# Toxicology of Chlorofluorocarbon Replacements

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Chlorofluorocarbons (CFCs) are stable in the atmosphere and may reach the stratosphere. They are cleaved by UV-radiation in the stratosphere to yield chlorine radicals, which are thought to interfere with the catalytic cycle of ozone formation and destruction and deplete stratospheric ozone concentrations. Due to potential adverse health effects of ozone depletion, chlorofluorocarbon replacements with much lower or absent ozone depleting potential are developed. The toxicology of these compounds that represent chlorofluorohydrocarbons (HCFCs) or fluorohydrocarbons (HFCs) has been intensively studied. All compounds investigated (1,1-dichloro-1-fluoroethane [HCFC-141b], 1,1,1,2-tetrafluoroethane [HFC-134a], pentafluoroethane [HFC-125], 1-chloro-1,2,2,2-tetrafluoroethane [HCFC-124], and 1,1-dichloro-2,2,2-trifluoroethane [HCFC-123]) show only a low potential for skin and eye irritation. Chronic adverse effects on the liver (HCFC-123) and the testes (HCFC-141b and HCFC-134a), including tumor formation, were observed in long-term inhalation studies in rodents using very high concentrations of these CFC replacements. All CFC replacements are, to varying extents, biotransformed in the organism, mainly by cytochrome P450-catalyzed oxidation of C-H bonds. The formed acyl halides are hydrolyzed to give excretable carboxylic acids; halogenated aldehydes that are formed may be further oxidized to halogenated carboxylic acids or reduced to halogenated alcohols, which are excretory metabolites in urine from rodents exposed experimentally to CFC replacements. The chronic toxicity of the CFC replacements studied is unlikely to be of relevance for humans exposed during production and application of CFC replacements. — Environ Health Perspect 104(Suppl 1):75–83 (1996)

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## Ozone, Chlorofluorocarbons, and the Environment

Stratospheric ozone plays an important role in reducing the amount of ultraviolet radiation reaching the surface of the earth (1–3). This protective shield in the stratosphere is generated by the interaction of molecular oxygen, UV-light, and particles. Ozone is created by the reaction of an oxygen atom, formed by photolytic dissociation of molecular oxygen, with another molecule of oxygen; this reaction is catalyzed by

particles. Ozone itself is cleaved by photolysis, and the formed oxygen atom recombines with another oxygen molecule to reform ozone. Ozone is removed by the reaction with oxygen atoms under constant conditions. The net result of these processes is a dynamic steady state in which the rate of formation of ozone is equal to the rate of ozone degradation (4). This steady state may be disturbed by a variety of factors such as solar radiation, meteorological effects, and the concentrations of chemically active species such as NO and halogen radicals. Halogen atoms such as chlorine catalytically reduce the steady-state concentration of ozone. Reaction of ozone with a chlorine radical forms chlorine monoxide and molecular oxygen; chlorine monoxide reacts with an oxygen atom thereby interfering with the catalytic formation of ozone, which results in a reduction of stratospheric ozone concentrations (Figure 1).

Among other natural sources of chlorine such as vulcanic eruptions, chlorofluorocarbons (CFCs) from anthropogenic emissions have been implicated in the observed reduction of stratospheric ozone. CFCs have been widely used since the 1930s because of their beneficial properties for many industrial and household applications, e.g., in propellants, refrigeration, and dry cleaning. CFCs are highly stable chemicals; thus, they are nonflammable, nontoxic, and inexpensive; they are ideally suited for refrigeration and metal degreasing. Ironically, these beneficial properties for commercial applications have made CFCs troublesome for atmospheric chemistry. Due to their high volatility, a significant amount of the produced CFCs was released into the atmosphere; due to their chemical inertness, CFCs are not degraded in the troposphere and reach the stratosphere. Under the intensive UV-light in the stratosphere, CFCs are photolytically cleaved to yield chlorine radicals, which then may participate in ozone destruction.

Besides participating in the destruction of stratospheric ozone, the release of CFCs may also contribute to global warming. Several anthropogenic compounds including CFCs influence the reflection of infrared radiation from the surface of the earth. Their increased release has been associated with the observed increases in average temperatures in the atmosphere. Global warming potential (GWP) expresses the increase in earthward infrared radiation flux due to the emission of a unit mass of a given compound and is expressed relative to a reference compound, usually carbon dioxide. CFCs are estimated to represent approximately 20% of the man-made greenhouse effect (5) (Table 1).

Due to the potential environmental and health effects of ozone depletion, e.g., increased incidences of skin cancer and cataracts, the use of CFCs has been reduced by international agreements.
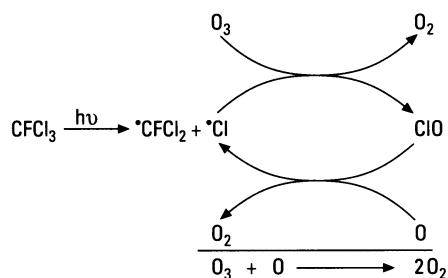


Figure 1. Mechanism of catalytic ozone destruction by chlorine radicals formed from chlorofluorocarbons in the stratosphere.

**Table 1.** Estimated participation of different anthropogenic gases to global warming.

| Chemical | Source | Contribution to global warming, % |
|---|---|---|
| Carbon dioxide | Energy generation, traffic, heating | 50 |
| Chlorofluorocarbons | Refrigeration, foam blowing | 20 |
| Methane | Agriculture | 15 |
| Tropospheric ozone | Solar radiation, locally increased by anthropogenic emissions | 7 |
| $N_2O$ | Agriculture | 5 |

Several developed countries have ceased the production of CFCs and banned their application; however, for many applications, CFCs are essential chemicals for industrialized countries. Thus, replacements for CFCs have to be developed (5). Nonhalogenated compounds, due to their flammability and other disadvantages, may only substitute for part of the chlorofluorocarbon applications. Chlorofluorohydrocarbons (HCFCs) and fluorohydrocarbons (HFCs) have been developed as replacements for CFCs. HCFCs will only be an interim solution because they retain some ozone-depleting potential and will be phased out by the year 2020. However, they are considered necessary as interim replacements for CFCs due to problems in adjusting several technologies to HFCs. HCFCs and HFCs show many beneficial properties of CFCs, e.g., low flammability, high volatility and suitability as refrigerants, but have a significantly lower potential for ozone depletion and global warming (6). The much lower potential for ozone depletion of HCFCs is due to the presence of hydrogen atoms in the molecules, which makes these compounds more readily degradable by hydroxyl radicals in the atmosphere, significantly reduces their atmospheric lifetime, and prevents them from reaching the stratosphere (7–9). Due to the absence of chlorine, hydrofluorocarbons are not expected to participate in ozone depletion, but depending on the content of fluorine, they exhibit a GWP. Their GWP depends on the number of fluorine atoms in the molecule and increases with higher fluorine substitution. With respect to GWP, a compromise with flammability has to be reached.

Due to the expected widespread application of HCFCs and HFCs with the potential of human exposure at the workplace and also in the general environment

(including the home), the toxicology of these groups of chemicals has been intensively studied. Many of these studies were performed by PAFT (Program of Alternate Fluorocarbon Toxicity Testing), a consortium of chlorofluorocarbon producers. Many of these studies have been published or are available as reports. This review will summarize the toxicology of selected compounds from the many potential CFC replacements. The compounds listed in Table 2 have been studied more intensively and have already been introduced in the market or are likely to be. In general, their ozone depletion potential and their GWP is significantly lower than those of CFCs (Table 2).

Other compounds are also considered for special applications such as 1,1,1,2,3,3,3-heptafluoropropane (HFC-227) for the generation of pharmaceutical aerosols; however, the information on potential toxic effects of these compounds is only partially available and is therefore not included here.

## Toxicology of Chlorofluorocarbons and Chlorofluorocarbon Replacements

Some general principles describing solvent toxicology are also applicable to CFCs and alternates. Although varying in chemical structure and physicochemical properties, most solvents produce a typical set of toxic effects upon acute administration of high doses (10). The most often induced effects are dysfunction of the central nervous system and, after sufficiently high exposure, narcosis. These effects, observed after acute exposure to most solvents, are based on the distribution into lipid membranes due to the lipid solubility of the solvents. The

effects are usually completely reversible if the exposure is survived. The characteristic effects of repeated solvent exposure are irreversible toxic effects such as organ necrosis or cancer. The magnitude of these chronic toxic effects and the organs affected are different between individual solvents and depend on the rate and site of solvent biotransformation to toxic metabolites. The interaction of these metabolites with cellular macromolecules may initiate irreversible changes in cellular function resulting in cell death or cancer.

Since most CFCs and their replacements are lipophilic, upon exposure they are rapidly taken up and distributed; narcosis is often the major toxic effect observed after acute exposure. The lack of chronic toxicity observed with CFCs is based on their lack of biotransformation reactions. The chronic toxic effects seen with CFC replacements are probably based on the ability of experimental animals, and presumably, humans, to metabolize these compounds. The formed reactive intermediates and their covalent binding to cellular constituents or, more likely, the interference of stable metabolites, which are formed with homeostasis of the organism, are assumed to be responsible for the chronic toxicity observed with some CFC replacements.

## Toxicology of Chlorofluorocarbons

No data on the environmental toxicology of CFCs are available, but due to their physiochemical properties, there is only a low potential for toxicity and no bio-accumulation is expected. The acute toxic effects of trichlorofluoromethane (CFC-11) and dichlorodifluoromethane (CFC-12) in animals are restricted to narcosis observed after inhalation of more than 20,000 ppm resp. 200,000 ppm and

**Table 2.** Comparison of chlorofluorocarbon replacements to trichlorofluoromethane.

| Compound | Intended use | ODP | GWP | Estimated atmospheric lifetime, years |
|---|---|---|---|---|
| Trichlorofluoromethane | Refrigeration, blowing agent | 1 | 1 | 144 |
| 1,1-Dichloro-1-fluoroethane (HCFC-141b) | Blowing agent, solvent | 0.1 | 0.12 | 11 |
| 1,1,1,2-Tetrafluoroethane (HFC-134a) | Refrigeration | – | 0.4 | 14 |
| Pentafluoroethane (HFC-125) | Refrigeration, fire extinguisher | – | 0.84 | 41 |
| 1-Chloro-1,2,2,2-tetra-fluoroethane (HCFC-124) | Blowing agent, refrigeration | 0.02 | 0.1 | 1.3 |
| 1,1,1-Trifluoro-2,2-dichloroethane (HCFC-123) | Refrigeration, blowing agent | 0.02 | 0.02 | 1.6 |

Abbreviations: ODP, ozone depletion potential; GWP, global warming potential.

cardiac sensitization to catecholamines after very high exposure concentrations. No adverse effects except eye irritation and slight neurotoxicity were seen in humans exposed to very high concentrations (>20,000 ppm CFC-11 and >40,000 ppm for CFC-12) as volunteers or in laboratory accidents and by application of CFCs as propellants in pharmaceutical aerosols (*11*). Both compounds are very weak irritants and did not show teratogenic effects in experimental animals. In subchronic and chronic toxicity studies, both CFC-11 and CFC-12 did not induce pathological changes in any of the organs examined in rats and they did not induce tumors in rats and mice (*12,13*). No adverse effects were observed in humans exposed for 4 weeks, 8hr/day to 1000 ppm of CFC-11.

Both CFC-11 and CFC-12 are not genotoxic in a variety of *in vitro* test systems. The lack of chronic toxicity of CFC-11 and CFC-12 is most likely due to the lack of biotransformation of these compounds in the mammalian organism. CFCs are rapidly taken up through the lung after inhalation exposure; inhaled CFCs are rapidly and quantitatively exhaled unchanged in experimental animals and in humans; metabolites formed were not detected in urine. Moreover, $^{14}CO_2$ was not exhaled after inhalation of $^{14}C$-labeled CFC-12 in humans (*11*). Only under the specific conditions of very low oxygen tension, metabolism of CFC-11 to chloride and CFC-12 was observed in liver microsomes of rats. The relevance of this observation for the *in vivo* situation is questionable because oxygen inhibited metabolite formation; therefore, this pathway may be a very minor contributor to the disposition of CFC-11.

## Toxicology of Chlorofluorcarbon Replacements

### The Role of Biotransformation Reactions in the Toxicity of CFC Replacements.
Parallel to the extensive toxicity studies on CFC replacements, several groups have been focusing on the metabolic transformations of HCFCs and HFCs. In principle, HCFCs and HFCs undergo identical biotransformation reactions, as has been elaborated for other halogenated hydrocarbons (*14–17*).

Briefly, HCFCs and HFCs have been shown to undergo cytochrome P450-catalyzed oxidation reactions of the C–H σ bond or, in the absence of oxygen, cytochrome P450-catalyzed reduction reactions, as demonstrated for some polyhalogenated

alkanes (*18–20*). The initial metabolites formed by the cytochrome P450-catalyzed oxidation reactions are geminal halohydrins, which may loose $HX$ (X = Cl,F) to form acyl halides or aldehydes. The acyl halides thus formed are rapidly hydrolyzed to give stable carboxylates, which are excreted in urine as final metabolites. The formed haloaldehydes may be excreted as conjugates, oxidized to carboxylates, or reduced to halogenated alcohols, which are excreted as such or as sulfate or glucuronide conjugates.
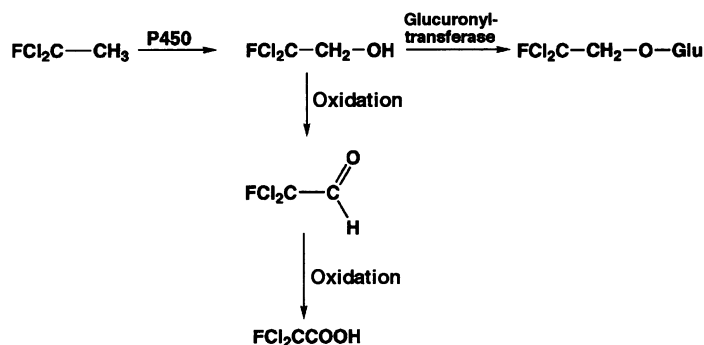
The cytochrome P450-catalyzed reduction of HCFCs yields haloalkanes with one halogen atom replaced by a hydrogen or haloalkenes formed by the dehydrohalogenation of haloalkanes. Haloalkanes and haloalkenes thus formed may also be oxidized by cytochrome P450 to give halogenated aldehydes or carboxylic acids as stable metabolites; fluoroalkenes may also undergo conjugation with glutathione. The formation of acyl halides represents a bioactivation reaction for HCFCs and HFCs because the formed intermediary acyl halides are electrophiles and may react with nucleophilic sites in macromolecules as demonstarted for halothane and some HCFCs (*21,22*). The reductive formation of haloalkenes may also represent a bioactivation reaction and contribute to HCFC toxicity. Some fluoroalkenes such as chlorotrifluoroethene are nephrotoxic; their nephrotoxicity is based on their biotransformation by glutathione conjugation followed by bioactivation of the derived cysteine *S*-conjugates by cysteine conjugate β-lyase (*23*). In addition to short-lived reactive intermediates, the formed stable metabolites may also inititate toxic effects of HCFs, especially under the conditions of chronic exposure. Under these conditions, halogenated carboxylic acids, which are only slowly eliminated due to a high degree of plasma protein binding, may accumulate to toxic concentrations. Some halogenated carboxylic acids have been shown to cause toxic effects. Trichloroacetic acid and dichloroacetic acid, for example, cause liver toxicity and induced hepatic carcinoma in rodents after long-term administration in drinking water (*24–26*). Repeated administration of trifluoroacetic acid to rats caused hepatomegaly and peroxisome proliferation in rats (*27*). Dichloroacetic acid and bromo- and dibromoacetate are testicular toxins (*28,29*). Moreover, some fluorinated aldehydes and alcohols are toxic in animals or show toxic effects *in vitro*. For example,

chlorodifluoroacetaldehyde inhibits esterases *in vitro* and trifluoroethanol causes intestinal toxicity in rodents (*30,31*).
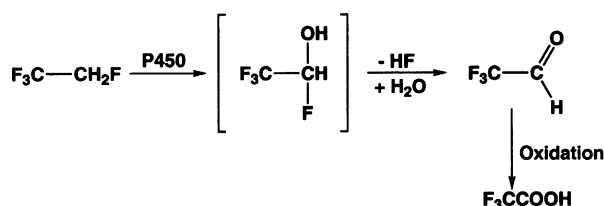
### Uptake, Biotransformation, and Elimination of Chlorofluorocarbon Replacements in Mammals.
Studies on the biotransformation of CFC replacements have been performed *in vitro* in rat liver subcellular fractions and in human liver microsomes and in rats *in vivo*. Cytochrome P450 enzymes have been shown to catalyze the enzymatic oxidation of the C–H bond in CFC replacements. The cytochrome P450 enzyme 2E1 has been demonstrated to play an important role in the oxidation of CFC replacements. This enzyme is present in rat and human liver and in rat kidney; its concentration in the liver is influenced by a variety of inducers such as organic solvents and ethanol but also by dietary factors and some drugs. The concentrations of this enzyme in human liver samples varies approximately 5-fold; therefore, differences in the extent of the biotransformation of CFC replacements can be expected between individuals (*32–34*).

In pyridine-induced rat liver microsomes, 1,1-dichloro-1-fluoroethane (HCFC-141b) is oxidized to 2,2-dichloro-2-fluoroethanol. In rats, HCFC-141b is rapidly absorbed after inhalation and undergoes a saturable uptake in the rat. In rats exposed by inhalation in a closed chamber to varying concentrations of HCFC-141b, low concentrations of the glucuronide of 2,2-dichloro-2-fluoroethanol were found as a metabolite in urine after 11,400 ppm. A linear relationship between HCFC-141b exposure concentrations and the urinary excretion of 2,2,-dichloro-2-fluoroethanol was observed. After exposure of rats to 40,000 ppm HCFC-141b, dichlorofluoroacetic acid was also found as a metabolite in urine (Figure 2). Overall, the level of biotransformation of HCFC-141b in rats is low (*35–37*).

The biotransformation of 1,1,1,2-tetrafluoroethane (HFC-134a) has been studied in rat liver microsomes, freshly isolated rat hepatocytes, in human hepatocytes, and in rats *in vivo*. In rat and human liver microsomes, HFC-134a undergoes limited cytochrome P450 2E1 metabolism to inorganic fluoride (*38,39*). The extent of biotransformation of HFC-134a in rats is also low. In studies with male and female Wistar rats exposed once to 10,000 ppm $^{14}C$-labeled HFC-134a, total radioactivity present in expired air, urine, and feces amounted to approximately 1% of the

$$FCl_2C-CH_3 \xrightarrow{P450} FCl_2C-CH_2-OH \xrightarrow{\text{Glucuronyl-transferase}} FCl_2C-CH_2-O-Glu$$

Oxidation

$$FCl_2C-\overset{O}{\underset{H}{C}}$$

Oxidation

$$FCl_2CCOOH$$

**Figure 2.** Metabolic pathway of HCFC-141b in rats. Dichlorofluoroacetic acid was only formed after exposure to very high concentrations (Glu = glucuronide).

$$F_3C-CH_2F \xrightarrow{P450} \left[ F_3C-\overset{OH}{\underset{F}{CH}} \right] \xrightarrow[+H_2O]{-HF} F_3C-\overset{O}{\underset{H}{C}}$$

Oxidation

$$F_3CCOOH$$

**Figure 3.** Biotransformation of HCFC-134a in rodents.

inhaled dose in both sexes. More than 60% of the inhaled dose was recovered within 1 hr after cessation of exposure as unchanged HFC-134a; the remaining radioactivity was excreted as carbon dioxide in exhaled air, as trifluoroacetic acid in urine, and as unidentified metabolites in feces (Figure 3) (40).

Pentafluoroethane (HFC-125) is metabolized very slowly in the rat in vivo. The major urinary metabolite has been identified as trifluoroacetic acid; the concentrations of this metabolite in the urine of rats comparatively exposed to identical concentrations of HFC-125, 1-chloro-1,2,2,2-tetrafluoroethane (HCFC-124), and 1,1-dichloro-2,2,2-trifluoroethane (HCFC-123) were lowest in HFC-125-treated rats. Concentrations of trifluoroacetic acid in urine were 10-fold higher in rats exposed to HCFC-124 and 40- to 50-fold higher in rats exposed to HCFC-123. These differences correlate well with differences seen in the extent of biotransformation of these three CFC replacements in rat liver microsomes, with the extent of hepatic protein trifluoroacetylation, and with the predicted enthalpies of activation for hydrogen atom abstraction within this series of compounds (41).

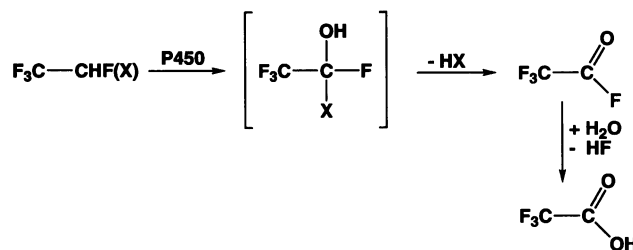Limited metabolic data have been published on the bioactivation of HCFC-124. Olson et al. (39) exposed rats to 10,000 ppm HCFC-124 for 2 hr and found both inorganic fluoride and trifluoroacetic acid, both products of an oxidative metabolism, in their urine. Increases in urinary fluoride levels in rats exposed by subchronic inhalation to HCFC-124 have also been reported (Figure 4).

The metabolism of HCFC-123 has been studied more intensively than that of other CFC replacements. The results suggest that HCFC-123 biotransformation is very similar to that of the anestetic halothane. As seen with halothane, trifluoroacetic acid is the major product of HCFC-123, both in rat and human liver microsomes and in rats in vivo. Figure 5 shows the pathways of HCFC-123 biotransformation in rodents (42–44).

Both halothane and HCFC-123 are biotransformed to trifluoroacetic acid, which has long been known as a major

halothane metabolite (45,46). The identification of trifluoroacetic acid as a major microsomal metabolite of HCFC-123 demonstrates that an identical reaction occurs with HCFC-123. Trifluoroacetate is formed by the oxidation of the carbon–hydrogen bond in HCFC-123 followed by the loss of hydrochloric acid to give trifluoroacetyl chloride. This reactive acylating agent may bind to proteins. Indeed, studies on the in vivo metabolism and covalent binding of HCFC-123 using immunoblotting with a hapten-specific antitrifluoroacetyl protein serum and $^{19}$F-NMR spectra identified $N^{\varepsilon}$-trifluoroacetylated lysine residues in proteins (21). However, studies on the microsomal metabolism also showed differences in the structures of metabolites formed from halothane and HCFC-123. Chlorodifluoroacetic acid is a metabolite formed from HCFC-123 but not from halothane. The mechanism for the enzymatic formation of chlorodifluoroacetetate is unclear; however, experimental evidence points to 1,1-dichloro-2,2-difluoroethene as an intermediate metabolite of HCFC-123. Chlorodifluoroacetate may be formed from HCFC-123 by a cytochrome P450-mediated oxidation of this potential metabolite. Cytochrome P450 has been shown to oxidize alkanes to olefins (47). Oxidation of this olefin by cytochrome P450 proceeds under rearrangement and gives chlorodifluoroacetate (48).

1,1-Dichloro-2,2-difluoroethene is also a likely intermediate metabolite of HCFC-123 formed in vivo in rats. After exposure to HCFC-123, a conjugation reaction of 1,1-dichloro-2,2-difluoroethene with glutathione (42,44) forms an S-conjugate, which is processed by the enzymes of mercapturic acid formation (49) to form urinary mercapturic acid, N-acetyl-S-(2,2-dichloro-1,1-difluoroethyl)-L-cysteine. This urinary mercapturic acid has been identified in the urine of rats and guinea pigs after exposure to low concentrations of HCFC-123.

$$F_3C-CHF(X) \xrightarrow{P450} \left[ F_3C-\overset{OH}{\underset{X}{C}}-F \right] \xrightarrow{-HX} F_3C-\overset{O}{\underset{F}{C}}$$

$$\xrightarrow[-HF]{+H_2O} F_3C-\overset{O}{\underset{OH}{C}}$$

**Figure 4.** Biotransformation of HCFC-124 (X = Cl) and HCFC-125 (X = F) in rodents.
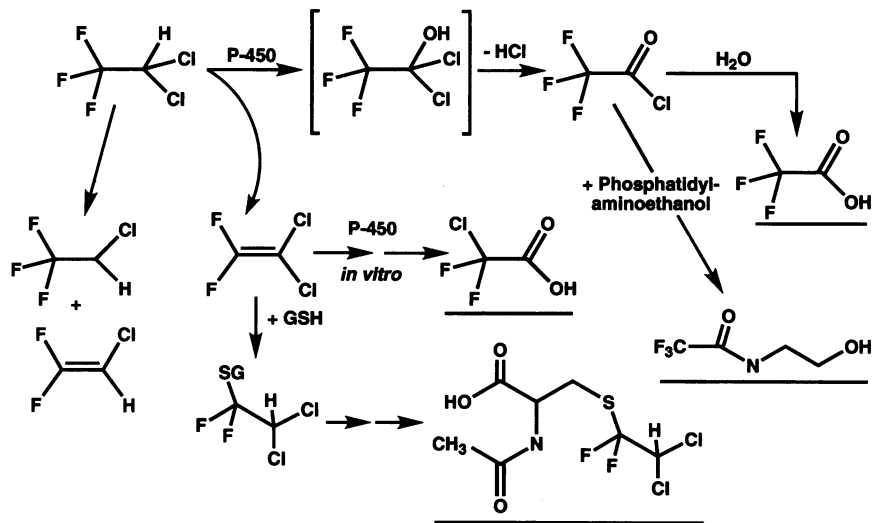
**Figure 5.** Metabolic pathways of HCFC-123 in rats *in vivo* and in rat liver microsomes. Metabolites identified [trifluoroacetic acid, *N*-trifluoroacetyl-2-aminoethanol, and *N*-acetyl-*S*-(1,1-dichloro-2,2-difluoro)-L-cysteine] are underlined.

Because the microsomal incubations did not contain glutathione, this metabolite was not observed *in vitro*. Instead, oxidation by cytochrome P450 to chlorofluoroacetic acid occurred. Cytochrome P450-catalyzed oxidation *in vivo* seems to occur only after 1,1-dichloro-2,2-difluoroethene doses that saturate the capacity of the glutathione *S*-transferases responsible for 1,1-dichloro-2,2-difluoroethene conjugation (*48*). Under reduced oxygen tension, reduction of HCFC-123 to give 1-chloro-2,2,-trifluoroethane and 1-chloro-2,2-difluoroethene has also been observed in low yields in rat liver microsomes (*50*) but not in rats *in vivo*.

**Acute and Subchronic Toxicity of Chlorofluorocarbon Replacements.** The acute toxicity of CFC replacements by inhalation or oral administration is low. The 15-min LC$_{50}$ values are usually above 500,000 ppm, and toxic effects consist of impairment of coordination of response reactions to external stimuli and other signs of depression of the central nervous system. Moreover, CFC replacements have a low potential for skin and eye irritation. Similar to other halogenated hydrocarbons, high concentrations of some CFC replacements cause the mammalian heart to react abnormally to adrenaline, which results in cardiac arrythmias. For example, such toxic effects are seen after exposure of Beagle dogs to 100,000 ppm of HFC-134a and 5,000 ppm of HCFC-141b (*37,51*).

Studies describing the subchronic toxicity of CFC replacements are available for

HCFC-141b, HFC-134a, HFC-125, HCFC-124, and HCFC-123 (*52–56*). In a 13-week inhalation study, Fischer 344 rats were exposed 6 hr/day, 5 days/week to HCFC-141b concentrations up to 20,000 ppm. Signs of central nervous system depression were observed after inhalation of the highest concentration. After both 4 and 13 weeks of exposure, plasma concentrations of cholesterol, triglycerides, and glucose were slightly raised in the rats exposed to 20,000 ppm. There were no changes in hematological or histopathological findings that could be attributed to exposure to dichlorofluoroethane.

The potential for subchronic toxicity of HFC-134a is also low. Rats were exposed for 13 weeks to concentrations up to 50,000 ppm HFC-134a (6 hr/day, 5 days/week). Ten males and 10 females from each group were killed in week 14 following their last exposure, and the remaining animals were killed in week 18 following a 4-week recovery phase. Small differences in body-weight gain and food consumption were noted between treated and control animals, which were not considered to be related to the exposure to HFC-134a. There were no significant differences in blood or urine clinical chemistry parameters, hematology parameters, or organ weights and no treatment-related macroscopic or microscopic findings (*57–61*).

Four groups of 20 male and 20 female Sprague-Dawley rats were exposed to HFC-125 (6 hr/day, 5 days/week) for 4 consecutive weeks at concentrations up to

15,000 ppm. Ten rats per sex at each concentration level were kept untreated for 4 weeks after the end of exposure. No mortality occurred; there were no compound-related effects on body weight, clinical signs, hematology, biochemistry, urinanalysis, organ weight, and tissue morphology. No increases in plasma and urinary fluoride concentrations were seen. Levels of peroxisomal β-oxidation activity of the liver were comparable to controls at all exposure concentrations (*62*).

In a 90-day inhalation toxicity study (*63*), both sexes of rats and mice were exposed to HCFC-124 for 6 hr/day and 5 days/week at concentrations up to 50,000 ppm. There were no compound-related effects on mortality, body weight, food consumption, clinical signs, hematology, organ weight, or tissue morphology at any exposure concentrations. During exposure to 50,000 ppm, rats were less responsive to stimuli compared to control rats. Relative to blood chemistry measurements, at a 45-day clinical evaluation interval, male rats and mice exposed to 15,000 or 50,000 ppm HCFC-124 had lower serum triglyceride concentrations than controls; female rats at 50,000 ppm showed increased alkaline phosphatase activity. At all exposure levels, fluoride concentrations in blood and urine were elevated. The mild diuresis observed at several sampling intervals in rats was considered a result of increased osmotic activity from the excreted fluoride ions rather than a direct effect of the compound.

The subchronic toxicity of HCFC-123 has been evaluated in four inhalation toxicity studies from 1 to 3 months in duration with exposure levels ranging from 300 to 20,000 ppm. Three of the studies used rats only; one study used both rats and dogs. Increases in relative liver weights were seen after inhalation of 1,000 ppm and higher HCFC-123; except for the 90-day exposure, minimal histopathological changes were noted. Clinical chemistry and biochemical investigations showed induction of peroxisomal β-oxidation, a decrease in serum cholesterol and triglyceride levels, and increased urinary fluoride levels (*58–60*).

**Chronic Toxicity and Tumorigenicity of Chlorofluorocarbon Replacements.** Chronic toxicity and carcinogenicity bioassays have been reported for HCFC-141b, HFC-134a, and HCFC-123 (*61,64,65*).

Sprague-Dawley rats were exposed (whole body) by inhalation 6 hr/day, 5 days/week for 104 weeks to concentrations up to 15,000 ppm HCFC-141b. The

highest exposure level was increased to 20,000 ppm after 17 weeks of exposure, in light of the absence of any toxicity signs up to this point. The survival rate in the exposed groups did not differ from those in the control groups, and no clinical signs of toxicity could be associated with exposure to HCFC-141b. There was a slight but statistically significant reduction in body-weight gain and food consumption in the high-dose group, particularly in the males. There were no intergroup differences in the incidence of ocular abnormalities or in any of the hematological parameters. There were no blood and urinary biochemical effects, although occasional decreases in serum triglycerides in high-dose group rats may have been related to treatment. Organ-weight effects were also not changed by exposure to HCFC-141b. There were no intergroup differences with respect to macroscopic and microscopic examinations at 52 weeks. After 104 weeks increased testicular weights in the highest exposure group were seen. Histological examinations did not show any treatment-related effects except a small increase in the number of vacuolated sinusoidal histiocytes of the cervical lymph nodes in the high-concentration group and a statistically significant increase of the incidence of hyperplasia and benign tumors of the testicular interstitial cells (Leydig cells) in the medium- and high-concentration groups (61).

Toxic effects on the testes were also observed in rats exposed to HFC-134a [by gavage of 300 mg/kg body weight (bw) HFC-134a in corn oil, 5 days/week for 52 weeks]. After 125 weeks in this study, HFC-134a did not increase the incidence of tumors in any of the organs from the treated group when compared with the control groups (13).

A combined chronic toxicity/carcinogenicity study was conducted by whole body inhalation exposures with concentrations up to 50,000 ppm HFC-134a for 6 hr/day, 5 days/week. All groups had a similar survival rate. The differences in body weight and food consumption reflected only biological variation and were not compound related. There was no evidence of toxicity effects at any exposure level in the clinical chemistry and hematology parameters investigated. The only treatment-related effect of toxicological significance was confined to the testes of male rats exposed to 50,000 ppm. There was a statistically significant increase in weight of the testes over controls and an increased incidence of Leydig cell hyperplasia and benign Leydig cell tumors.

In a chronic toxicity/carcinogenicity study, male and female rats were exposed to concentrations of up to 5,000 ppm HCFC-123 for 24 months. The exposed rats showed an increased survival rate and a decreased incidence of a variety of age-related lesions. In addition, dosed animals had decreased serum triglyceride and glucose concentrations, lower body weights, and lower body-weight gains. At the end of the study, increased incidences of hepatocellular adenomas were seen in the high-dose males and in females exposed to 300, 1,000, and 5,000 ppm. Hepatic cholangiofibromas were also observed in females in the highest dose group, and increased incidences of pancreatic acinar cell carcinoma were seen at all doses in males. In addition, the incidence of testicular interstitial adenomas was increased in all groups of dosed males.

**Genetic Toxicology of Chlorofluorocarbon Replacements.** The mutagenic properties of CFC replacements were investigated in bacterial assays, chromosomal abberation assays in cultured mammalian cells *in vitro*, and in *in vivo* micronucleus assays. In most studies with pure CFC replacements, negative responses were obtained, both in *Salmonella typhimurium* and in *Escherichia coli*. Moreover, CFC replacements did not show evidence of clastogenic activity in cultured Chinese hamster ovary cells or in human lymphocytes with and without metabolic activation by rat liver S-9. *In vivo*, the majority of tests for clastogenic activity did not indicate an effect of exposure to high CFC replacement concentrations. In summary, the data of the mutagenicity studies suggest that CFC replacements do not have mutagenic properties (53–56).

**Reproductive Effects and Teratogenicity of CFC Replacements.** There was no evidence of teratogenic or embryotoxic effects in pregnant rabbits exposed to concentrations up to 12,600 ppm or in pregnant rats exposed to 3,200 or 7,900 ppm of HCFC-141b, although signs of maternal toxicity were observed at the higher dose in rats and above 4,200 ppm in rabbits. A two-generation inhalation study in rats demonstrated a no-observed effect level of 8,000 ppm for reproductive parameters (66). At a higher concentration, 20,000 ppm, a nonreproducible decrease was observed in the number of litters and the number of pups per litter; there was also some retardation of sexual maturation of male pups that may have been caused by the slight body-weight growth retardation.

HFC-134a showed no adverse effects on fertility in a limited study in mice. It was not teratogenic in rats and rabbits. Only nonspecific effects on fetal maturation in the form of delayed ossification in the rat were observed at 50,000 ppm and above. Developmental toxicity studies with HCFC-125 by inhalation were carried out both in rats and rabbits. No evidence of embryotoxicity or teratogenicity was seen, even at exposure levels as high as 50,000 ppm. No evidence of embryotoxicity or teratogenicity of HCFC-124 was seen in developmental studies by inhalation in rats and rabbits at exposure levels as high as 50,000 ppm. Minimal evidence of maternal toxicity was seen at concentrations of 15,000 ppm and above in each of these studies. When pregnant rats and rabbits were exposed to HCFC-123 at very high concentrations during a critical part of their gestation, there was no evidence of teratogenicity or embryotoxicity and evidence of only slight maternal toxicity.

**Relevance of Tumorigenic Effects of Chlorofluorocarbon Replacements in Animals for Human Risk Assessment.** Due to low acute and subchronic toxicity of CFC replacements, the toxic effect of concern for human risk assessment is the tumorigenicity observed in rodents after long-term inhalation of high concentrations of CFC replacements. Benign testicular tumors were observed after inhalation of HCFC-141b, HFC-134a, and HCFC-123. In addition, HCFC-123 caused hepatic and pancreatic tumors. Because a variety of *in vitro* and *in vivo* test systems have not demonstrated genotoxic activity of these CFC replacements, tumor initiation is probably caused by nongenotoxic mechanisms.

Benign tumors of the testicular interstitial cells (Leydig cell adenoma) are common in the aging rat. Benign Leydig cell tumors appear late in life and are not life threatening to rats. They are associated with the senescence process. The spontaneous incidence of this tumor type varies from one strain to another, ranging from a few percent in Sprague-Dawley rats up to 100% in some Wistar-derived and in Fischer 344 rats (67). These tumors do not usually progress to malignancy in the rat (e.g., no malignant Leydig cell tumors were found in several thousand control Fischer rats) (68,69). Leydig cells secrete sex hormones (e.g., testosterone, dihydroandosterone, estradiol). The high incidence of hyperplasia and tumors of these testicular cells in old rats may be related to senile

endocrine disturbance (70). Leydig cell tumors in the rat are induced by a variety of different chemical structures. Their formation is associated with hormonal imbalances, especially imbalances of sex hormones, and is also observed after treatment with estrogens. It is therefore assumed that HCFC-141b and HFC-134a exaggerate hormonal disturbances linked with senility resulting in Leydig cell hyperplasia and tumor formation. The role of parent HCFC or of metabolites formed by biotransformation in the testicular toxicity is not defined. However, fluorinated aldehydes such as trifluoroacetaldehyde are testicular toxins; their formation may contribute to the observed toxic effects (71).

The pathways of HCFC-123 metabolism and the lack of genotoxicity of HCFC-123 and the identified metabolites suggest that nongenotoxic effects are most likely responsible for the tumor induction observed after long-term inhalation of HCFC-123. Moreover, the lack of covalent binding of HCFC-123 in testes and pancreas (42), two target organs of HCFC-123 tumorigenicity, and the lack of detectable cytochrome P450 2E1-expression in these organs indicate that metabolic activation reactions do not play a role in HCFC-123 toxicity to these organs. However, the mechanisms responsible for the tumor induction by HCFC-123 are unclear. HCFC-123 and its major metabolite, trifluoroacetic acid, induce hepatomegaly; HCFC-123 also induced an increase in hepatic beta-oxidation, a marker for hepatic peroxisome proliferation. Peroxisome proliferation has been demonstrated to be causally linked to the induction of liver tumors in rodents by

nongenotoxic, receptor-mediated mechanisms involving cell proliferation (72–74); however, an increased cell proliferation in the liver could not be demonstrated after 12 months of HCFC-123 exposure in rats. Moreover, the relationship between the disturbances in lipid metabolism, peroxisome proliferation, and hepatic tumor induction is unclear. The induction of testicular interstitial cell-adenomas by HCFC-123 may also be related to hepatic peroxisome proliferation. Perfluorooctanoate, a perfluorinated carboxylic acid, that is structurally related to trifluoroacetic acid (the major metabolite of HCFC-123), also causes testicular adenomas, probably by induction of hormonal imbalances (75). The minor HCFC-123 metabolite 1-chloro-2,2,2-trifluoroethane (76) is a testicular toxin; its testicular toxicity likely is based on its biotransformation to trifluoroacetaldehyde (77). The formation of this metabolite could also contribute to toxic effects on the testes after long-term administration. Peroxisome proliferation or disturbances of lipid metabolism may also be causative factors in the induction of pancreatic tumors by HCFC-123; however, a more in-depth understanding of the issues needs to be developed.

*Environmental Toxicology of Chlorofluorocarbon Replacements.* Most CFC replacements have been only partly tested for toxic effects on organisms in the environment. HCFC-141b, HFC-134a, and HCFC-123 have only a low acute toxicity to aquatic organisms. The low octanol/water coefficients of these compounds and their high volatility make bioaccumulations very unlikely. Based on the anticipated production volumes and

the mechanisms of atmospheric degradation, the contribution of released fluoride from these compounds to total atmospheric flux is calculated to be very low. The formation of hydrochloric and hydrofluoric acid from HCFC replacements and their collection in rainwater is also expected to represent only a very minor contribution to the generation of acid rain. At the anticipated production volumes and the amounts expected to be released, trifluoroacetic acid formed by atmospheric degradation of HCFC-123, HCFC-124, and HCFC-125 also will contribute only in a very minor way to acid rain (78).

## Conclusions

Due to anticipated widespread use of CFC replacements, there has been a major effort to characterize their direct toxic effects on humans and their effects on the environment. The selected CFC replacements are characterized by a low potential for acute and chronic toxicity and can be handled safely, with a much-reduced environmental impact compared to CFCs. The chronic toxic and tumorigenic effects observed with selected CFC replacements are not likely to be of relevance for human risk assessment of CFC-replacement exposure during production and application. Based on the available data and the lack of genotoxicity of CFC replacements, the observed effects, with the exception of HCFC-123, likely occur only after application of very high doses not expected to be encountered by humans. Further research is required for a better understanding of the chronic toxicity of HCFC-123 and the mechanisms involved.

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