-1,1,1-triflluoroethane (HCFC-133a) and to 2-chloro-1,1-difluoroethene in the presence of lowered oxygen concentrations (*20,21,41,42*). This reaction is thought to proceed via a cytochrome P-450-catalyzed one-elecctron reduction to an intermediate radical anion, which loses bromine to give the 1-chloro-2,2,2-trifluoroethyl radical. Abstraction of a hydrogen atom from polyunsaturated lipids would yield the observed metabolite 2-chloro-1,1,1-trifluoroethane, whereas the homolytic elimination of fluorine would afford the alkene 2-chloro-1,1-difluoroethene, also a known metabolite of halothane. By analogy with the metabolism of halothane, the reductive metabolism of HCFC-123 may yield 2-chloro-1,1,1-trifluoroethane and 2-chloro-1,1-difluoroethene (Fig. 2); the reductive metabolism of HCFC-124 and 125 may yield 1,1,1,2-tetrafluoroethane (HFCF-134a) and trifluoroethene (Fig. 2). Whereas it is possible to make qualitative predictions about the metabolic fate of HCFC-123, 124, and 125, there is insufficient information available to predict their rates of metabolism, although metabolism usually decreases with increasing fluorination.

HCFC-132b. The lowest observed lethal concentration of HCFC-132b is 20,000 ppm/4 hr in the rat (*43*). HCFC-132b undergoes dechlorination (1.2%) when incubated with rat hepatic microsomes (*44*). Rats exposed by inhalation to HCFC132b (500, 2,000, or 5,000 ppm for 6 hr/day, 5 days/week for 13 weeks) showed proliferation of bile duct epithelial cells, testicular damage, elevated liver-, heart-, kidney-, and lung-to-body weight ratios, decreased brain and testes weights, and low activity and responsiveness to noise (*45*).

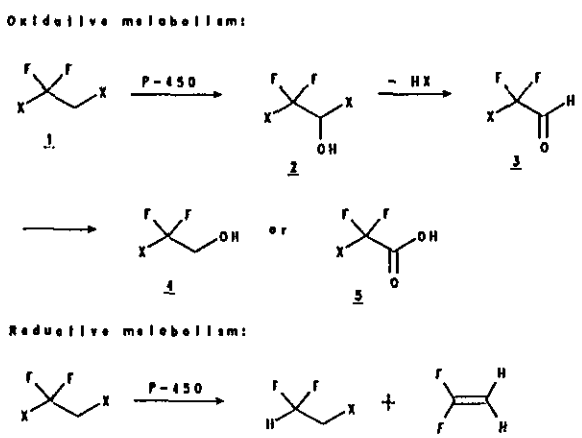

FIGURE 3. Pathways for the oxidative and reductive metabolism of HCFC-132b (x = Cl) and HCFC-134a (x = F). P-450, cytochromes P-450.

HCFC-132b undergoes extensive metabolism in the rat (*46*). The major metabolite was identified as 2-chloro-2,2-difluoroethyl glucuronide. Chlorodifluoroacetic acid was present in the urine of HCFC-132b-treated rats along with chlorodifluoroacetaldehyde (Fig. 3*A*, X = Cl) may undergo oxygenation to yield 1,2-dichloro-2,2-difluoroethanol (Fig. 3*B*, X = Cl), which may lose HCl to give chlorodifluoroacetaldehyde (Fig. 3*C*, X = Cl). 2-Chloro-2,2-difluoroethanol undergoes conjugation to form a glucuronide and a sulfate ester. No detectable alkylation of hepatic proteins was seen (*46*). These results are consistent with the generalization that protein acylation may be associated with the metabolism of geminal dihaloethanes to acyl halides; HCFC-132b should not be metabolized to an acyl halide and would not, therefore, be expected to acylate proteins. The toxicity of 2-chloro-2,2-difluoroethanol has not been investigated, but the fluorinated analog 2,2,2-trifluoroethanol is toxic (*47*).

The cytochrome P-450-dependent reductive metabolism of 1,1,1,2-tetrachloroethane yields 1,1-dichloroethene as the major metabolite (*23*) and 1,1,2-trichloroethane as a minor metabolite (*22,48*). These results are consistent with the generalization that chloroethanes with vicinal chlorine substituents may be reductively metabolized to haloalkenes by didechlorination (*48*). If these generalizations apply to HCFCs, HCFC-132b may be metabolized to 1,1-difluoroethene and 2-chloro-1,1-difluoroethane (Fig. 3). 1,1-Difluoroethene induces the formation of preneoplastic foci in rats, although the compound is much less potent than chloroethene (*49*).

HCFC-134a. HCFC-134a has been tested as an inhalational anesthetic agent and reported to have no direct toxic effects (*50*). HCFC-134a is not mutagenic in the Ames test (*35*). The cytotoxicity of HCFC-134a has been studied in isolated rat hepatocytes (*51*). Head-space HCFC-134a concentrations $\leq$75% did not increase lactate dehydrogenase release from hepatocytes. In hepatocytes isolated from fed rats, HCFC-134a ($\geq$12.5%) increased glycolysis and the lactate/pyruvate ratio and decreased glucose production; in hepatocytes from fasted rats, HCFC-134a inhibited gluconeogenesis.

Recent studies on the metabolism of HFCF-134a have been reported (*52,53*). HCFC-134a undergoes metabolism, as measured by the release of inorganic fluoride, in isolated rat hepatocytes (*52*) and in rat hepatic microsomes (*53*). HCFC-134a defluorination in hepatocytes is proportional to the head-space HCFC-134a concentration; with 50% HCFC-134a, fluoride release amounted to 12 nmole F^-/mg protein/ 2 hr. HCFC-134a defluorination is decreased in heat-treated hepatocytes, is inhibited by pyrazole and halothane, and is increased in hepatocytes isolated from phenobarbital-treated rats. In hepatic microsomes, HCFC-134a defluorination is also proportional to the head-space HCFC-134a concentration; with 50% HCFC-134a, fluoride release amounted to 6 nmole F^-/mg protein/15 min. The microsomal metabolism of HCFC-134a is inhibited by carbon monoxide, is decreased in the presence of low oxygen concentrations, and is increased in microsomes isolated from Aroclor-treated rats. These results indicate that HCFC-134a undergoes a cytochrome P-450-catalyzed defluorination reaction, and recent studies implicate cytochrome P-450 isoform IIE1 in the reaction (*54*).

Although reaction mechanism studies have not yet been reported, the cytochrome P-450-dependent oxidative metabolism of HCFC-134a may yield 1,2,2,2-tetrafluoroethanol, which may lose HF to give trifluoroacetaldehyde; fluoride is a known metabolite of HCFC-134a (*52,53*). Trifluoroacetaldehyde may be reduced to 2,2,2-trifluorethanol or oxidized to trifluoroacetic acid (Fig. 3). The reductive metabolism of HCFC-134a may yield 1,1-difluoroethene and 1,1,2-trifluoroethane as metabolites (Fig. 3). The possible formation of 2,2,2-trifluoroethane as a metabolite of HCFC-134a is of particular interest in view of the observed gastrointestinal toxicity and hematotoxicity of 2,2,2-trifluoroethanol (*47*).

HCFC-141b. The mammalian toxicity of HCFC-141b has not been studied. HCFC-141b undergoes dechlorination (1.0%) when incubated with rat hepatic microsomes (*44*). The chlorinated analog 1,1,1-trichloroethane is metabolized to 2,2,2-trichloroethanol, which may be excreted as its glucuronide conjugate, and to trichloroacetic acid in rats and humans (*55,56*). With rat hepatic microsomal and nuclear fractions as the enzyme sources, 1,1,1-trichloroethane is metabolized to 2,2,2-trichloroethanol (*57,58*). Based on these precedents, expected metabolites of HCFC-141b include 2,2-dichloro-2-fluoroethanol, which may be further oxidized to dichlorofluoroacetaldehyde and dichlorofluoroacetic acid (Fig. 4). Preliminary studies with ^{19}F-NMR in rats exposed by inhalation to HCFC-141b showed the presence of a single fluorine-containing metabolite in urine in low concentrations, which has tentatively been identified as 2,2-dichloro-2-fluoroethyl glucuronide (*59*). The toxicity of 2,2-dichloro-2-fluoroethanol has not been reported, but, as noted, above, the analog 2,2,2-trifluoroethanol is toxic (*47*).

HCFC-142b. The lowest observed lethal concentration of HCFC-142b in the rat is 50 pph/30 min (*60*). HCFC-142b undergoes dechlorination (0.6%) when incubated with rat hepatic microsomes (*44*). HCFC-142b is mutagenic in the Ames test with *S. typhimurium* TA1535 or TA100 as the test organisms (*35*) and in a BHK21 cell-transformation assay (*34,35*). Rats exposed to HCFC-142b by inhalation (100, 10,000, or 20,000 ppm for 6 hr/day, 5 days/week for 13 or 15 weeks) did not show compound-related effects in a bone marrow cytogenetic assay or in a dominant lethal assay (*61*). No treatment-related effects were observed in a 90-day study of rats and dogs exposed by inhalation to HCFC-142b (1,000 or 10,000 ppm for 6 hr/day, 5 days/week for 90 days) (*62*) or in a 104-week study of rats exposed by inhalation to HCFC-142b (1,000, 10,000, or 20,000 ppm for 6 hr/day, 5 days/week for 104 weeks) (*61*).

Apart from the observation that HCFC-142b undergoes dechlorination (*44*), the metabolism of HCFC-142b has not been investigated. The cytochrome P-450-dependent oxidative metabolism of HCFC-142b may yield 2-chloro-2,2-difluoroethanol as the initial metabolite, and chlorodifluoroacetaldehyde and chlorodifluoroacetic acid may be formed by the further metabolism of the trihaloethanol (Fig. 4). It should be noted that the toxicity of alcoholic metabolites may warrant investigation because of the observation that the analog 2,2,2-trifluoroethanol is toxic (*47*).

Future Research Needs

Toxicology

With the exception of HCFC-22, whose toxicity has already been extensively investigated, there are limited published toxicological data available about the HCFCs that have been proposed as replacements for CFCs. Hence *in vivo* toxicity studies on HCFCs are needed; these studies should include short- and long-term toxicity studies, including, at least, developmental and reproductive toxicity, neurotoxicity, carcinogenic potential, mutagenicity, and immunotoxicity. The objective of these studies should be to identify target organs that are affected and that may signal potential human health hazards. Toxicity studies in *in vitro* systems (freshly isolated cells and cultured cell lines) should be conducted in parallel with *in vivo* toxicity studies, and the choice of cells or cell lines to be used should reflect the target organs observed in *in vivo* studies. These *in vitro* studies should also include mutagenicity studies in submammalian test systems. Finally, *in vitro* systems may find utility in exploring possible mechanisms of toxicity.

As noted above, recent studies indicate that metabolites of HCFC-123 acylate hepatic proteins; because modification of cellular macromolecules may be associated with cytotoxicity, future studies should examine fully the extent and nature of the interactions of HCFC metabolites with cellular macromolecules. In addition, preliminary studies also indicate that 2-chloro-2,2-difluoroethanol is a metabolite of HCFC-132b; because the analog 2,2,2-trifluoroethanol is toxic (*47*), future studies should address the potential toxicity of 2,2,2-trihaloethanol metabolites of HCFCs.

Strategies for toxicity testing CFC replacements have been proposed (*63*). Also, toxicity studies are currently underway; the multi-industry Program for Alternative Fluorocarbon Toxicity Testing (PAFTT) is developing toxicity profiles for several HCFCs (*8*).

Metabolism

Limited metabolic data are available for most HCFCs proposed as replacements for CFCs. Hence metabolism studies should include investigations on the pharmacokinetics of uptake and elimination of HCFCs as well as investigations on the *in vivo* metabolic fate of HCFCs, including identification of metabolites,

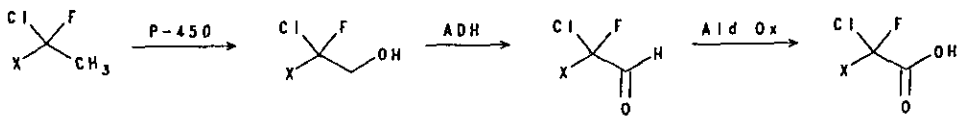

FIGURE 4. Pathway for the oxidative metabolism of HCFC-141b (X = Cl) and HCFC-142b (X = F). P-450, cytochromes P-450; ADH, alcohol dehydrogenase; Ald Ox, aldehyde oxidase.

their possible interaction with cellular constituents, and the mechanisms and routes of clearance of metabolites. *In vivo* studies on the metabolism of HCFCs may exploit the utility of ^{19}F-NMR spectrometry in identifying free and tissue-bound metabolites, as noted above (*37,46,59*). The objective of *in vitro* metabolism studies, particularly in identified target organs or derived cell systems, should be to identify the enzymes catalyzing the metabolism of HCFCs, possible associations between metabolism and toxicity, and interactions of HCFC metabolites with cellular macromolecules. Ultimately, studies on HCFC metabolism should be conducted with purified enzymes or enzyme systems so that reaction mechanisms can be explored in detail. Because the HCFCs constitute a group of closely related analogs, computational analysis of data from structure–metabolism studies may be used to improve predictability of the metabolic fate of HCFCs, interactions of HCFC metabolites with cellular macromolecules, and relationships between HCFC metabolism and toxicity.

Although there is a sense of urgency to develop HCFCs as replacements for CFCs because of mandated reductions in CFC use, it is important to note that CFC replacements will likely enjoy widespread commercial use, which may be associated with human exposure to HCFCs. Therefore, careful toxicological and metabolic evaluation of HCFCs should not be compromised to allow early introduction of HCFCs into commerce.

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