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FLUORINATED HYDROCARBONS USED AS REFRIGERANTS – TOXICOKINETICS AND EFFECTS IN HUMANS

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The photograph on the front page was taken by Bo Sahlberg.

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”Målet är ingenting vägen är allt
- emellan Himmel och Jord”

Robert Broberg

Till Melker

ABSTRACT

Hydrofluorocarbons (HFCs) and hydrochlorofluorocarbons (HCFCs) have replaced the chlorofluorocarbons (CFCs) in refrigeration installations and air-conditioning applications. As substitution progressed, some refrigeration mechanics experienced symptoms of inflammation. The new refrigerants were well studied in animals, but data on toxicology and toxicokinetics in humans were scarce. The aim of this thesis was to study human toxicokinetics and effects of HFC-134a and HFC-143a. Moreover, autoimmune responses due to exposure to halogenated hydrocarbons used as refrigerants were investigated and a case of myocardial infarction was discussed.

Toxicokinetics and Effects (Study I and II): Ten male volunteers were exposed to 500 ppm HFC-134a and/or HFC-143a in an exposure chamber (2 h, workload of 50 W). Blood, exhaled air, and urine were collected up to 19 h post-exposure and analysed for HFC with gas chromatography. The experimental data was described by a physiologically-based toxicokinetic (PBTK) model. Markers of inflammation (C-reactive protein, serum amyloid A protein, D-dimer, fibrinogen) and uric acid were analysed in plasma collected before and 21 h post-exposure. The volunteers rated symptoms related to irritation and CNS-symptoms on a visual analogue scale before, during and after exposure.

The increase in blood upon exposure to HFC was fast, and apparent steady-states (9.4 µM for HFC-134a, 4.8 µM for HFC143a) were reached within minutes. The post-exposure decrease in blood was fast as well and parallel to that of exhaled air. The urinary excretion of HFC-134a and HFC-143a was less than 0.002% of the inhaled amount with half-times of 58 and 53 min. The experimental data fitted well within a PBTK framework, and the relative respiratory uptake was estimated to 3.7 % for HFC-134a and 1.6% for HFC-143a. No effect due to exposure was seen on symptom ratings or in the electrocardiographic recordings. Fibrinogen plasma concentration had increased after exposure to both HFCs, whereas none of the other inflammatory markers or uric acid had increased significantly. Further studies are needed to confirm or reject this finding.

Autoimmune Response (Study III): Serum samples from 44 Swedish men, occupationally exposed to refrigerants, were screened for antibodies against CYP2E1 with enzyme-linked immunosorbent assay. The men participating in the study were selected from a cohort of 280 refrigeration mechanics. One group comprised 30 men with asthma, joint or influenza-like symptoms and another exposed group comprised 14 men with no such symptoms. Unexposed, healthy subjects from Sweden (n=35) and Italy (n=26) constituted control groups. No increase in CYP2E1 antibody titer was seen among occupationally exposed persons compared to unexposed controls. Neither were there any differences between exposed, symptomatic subjects and healthy exposed or unexposed ones. Further, no relation between antibody titer and severity of symptoms, smoking, inhaled anesthetics, age, or year in occupation was detected.

Case Report (Study IV): A case of myocardial infarction was reported. Possible associations between welding, exposure to decomposed HCFC-22, respiratory symptoms and the myocardial infarction were discussed.

In conclusion, the respiratory uptake of HFC-134a and HFC-143a is low and the post-exposure decrease in blood and exhaled air is fast. There may be an inflammatory response at 500 ppm exposure level (i.e. at the Swedish occupational exposure limit, 8-h time-weighted average), but further studies are needed to confirm this response. Exposure to halogenated hydrocarbons could not be associated to CYP2E1-related autoimmunity.

LIST OF PUBLICATIONS

I. Toxicokinetics of 1,1,1,2-tetrafluoroethane (HFC-134a) in male volunteers after experimental exposure. Toxicol Lett; 167:54-65.

II. Experimental exposure to 1,1,1-trifluoroethane (HFC-143a): Uptake, disposition and acute effects in male volunteers. Submitted.

III. Non-positive autoimmune responses against CYP2E1 in refrigeration mechanics exposed to halogenated hydrocarbons. Submitted.

IV. Inhalation of decomposed chlorodifluoromethane (Freon-22) and myocardial infarction. Scand J Work Environ Health; 28(3):205-207.

CONTENTS

LIST OF ABBREVIATIONS

Anti-CYP2E1	Antibody against CYP2E1
AUC	Area under the concentration time curve
BMI	Body mass index
BW	Body weight
CFC	Chlorofluorocarbon
CLi	Intrinsic metabolic clearance
CNS	Central nervous system
CRP	C-reactive protein
CYP2E1	Cytochrome P-450 2E1
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
HCFC	Hydrochlorofluorocarbon
HCl	Hydrogen chloride
HF	Hydrogen fluoride
HFC	Hydrofluorocarbon
IgG	Immunoglobulin G
LD_{50}	Lethal dose, 50%
PBPK model	Physiologically based pharmacokinetic model
PBTK model	Physiologically based toxicokinetic model
PC	Partition coefficient
PCblair	PC between blood and air
PCfatbl	PC between fat and blood
PClivbl	PC between liver and blood
PCmbl	PC between muscle and blood
PCvrg	PC between vessel-rich groups and blood
RA	Rheumatoid arthritis
SAA	Serum amyloid A protein
SD	Standard deviation
SLE	Systemic lupus erytematosus
UV-light	Ultraviolet light
VAS	Visual analogue scale
VPR	Ventilation-perfusion ratio
W	Watt

1 INTRODUCTION

Hydrofluorocarbons (HFC) were introduced during the 1990s as substitutes to chlorinated hydrocarbons like chlorofluorocarbons (CFCs) that contribute to depletion of stratospheric ozone. Refrigeration and air-conditioning are typical applications for the HFCs.

Keeping foods cold has always been a priority for society. From ancient times and onwards ice has been used, and in the late 19th century, harvesting, transportation and storage of ice was a large industry. A thorough description of refrigeration history is given by Thévenot [119]. The first actual refrigerant was introduced in the 1830s, when a vapour-compressor machine was constructed with cauotchoucine or sulphuric (ethyl) ether as refrigerant [21]. After that, a number of chemicals has been suggested as refrigerants and tested with varying success [21]. When the CFCs were developed in the 1930s, the ultimate solution for cooling was believed to have been found. These substances were non-flammable, non-toxic and inexpensive, and also very suitable as refrigerants [29]. Not until 40 years later, when Molina and Rowland predicted that the discharge of chlorofluorocarbons would influence the ozone layer by destruction of stratospheric ozone, were there any concern about these substances [86]. The theory was confirmed in 1985 when loss of ozone was measured over Antarctica [44].

Paradoxically, it is the properties that make the CFCs suitable as refrigerants that also make them disposed to deplete the ozone layer [29]. The chemical inertness makes them less prone to be degraded in the troposphere and the high volatility facilitates reaching of the ozone layer. Owing to ultraviolet light (UV-light) CFCs are cleaved by photolysis to chlorine radicals that will influence the ozone destruction.

There were growing concerns about effects on the environment and on human health owing to the reduced ozone layer. Due to international agreements (The Montreal Protocol) [122], there were restrictions in CFC use and replacement chemicals were developed. Hydrofluorocarbons (HFCs) and hydrochlorofluorocarbons (HCFCs) are two groups of CFCs substitutes used in applications, e.g. refrigeration and air-conditioning. Their C-H bonds make both of them more prone to degrade in the atmosphere [45]. Substitution to HCFCs was only a temporary solution since these substances still have some ozone depleting potential. HFCs on the other hand contain no chlorine and consequently, they have no potential to deplete the ozone layer [29]. The use of HFCs is not regulated in the Montreal Protocol. They have an effect on global warming, though [46], which is of growing concern today.

The ideal refrigerant should have the following properties: First, its thermodynamic qualities have to be considered since moderately high as well as low pressures are required. It should be non-flammable, stable within a closed cooling system, and self-lubricated. Last, but not least, it should be non-toxic and environmentally safe [20]. The new generation of fluorinated refrigerants fitted largely this description.

In 1998 when substitution was very common in Sweden, some Swedish refrigeration mechanics reported symptoms like headache and joint problems [4, 56]. The

refrigeration mechanics are as a daily mean moderately exposed to refrigerants at work. Despite that, peak exposures may occur, especially during evacuation of cooling systems, drainage and refilling of compressor oils [52, 56]. In a cross-sectional questionnaire study, an increased incidence of asthma, pain in joints and influenza-like symptoms were observed among persons occupationally exposed to refrigerants [4]. Mineral oils were used for greasing the compressors in systems with CFC and HCFC as refrigerants. Ester oils are used for the same purpose in the currently used HFC systems [56]. Exposure to mineral oil, but not ester oils, was related to swollen joints in a nested case-control study of refrigeration mechanics [73]. A relation between exposure to mineral oils and rheumatoid arthritis was observed in a case-control study of patients with rheumatoid arthritis and randomly selected controls [116].

In general, the acute and chronic toxicity of HFCs are low [29]. However, there are scarce human data on the toxicokinetics and toxicology of HFCs. The main focuses in this thesis are toxicokinetics and effects in humans exposed to HFCs or other halogenated hydrocarbons used as refrigerants.

2 HALOGENATED HYDROCARBONS USED AS REFRIGERANTS

There are five HFCs used in Sweden; HFC-32 (difluoromethane), HFC-125 (pentafluoroethane), HFC-134a (1,1,1,2-tetrafluoroethane), HFC-143a (1,1,1-trifluoroethane), and HFC-152 (1,1-difluoroethane). They are all used as refrigerants, but some of them could also be used as dry etching agents, blowing agents, and as propellant in aerosol inhalers [121]. We have studied two of them more closely; HFC-134a and HFC-143a.

2.1 NOMENCLATURE OF OZONE-DEPLETING SUBSTANCES AND THEIR SUBSTITUTES

The HFC, HCFC, CFCs are given names according to a system used worldwide, (Table 1). In the prefix (CFC, HCFC and HFC) “C” is for chlorine, “F” is for fluorine, “H” is for hydrogen, and the last “C” is for carbon. As an alternative, the refrigerants can also be named with the prefix “R” or “Refrigerant”, e.g. R-134a or Refrigerant 134a. The following numbers denotes the number and placement of halogens. Finally, an additional letter is added to identify isomers. For the methanes, there is only a single carbon and consequently no isomers (e.g. HCFC-22, chlorodifluoromethane). The isomer with the smallest mass difference between the carbons has no letter, but as the mass difference increases, “a”, “b”, and “c” are added to the name. The ethanes can have “a”, the 3-carbon molecules (propane-derived) can have an “a” or a “b”, and so on. The name HFC-134a means that there is hydrogen, fluoride, and carbon in the compound. There is no double bond, but two carbon atoms (2-1), two hydrogen atoms (3-1), and four fluorine atoms. There is no bromine in this compound, and the additional letter “a” means that the compound has the second least mass difference between the two carbon atoms. Blends are given names serially. The first zeotropic and azeotropic blends were named R-400 and R-500, respectively. Different amounts of the components of the blends are differentiated by a capital letter.

Table 1. Naming system of CFC, HCFC and HFC.

Prefix	
1st C	Chlorine
F	Fluorine
H	Hydrogen
2nd C	Carbon
Numerical	
1st	No. of double bonds (omitted if it is zero)
2nd	Carbon atoms – 1 (omitted if it is zero)
3rd	No. of hydrogen atoms + 1
4th	No. of fluorine atoms
5th	No. of chlorine atoms replaced by Bromine (a "B" prefix will also be added)
Additional letter	This letter is added to the name to state the isomer situation. No letter is the "normal" isomer with the smallest mass difference between the carbons. As the mass difference increases, a, b, and c are added to the name.

Refrigeration mechanics

There are about 700 refrigeration mechanics in Sweden according to the Swedish Building Workers´ Union [115]. This number is probably an underestimation since some workers will not belong to a union and others will work with refrigerants although belonging to other sectors of the union. The majority of the refrigeration mechanics are men. Typical work tasks of the refrigeration mechanics are installations of new refrigeration systems, service and repair of existing systems, or they could be involved in both [56]. New installations may require soldering and welding to install new pipes, electrical installations, and glueing [73]. When servicing and repairing old systems, compressors and compressor oils are changed, leaks are being searched for and repaired, and refrigerants are being exchanged [73]. Sometimes soldering and welding is required, which may result in exposure to decomposed refrigerants. The refrigeration installations mainly occur in retail trade, at homes, in large industrial installations and in air-conditioning systems [4, 56, 73], i.e. the refrigeration mechanics could work at many different places. The ergonomic situation in these places can be problematic and symptoms related to bad ergonomics have been seen among refrigeration mechanics [4, 32, 56].

The refrigeration mechanics are moderately exposed to refrigerants at work expressed as daily mean values. High short time exposures to refrigerants has been observed, but the overall exposure is low [8, 52, 74, 97]. The peaks are associated with certain activities like evacuation of cooling systems, drainage and refilling of compressor oils and repairs of leakages. The Swedish short-term occupational exposure limit (OEL) may be exceeded, whereas the time-weighted average is not exceeded. Permonius [97] stated that the high levels (up to 35 000 ppm CFC-12) were reached since the installations were not sufficiently drained before the operations and that excess refrigerant was not evacuated from the environment. Gjølstad et al. [52] measured

short-time exposure levels of up to 42 434 mg/m^3 of HCFC-22. The exposure situation is ruled by the amount of refrigerant released, the possibility to exhaust it, the size of the room and whether the worker can leave the work place temporarily or not [74]. Working tasks like welding and soldering can give exposure to degradation products like hydrogen chloride (HCl) from CFC and hydrogen fluoride (HF) from HCFC and HFC as well as welding and soldering smoke and nitrogen oxides.

Cardiac arrhytmia, feeling of intoxication, nausea, headache, lightheadedness and palpitations have been reported in connection to exposure to the HFC predecessors [9, 23, 33]. However, increased mortality owing to cardiovascular disease have not been found among refrigeration mechanics (Szmith et al., 1981 [117] cited by Edling et al., 1990 [33]). There have been some accidental exposures to refrigerants, though. Liver diseases have been reported after an accidental exposure to HCFC-123 (1,1-dichloro-2,2,2-trifluoroethane) and HCFC-124 (1-chloro-1,2,2,2-tetrafluoroethane) [61]. Moreover, trifluoroacetyl-adducted liver proteins and antibodies against CYP2E1, (found in many patients with halothane hepatitis) were detected in samples from these workers. Another accidental exposure to HCFC-123 caused severe liver dysfunction in workers who produced a container and filled it with HCFC-123 [118]. Except for liver injuries, some workers lost their appetite and had abdominal pains. One of them also had tightness in the chest. Liver function tests were back to normal levels again after 1.5 months.

3 TOXICOKINETIC MODELS

3.1 BASIC DEFINITIONS

Toxicokinetics is the mathematical description of uptake and disposition (absorption, distribution, biotransformation/metabolism, and excretion) of a chemical in the body [63, 83].

Absorption is the process by which a chemical enters the blood stream by crossing membranes of the body. It mainly occurs via the gastro-intestinal tract, the lungs and the skin. In the work environment, the main routes are through inhalation and dermal absorption [112]. Substances absorbed by the lungs are usually gases, volatile liquids or aerosols. Subsequently, the chemical is distributed to various tissues of the organism, a process ruled by blood flows, how the chemical will partition between blood and different tissues, and the rate of elimination.

Elimination denotes removal of a substance from the body. A toxic substance is eliminated from the body by excretion and metabolism/biotransformation and/or diffusion from the lung.

The purpose of metabolism is to convert chemicals from being polar and fat-soluble to a more water-soluble state that can more readily be excreted. The dominant site of metabolism is the liver. The metabolism is divided into two phases. Phase 1 includes oxidation, reduction, hydrolysis, and hydration reactions, and the water-solubility is only increased to some extent. Phase 2 reactions (glucuronidation, sulfonation, acetylation, methylation, conjugation with glutathione or amnio acids) results in water-soluble products that can be excreted via urine or bile. The two phases are linked since products of phase 1 in many cases are substrates of phase 2 reactions [71].

There are several routes of excretion of chemicals. The most important excretion route is via the kidney, where many chemicals are excreted via urine after glomerular filtration, tubular excretion by passive diffusion, and active tubular secretion. However, chemicals that primarily exist in gas phase at body temperature are mainly excreted via the lungs, by simple diffusion. The quantity of the substance eliminated that way is ruled by the chemical´s vapour pressure. Gases that easily solve in blood will be eliminated more slowly than those with low solubility in blood, which will be more rapidly excreted as elimination of gases is almost inversely proportional to the absorption rate. Also, volatile substances with high solubility in fat tissues will be more slowly eliminated since diffusion from fat to blood and further to the alveolar space is a more time consuming process.

Elimination of a substance can be expressed as the half time ($t_{1/2}$), which is the time to reduce the amount of substance from an organ, tissue or tissue group by 50%. The area under the concentration-time curve (AUC), i.e. the integral of concentration of chemical over time, is a measure of the uptake and disposition of a chemical, and also proportional to the amount absorbed.

3.2 PHYSIOLOGICALLY-BASED TOXICOKINETIC (PBTK) MODELS

A physiologically-based toxicokinetic (PBTK) model (or physiologically-based pharmacokinetic (PBPK) model) can be used to predict the concentration of drugs or chemicals in blood and tissues as a function of time. For this purpose, the body is divided into compartments that represent specific organs or groups of organs or tissues. Blood flows between the compartments and the chemical will go in and out of the different compartments in a manner ruled by physiological (cardiac output, respiratory rate, organ weights, and blood perfusion rates), biochemical (metabolic rate in tissues) and physiochemical (the amount of chemical partitioning to different tissues) data. The procedure is described by differential equations based on mass balance. An example of a PBTK model, used in Study I and II, is presented in Figure 1.

Selection and definition of the model is essential. A lung compartment is required when building a model for chemicals in gas phase, like the HFCs, since inhalation is the main uptake route. The fewer compartments, the better is a rule of thumb since the model structure should not be more complex than required for description of a specific set of data and exposure predictions. Then you insert experimental data into the model, make the predictions, and compare the result to experimental data. The model has to be refined until the fitting to experimental data is satisfactory [7].

Like all models, PBTK models are simplifications of the real world. However, they can be used to clarify complicated relationships like the concentration and elapse of a chemical within different tissues and organs in the body.

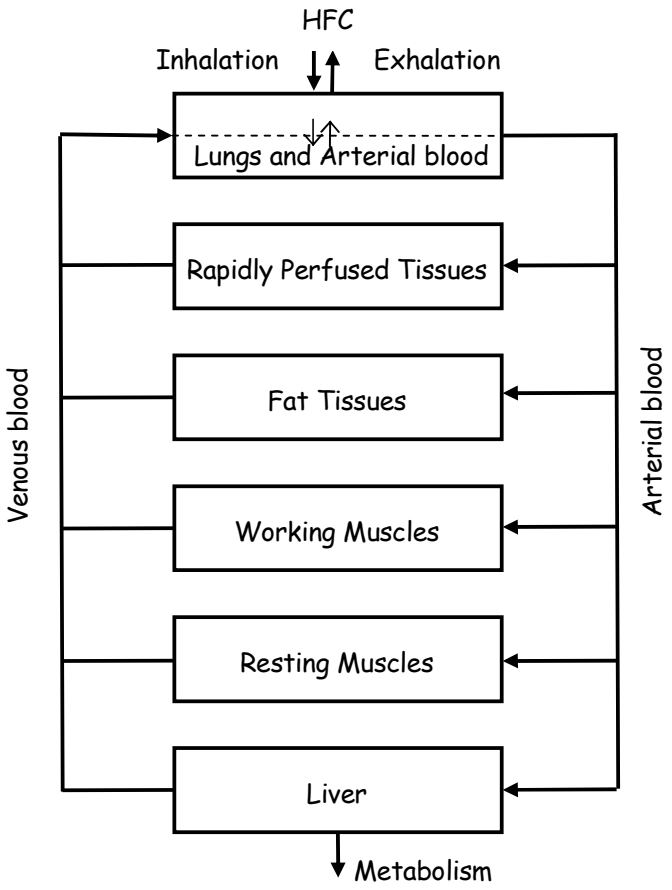


Figure 1. The physiologically-based toxicokinetic model used to simulate the profiles of HFC-134a and HFC-143a in blood and exhaled air (Study I and II).

4 THE IMMUNE SYSTEM

The immune system defends us against invading non-self entities, like bacteria, viruses, and fungi. It is made up of two parts; the innate and the adapted response. The innate immune response is not very specific and consists of (1) a non-specific barrier and (2) cells with receptors that recognize some pattern common to many pathogens. It is a fast response with no memory, i.e. repeated exposure for the same antigen will not be recognized. It only recognizes and responds to microbial antigens. The innate immune system is composed of epithelial barriers, phagocytes, natural killer cells, and the complement system.

The adaptive (acquired) response is highly specific and recognises the exact part that triggered the response in the first place. If the immune system gets in contact with the antigen again, the adaptive system will respond quicker and with more strength than the first time. The adaptive immune system consists of T-lymphocytes and antigen-presenting cells. Memory, specificity and ability to differentiate “self” and “non-self” are specific properties of the adaptive immune system [69].

The functions of the immune system are described in textbooks like e.g. “Basic immunology : functions and disorders of the immune system” by Abul K. Abbas and Andre H. Lichtman [1] or “Immunology” by Richard A. Goldsby et al. [53].

The Inflammatory Response

Inflammation is a general response to trauma, ionizing and non-ionizing radiation, chemical agens, and biological agens (viruses, bacteria, and fungi). Redness (rubor), swelling (tumour), heat (calor), and pain (dolor) are the cardinal symptoms of the inflammatory response. The cells of the immune system and many mediators are involved in the inflammation process. It is a typical process of the innate immune system. However, an inflammation may also occur when the tissues are attacked by the body´s own immune system, as in autoimmunity [53].

The inflammatory response, caused by e.g. tissue damage, is initiated by a series of actions by mediators. The mediators are for instance released from cells in affected tissues, products from white blood cells that participate in the inflammation, or derived from microorganisms.

Major events of the inflammatory response are:
(1) Vasodilation (the diameter of the blood vessels increases) of capillaries close to the inflammation site as vessels transporting blood from the affected area constricts. Tissue redness and increased tissue temperature are results of engorged capillaries. (2) Cells and fluids from the capillaries are more easily transported to the tissue due to increased permeability of the capillaries. Fluid with high protein concentrations will accumulate and cause swelling. (3) Influx of phagocytes from the capillaries will also be facilitated by the increased permeability.

Activated macrophages secrete peptides and proteins important for immune responses, e.g. cytokines like interleukin-1 (IL-1), which promotes the inflammatory response, and interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) which promotes the innate immune system. IL-1 and TNF-α activates IL-6, which stimulates the liver.

The release of acute phase proteins increases dramatically in response to tissue damage. C-reactive protein (CRP) is one of the major acute phase proteins, and it is produced by the liver in response to tissue damage. CRP was named after its ability to bind to the C-polysaccharide cell-wall of pneumococci. The binding will activate the complement system resulting in complement-mediated lysis of pathogens or increased phagocytosis. CRP is one of the fastest acute phase proteins. Increased plasma concentrations (up to 100 times the normal value) may be seen about 8 h after tissue damage [49]. Serum amyloid A (SAA) protein and fibrinogen are other acute phase proteins produced in the liver [49, 82]. CRP, SAA, fibrinogen and D-dimer (a degradation product of fibrinogen) are valuable as markers of inflammation and has been used in Study I and II in this thesis.

Due to vasodilation and increased permeability of the capillaries, the blood-clotting systems may also enter the damaged tissue, initializing a cascade of enzymes with deposition of fibrin strands as a result. Fibrin is the main product of blood clotting, and its presence separates the injured area from the rest of the body.

Inflammation and coronary heart diseases

During the last decade, inflammation has been related to the occurrence of atherosclerosis by additional data [40, 72, 102, 104]. Some specific inflammatory diseases, e.g. chronic bronchitis, have also been linked to occurrence of coronary heart disease [57, 64]. Also, higher concentrations of inflammatory markers in the blood (e.g. fibrinogen and CRP) have been associated with coronary heart disease in many epidemiological studies (e.g. [27, 28]).

Autoimmunity

The adaptive immune system acquires the ability to different self from non-self. Autoimmunity is an immunological response against components of our own body which are seen as foreign by the immune system, i.e. the self tolerance has failed. B and T-cells of the peripheral immune system may react with self-antigens and thus cause severe damage to tissues and cells. If sustained, tissue damage may occur. Type I diabetes (insuline-dependent diabetes mellitus) and thyroiditis are examples of autoimmune diseases that affects specific organs (organ-specific autoimmunity), whereas systemic lupus erythematosus (SLE) and scleroderma are examples of systemic autoimmune diseases which affects several organ systems [53]. The incidence of autoimmune diseases in Europe and North America is about 3 % [62].

Circulating lymphocytes which react with self-antigens may be present in healthy individuals without causing autoimmune diseases, i.e. their activity is regulated in normal cases. Genetic predisposition, hormones and environmental exposure are factors behind the progress of autoimmune diseases.

Exposure to chemicals may result in interaction with activities of the immune system. Suppression may cause prolonged infections and development of cancer, whereas enhancement may lead to diseases mediated by the immune system as hypersensitivity and autoimmune diseases [71]. Exposure to various chemicals can result in autoimmune reactions and diseases [13, 54]. The exposure may not only induce the disease as such, but it may also accelerate an underlying process [99]. There are potential links between environmental agens and autoimmune diseases and responses.

Halothane

Halothane (1-bromo-1-chloro-2,2,2-trifluoroetane) is an anesthetic that has the ability to cause autoimmune diseases (halothane hepatitis) and autoimmune responses (e.g. auto-antibodies that react with the enzyme CYP2E [16, 35]). Halothane is structurally similar to the HCFCs and HFCs, and like these compounds it is a substrate of CYP2E1 [29, 68, 111]. Halothane was introduced in 1956 and for several years it was the most commonly used anesthetic [100]. Only 2 years after its introduction, there were reports of liver necrosis after operations where halothane had been used, reviewed in [100]. A mild and a severe form of liver damage mediated by biotransformation of halothane has been established [100]). The mild one, occurring in about 20 % of the halothane anesthetics, is presumed to be mediated by some toxic mechanism [11]. In about one out of 6 000-35 000 anesthetics, severe hepatic necrosis, halothane hepatitis, occurs [100]. In 75 % of these cases, the outcome was fatal [26, 100]. Typical clinical signs of halothane hepatitis are non-specific gastrointestinal upset (e.g. nausea), delayed pyrexia, jaundice, eosinophilia, auto-antibodies in serum, rash and arthralgias [81, 100].

It is more common to obtain severe liver damage after repeated exposure to halothane, especially if the time between administrations is less than about one month. However, no safe interval between halothane exposures seem to exists since there are reports of liver damages presumably caused by recurrent exposure that occurred many years after the first exposure (e.g. [14]), and as far as 28 years after the first administration [80].

Reactive halogenated acetyls and acetaldehydes are formed during the metabolism of halothane as well as during metabolism of HFCs and HCFCs [6, 48]. These intermediates may react with water to form trifluoroacetic acid, or bind to a liver protein to form a trifluoroacetyl-adducted protein, e.g. trifluoroacetyl-CYP2E1. These adducts may subsequently be seen as antigens and induce an autoimmune response resulting in antibodies against trifluoroacetyl-CYP2E1. Due to a decreased tolerance, caused by the trifluoroacetyl-CYP2E1 complex, antibodies against native CYP2E1 will also be elicited.

Enflurane, sevoflurane and isoflurane are other anesthetics with similar structure as that of halothane and with the ability to cause hepatocellular injury in humans. Since the damage is preceded by reactive intermediates and immunocomplexes (proteins modified by acetylation, neoantigenes) which occur due to biotransformation of the anesthetics, the extent of metabolism of the respective anesthetic will rule the autoimmune response caused by each anesthetic [101]. Consequently, the incidence of autoimmune reactions and damages is less common after exposure to the compounds that are less inclined to be metabolised. However, cross-reactions between e.g. halothane and enflurane has been reported [106, 108], i.e. a previous exposure to

halothane may result in presence of autoantibodies that causes an autoimmune responses after exposure to another anesthetic. A similar phenomenon would thus be possible with inhaled anesthetics and refrigerants.

5 AIMS OF THIS THESIS

The primary aim of this thesis was to study the toxicokinetics and some effects of human exposure to HFC. The most common HFC (HFC-134a) and one of the least studied ones (HFC-143a) were selected for that purpose. In addition, effects of exposure to halogenated hydrocarbons used as refrigerants and their decomposition products were investigated.

The more specific aims were to

1. determine the uptake, disposition, elimination and metabolism of HFC-134a and HFC-143a in humans,

2. investigate some effects of exposure to these substances,

3. establish whether there are autoimmune effects (autoimmune responses to CYP2E1) because of exposure to the halogenated hydrocarbons used as refrigerants, and

4. discuss possible associations between welding, exposure to decomposed HCFC-22, respiratory symptoms and myocardial infarctions.

6 HFC-134A AND HFC-143A

6.1 CHEMICAL AND PHYSICAL PROPERTIES

HFC-134a (1,1,1,2-tetrafluoroethane) and HFC-143a (1,1,1-trifluororethane) are colourless gases with faint, ethereal smells [31, 47]. Their common names, structural formulas, and chemical and physical data are given in Table 2. HFC-134a is non-flammable [31], whereas HFC-143a is flammable at concentration of about 70 000 ppm [18].

6.2 OCCURRENCE

There are no natural sources of HFC-134a [31] and HFC-143a [39]. HFC-134a is being used as refrigerant, as propellant in metered dose inhalants, for foam blowing, and as dry etching agent [31, 121], whereas HFC-143a is only used as refrigerant [121]. As refrigerant, HFC-134a appears as single component (HFC-134a) or in blends (e.g. 4% in R404a). HFC-143a only appears in blends (e.g. 52% in R404a and 50% in R507). Other HFCs used as refrigerants are HFC-32, HFC-134, HFC-152a, HFC-227ea, HFC-236ea, HFC-236fa, HFC-245ca, and HFC-245fa [121].

In 2005, 518 600 kg HFC-134a and 43 400 kg HFC-143a was used (imported and produced) in Sweden [66]. The annual use of HFC-134a and HFC-143a in Sweden between 1992 and 2005 is presented in Figure 2. The world sales of HFC-134a and HFC-143a in 2004 were 173 851 and 14 053 tons, respectively [3].

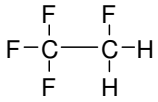
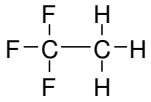
The atmospheric lifetimes of HFC-134a and HFC-143a are 14 and 52 years, respectively. None of them have any ozone depletion potential (i.e. ODP=0), and their global warming potentials (GWP) (related to CO_2=1) are 1300 for HFC-134a and 4300 for HFC-143a [22]

6.3 OCCUPATIONAL EXPOSURE

Humans may be exposed to HFC-134a via inhalation from accidental leaks from refrigerators and air-conditioning systems, from spills and industrial use, and from metered-dose inhalers used to deliver medication e.g. for treatment of asthma [85]. Exposure to HFC-143a may occur during service and repairs of refrigeration systems [39].

Occupational exposure related to refrigeration repair work is usually moderate although peak exposures of HFCs and HCFCs may occur [52], especially if an open system is used during maintenance and/or repair of the refrigeration system [32, 74, 97]. There are few measurements of exposure to HFCs in the work environment. However, Gjølstad et al. measured peak concentrations of about 1400 ppm HFC-134a and 350 ppm HFC-143a in the work environment of Norwegian refrigeration mechanics [52]. Those peaks lasted for less than 20 min and were associated with certain working operations.

Table 2. Chemical and physical properties of HFC-134a and HFC-143a.

	HFC-134a	HFC-143a
Common synonyms	1,1,1,2-tetrafluorethane, R134a, HFA 134a, FC-134a, Fluorocarbon 134a, etan-1,1,1,2-tetrafluorid	1,1,1-trifluorethane, methylfluoroform, 1,1,1-trifluoroform, HFC143a, FC143a, R143a
CAS number	811-97-2	420-46-2
Chemical structure		
Chemical formula	$C_2H_2F_4$	$C_2H_3F_3$
Molecular weight (g/mol)	102.03	84.04
Boiling point (°C)	-26.5	-47.5
Melting point (°C)	101.0	111.3
Vapour pressure (kPa, 25 °C)	630	1267
Conversion factor (20 °C, 101.3 kPa)	1 ppm = 4.25 mg/m^3 1 mg/m^3 = 0.24 ppm	1 ppm = 3.49 mg/m^3 1 mg/m^3 = 0.287 ppm

6.4 TOXICOKINETICS

HFC-134a

The respiratory uptake of HFC-134a is fast in animal studies. HFC-134a is absorbed rapidly in rats and dogs and a plateau is reached within 15 min of exposure [5]. The half-lives were 4 to 7 min, respectively. In rats exposed for 10 000 ppm HFC-134a for 1 h 1 % was recovered in urine, feces and exhaled air. One third of this was exhaled as CO_2, excreted as trifluoroacetic acid in urine, or as an unidentified metabolite in faeces [36].

There are a few studies of uptake and elimination of HFC-134a in humans. In most of these studies the substance was administered via metered dose inhalers [58, 87, 123], which makes the level and duration of the exposure difficult to evaluate. However, one study reports data from a chamber exposures study where eight volunteers (4 men, 4 women) were exposed for 1 h to 1000, 2000, 4000, and 8000 ppm HFC-134a at different occasions [38] The maximum concentrations in blood were similar for men and women except at the highest exposure level (8000 ppm) where it was higher in men than in women (70 and 59 µM, respectively).

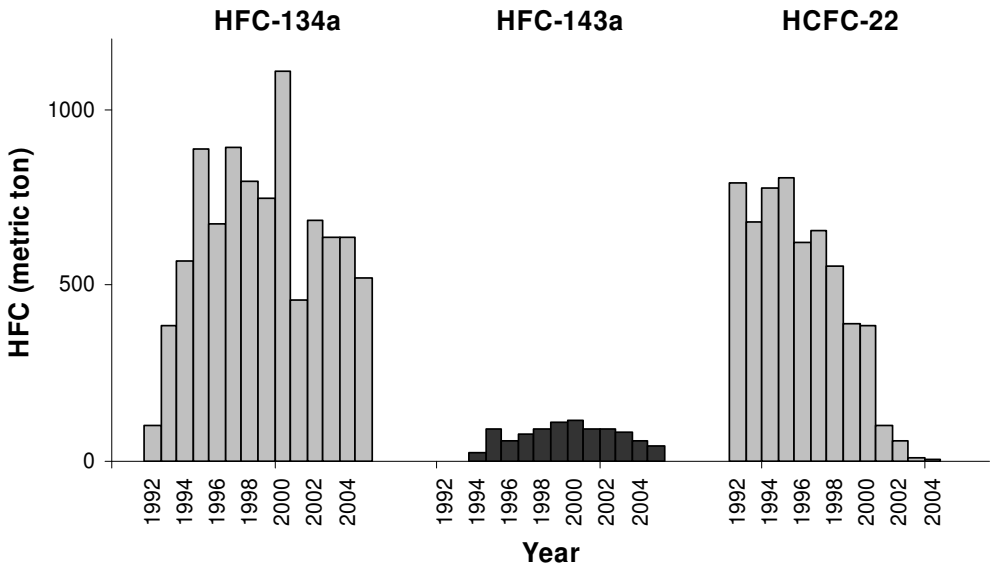


Figure 2. Imported and produced amounts of HFC-134a, HFC-143a and HCF-22 in Sweden between 1992 and 2005 [66].

HFC-134a is rapidly eliminated in humans. Five min after exposure (via metered dose inhaler) about 10 % was retained in the body. After 6 h it had declined to less than 1 % and the terminal half-life was 3.2 h [98]. Biphasic elimination was reported in the study by Emmen et al. [38] after exposures to 1000 – 8000 ppm HFC-134a. The average half-lives were less than 11 min and 42 min.

These studies suggest that most of the inhaled HFC-34a is exhaled unchanged. Less than 0.006 % of the inhaled dose (after a single-breath inhalation) was retained in urine sampled 2 h after the inhalation [98].

Oxidative defluorination of HFC-134a via CYP2E1 has been demonstrated in human liver microsomes *in vitro* [95, 113]. The rate of metabolism is lower in human liver microsomes compared to that of liver microsomes from rats and rabbits [95]. Most of the inhaled HFC-134a is immediately exhaled, and less than 0.0005 % of the inhaled dose is metabolised to trifluoroacetic acid, the only fluorinated metabolite found in urine after exposure [87].

HFC-134a a has been suggested to be metabolised to 2,2,2-trifluoroethanol, further to trifluoroacetaldehyde and then to trifluoroacetic acid which will be excreted via the urine [95]. The suggested metabolic pathway of HFC-134a is presented in Figure 3.

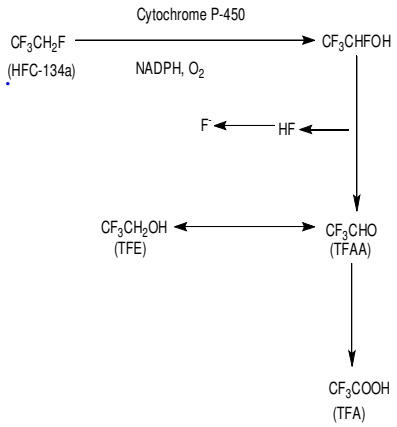


Figure 3. The suggested route of metabolism of HFC-134a. TFE=trifluoroethanol, TFA=trifluoroacetaldehyde, TFAA=trifluoroacetic acid. After Monté at al. [6, 87].

HFC-143a

There are no studies of the toxicokinetics of HFC-143a in animals or humans. However, the uptake in rats is low, as measured in rats exposed for 5 500 – 30 000 ppm HFC-143a in an exposure chamber [75]. The *in vivo* metabolic rate constant was determined to 1.17 h^{-1} kg^{-1} with a PBTK model [75].

6.5 EFFECTS IN ANIMALS

HFC-134a

The acute and chronic toxicities of HFC-134a are low, and NOEL (no effect level) has been estimated to >49 500 ppm [78]. However, a slight maternal toxicity, expressed as lower rate of body weight gain, was seen in pregnant rabbits at exposure levels of 10 000 and 40 000 ppm HFC-134a, but not at 2500 ppm [24].

Irritation

Slight signs of irritation were seen after application of liquid HFC-134a to eyes and skin in rabbit, but no skin sensitization was induced in guinea pigs after application (Mercier, 1989, 1990), cited by Mitchell [85]).

Single exposure

HFC-134a has a low acute toxicity. LC_{50} for rats exposed to HFC-134a for 4 hours was >500 000 ppm [120]. No lethal effect was seen in dogs exposed to 700 000 – 800 000 ppm for 3-5 h [107]. However, an anesthetic effect was seen as inhibition of the blinking reflex. The threshold for cardiac arrhythmias was 75 000 ppm in dogs given

adrenaline after 5 min exposure to HFC-134a [120]. No such reaction was seen at exposure concentrations of 50 000 ppm.

Exposure to 40 000 to 140 000 ppm HFC-134a (≤30 min) induced neurobehavioral effects (motion inability due to forelimb and hindlimb dysfunctions, inability to maintain an upright position under gravitational forces) in rats [103]. Also, anesthesia and occasional convulsions were seen at these exposure levels.

Repeated exposure
Rats and mice were exposed 7 days a week to up to 810 000 ppm HFC-134a (with oxygen supplementation) with no effects on mortality, body weights or consumption of food and water [5]. In another study, no significant adverse effects were seen in rats after repeated exposure (exposed for all their life) to ≥ 50 000 ppm HFC-134a [120].

Dogs exposed to 40 000 and 80 000 ppm HFC-134a for 1 h did not show any clinical signs related to exposure. At exposure to 160 000 ppm, head-shaking, salivation and struggling was seen, and at 320 000 ppm these effects were more severe [5].

Neurobehavioral effects, deficits on operant training, were seen after repeated exposure of rats to up to 140 000 ppm HFC-134a [103].

HFC-143a

Single exposure
No tissue damage was obtained in rats exposed for 3 h for up to 30 000 ppm HFC-143a measured as activities of glutamate dehydrogenase, sorbitol dehydrogenase, and lactate dehydrogenase in the sera of exposed rats [75].

Rats exposed to 97 000 or 540 000 ppm HFC-143a lost weight temporarily, but no deaths were seen [18]. There were tendencies to cardiac arrhythmias in dogs given epinephrine before and in the middle of a 10-min exposure to 300 000 ppm HFC-143a. No effect was seen at an exposure level of 250 000 ppm [18].

Repeated exposure
Rats were exposed to 2000, 10 000 or 40 000 ppm HFC-143a (6 h/day, 5 days/week) for 4 weeks (nose only) or 90 days (whole-body) [18]. When exposed via the nose, males lost weight and had degenerative changes in the testes at all exposure levels, probably caused by stress due to extreme temperature conditions. No effects were seen in female rats. Whole-body exposure did not result in any effect related to exposure.

6.6 REPRODUCTIVE TOXICITY

HFC-134a
No effect on fertility was observed in mice exposed 6 h/day for 5 days for up to 50 000 ppm (5 days, 6 h/day). Neither were any effects on reproduction seen in rats (males and females) exposed 1 h/day to up to 50 000 ppm HFC-134a [5]. No

teratogenic or adverse developmental effects were seen in rabbits exposed to up to 40 000 ppm HFC-134a [24].

HFC-143a

Rats and rabbits were exposed to 2000, 10 000, or 40 000 ppm HFC-143a for 6 h/day on day 6-15 of gestation for rats and on day 6-18 for rabbits. Increased visceral and skeletal anomalies due to a retarded development were seen for all concentrations in rats and for the high and low concentrations in rabbits [18].

6.7 GENOTOXICITY AND CARCINOGENICITY

HFC-134a

HFC-134a had no mutagenic potential in reverse mutation tests with *Salmonella typhimurium* and *Escherichia coli* with and without metabolic activation [76, 105]. No activation was seen in chromosome aberration studies with human lymphocytes [24] or hamster lung cells (Asakura, 1991, cited by Rusch [105]). No signs of gene mutations were seen in *E. coli* or in lymphoma cells from mice [5]. Neither were there any signs of genotoxicity in a series of *in vivo* tests (bacterial reverse mutation assays with *S. typhimurium* and *E. coli*, cytogenetic assays in cultured mammalian cells (Chinese hamster lung cell line) or human lymphocytes, all assays performed both in the presence and absence of metabolic activation) [24]. No tumors were induced in rats given HFC-134a dissolved in corn oil (3 % w/v solution) for 52 weeks (5 days/week), followed by 73 weeks of no exposure [76].

HFC-143a

Mutagenic effects in 2 out of 4 strains of *S. typhimurium* has been reported when tested *in vitro* with and without metabolic activation [76]. In a reverse mutation assay (Ames assay) no mutagenic effect was detected in 4 strains of *S. typhimurium* and 2 strains of *E. coli* with and without addition of liver fractions for metabolic activation [18]. No mutagenic effects of HFC-143a exposure was seen in cell transformation test with mammalian cells (hamster kidney fibroblasts) [76]. No increases in chromosomal aberrations were seen in human lymphocytes either with or without metabolic activation, when exposed to HFC-143a [18]. Neither were there an increase in micronuclei in bone marrow in mice exposed to 2 000 – 40 000 ppm HFC-143a for 6 hours/day [18]. No tumors were induced in rats given HFC-143a dissolved in corn oil (3 % w/v solution) for 52 weeks (5 days/week), followed by 73 weeks of no exposure [76].

6.8 HEALTH EFFECTS IN HUMANS

HFC-134a

There are few data on health effects in humans. An inhalation study was stopped after 4 and 10 minutes exposure to 4 000 ppm HFC-134a as the subjects had circulatory problems (bradycardia/hypotension and tachycardia/hypertension, respectively) [125]. However, no circulatory problems (pulse, blood pressure, and cardiac rhythm) were seen in another inhalation study where male and female volunteers were exposed to up

to 8 000 ppm HFC-134a [38]. Neither were there any effects on the central nervous system or as irritation in the upper respiratory tract.

No adverse effects (measured as blood pressure, heart rate, electrocardiograms, pulmonary function, haematology or serum chemistry) where seen in humans who had inhaled HFC-134a via metered-dose inhalers [58, 98]. Neither was there any effect on cardiac activity (dose unknown) [30]. However, rash or nausea and vomiting was reported by 2 subjects in that inhalation study [30].

Nausea and confusion were experienced during an aerial ambulance flight as the crew was exposed to HFC-134a. Also, poly ether oils were lost from the system. The pilot could not focus his eyes and did not remember the landing. Several months after this incidence, the crew suffered from symptoms like headache, increased blood pressure, reduced concentration level, and anxiety [10].

HFC-143a
No data of health effects in humans are available [88].

6.9 CRITICAL EFFECTS

The critical effect of HFC-134a, based on animal studies, are cardiac sensitization in dogs [78]. Since there were no data on human exposure to HFC-143a, no critical effect of occupational exposure was established [88].

6.10 OCCUPATIONAL EXPOSURE LIMITS (OEL)

The present Swedish 8-h time-weighted occupational exposure limit of HFC-134a is 500 ppm or 2 000 mg/m^3. The short time value (15-min time-weighted average) is 750 ppm or 3 000 mg/m^3 [114]. No OEL for HFC-143a is available in Sweden (April 2007).

The OEL (8-h time-weighted average) for HFC-134a is 1000 ppm in Germany [19] and United Kingdom [59].

7 METHODS

A summary of the methods used are given here. However, all details are further described in the original papers.

The exposure (Study I-II) and autoimmunity (Study III) studies were performed after informed consent and according to the Declaration of Helsinki. They were approved by the Regional Ethics Committee at Karolinska Institutet, Stockholm, Sweden. Sampling and analysis of serum from the Italian subjects included in Study III was performed after informed consent and according to the guidelines of the local ethics committee at the University of Piedmont, Novara, Italy.

7.1 EXPOSURE CHAMBER (STUDY I AND II)

7.1.1 Study design

Healthy, male volunteers, occupationally exposed to HFCs and related compounds, were exposed one or two at a time during 2 h to HFC-134a (n=10, Study I) and/or HFC-143a (n=9, Study 2) in a 20-m^3 stainless steel exposure chamber (Figure 4). They were experimentally exposed to both HFCs, except for one volunteer who was only exposed to HFC-134a. The target chamber concentrations were 500 ppm, equivalent to the Swedish OEL for HFC-134a. During exposure, the volunteers performed light physical work (50 W) on computer-controlled ergometer bicycles. The pedal frequency and actual work load was recorded every 20 s. The electrocardiograms of the volunteers were monitored during exposure. The average temperatures in the chamber were 20.3-21.5 °C, the average relative humidity was 20.8-33.7 %, and the chamber air was exchanged 20.6-21.5 times per h on average. (406.4-429.9 m^3/h). The climate in the chamber was logged every minute.

Gaseous HFC was introduced to the chamber via the inlet air stream. The container with HFC was heated in a water-bath to facilitate outflow of gas, which was restricted by a pressure regulator and a mass flow controller. The concentration of HFC in the chamber was analysed with a gas chromatograph equipped with a flame ionizing detector between 7 and 12 times each exposure occasion. To avoid leakages of HFC from the exposure chamber to the surrounding laboratory, the inlet airflow rate was lower than the outlet rate generating a lower pressure within the chamber than outside.

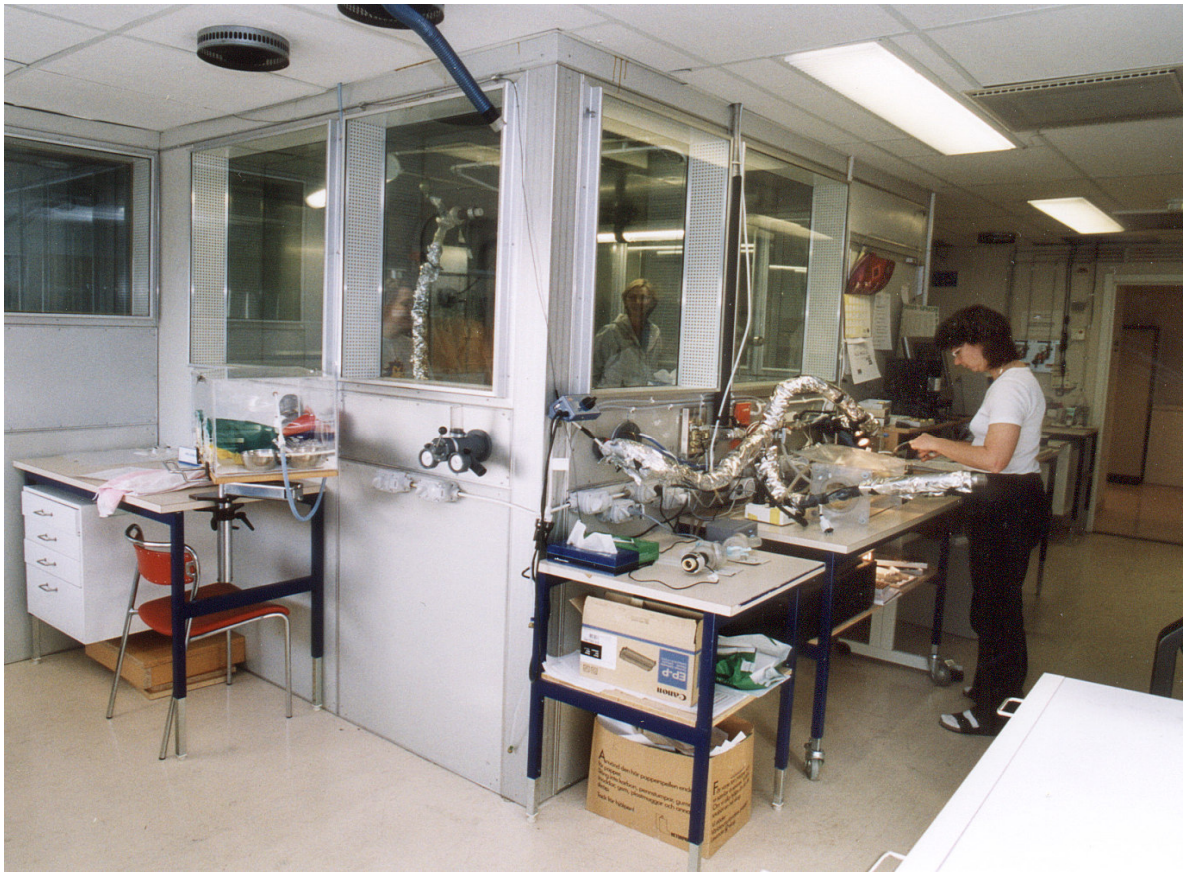


Figure 4. The exposure chamber. At the starting time of the study it was located at the Swedish National Institute for Working Life in Solna.

7.2 TOXICOKINETICS

Exhaled air

To set the respiratory excretion of HFC-134a and HFC-143a, mixed exhaled air was sampled once before exposure, 4 times during exposure, and 7 times post exposure via a mouthpiece connected to a valve with separate inlet and outlet (Figure 5). HFC in exhaled air was analysed in the same way as analyses of chamber air.

Blood (200µl) was sampled 21 times from the volunteers´ finger tips before, during and up to 22 h after HFC exposure (Figure 5). All urine was collected before exposure and until 6 h post exposure for analyses of HFC-134a or HFC-143a. The volumes were noted and thereafter the amount of HFC-134a or HFC-143a was analysed in the same way as the blood samples. The blood and urine samples were analysed by head-space gas chromatography with flame ionising detection.

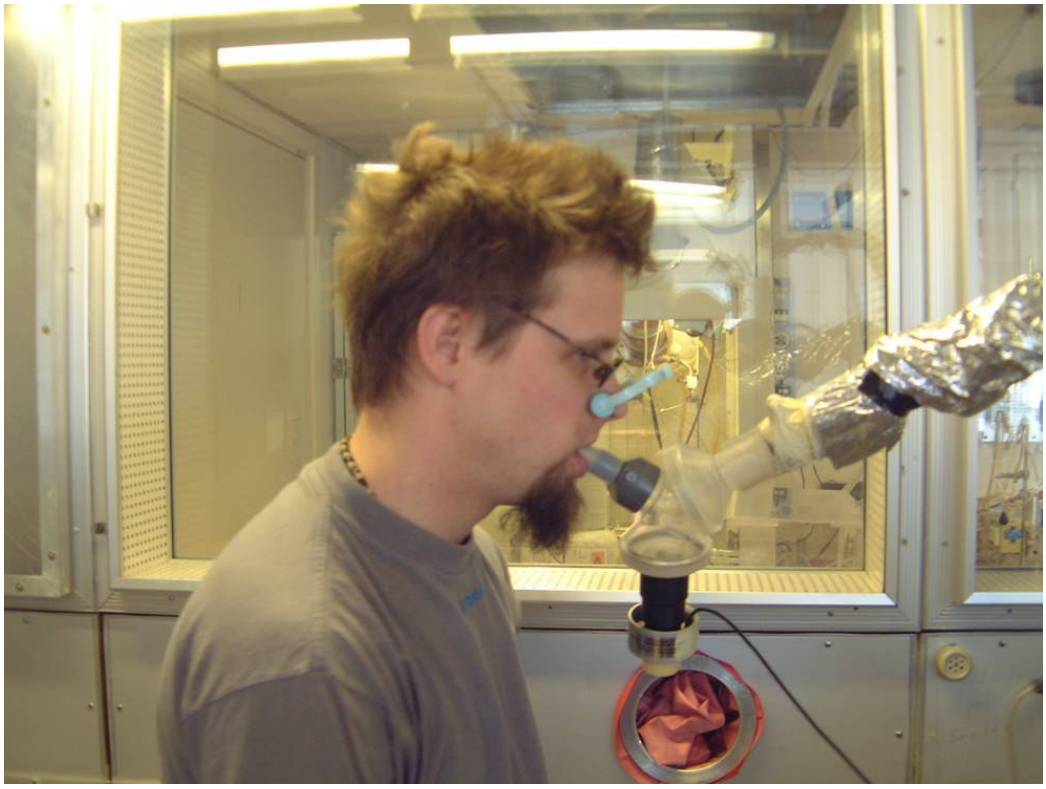


Figure 5. Sampling of mixed exhaled air during exposure to 500 ppm HFC-134a or HFC-143a.

Fluoride ions (not presented in any of the studies)

Fluoride in plasma sampled before exposure and 19 min post exposure and in urine sampled before and until the afternoon 2 days post exposure were analysed with ion selective electrodes (PHM85 Precision pH-meter with ISEC301F Combined Fluoride Electrode, Radiometer, Copenhagen, Denmark and 390 pH/mv/ISE Meter, Beckman, CA, respectively). The detection limits for fluoride was 2 µM in plasma and 1.1 µM in urine. The analytical error was 5 % for fluoride in plasma and 2.5 % for fluoride in urine.

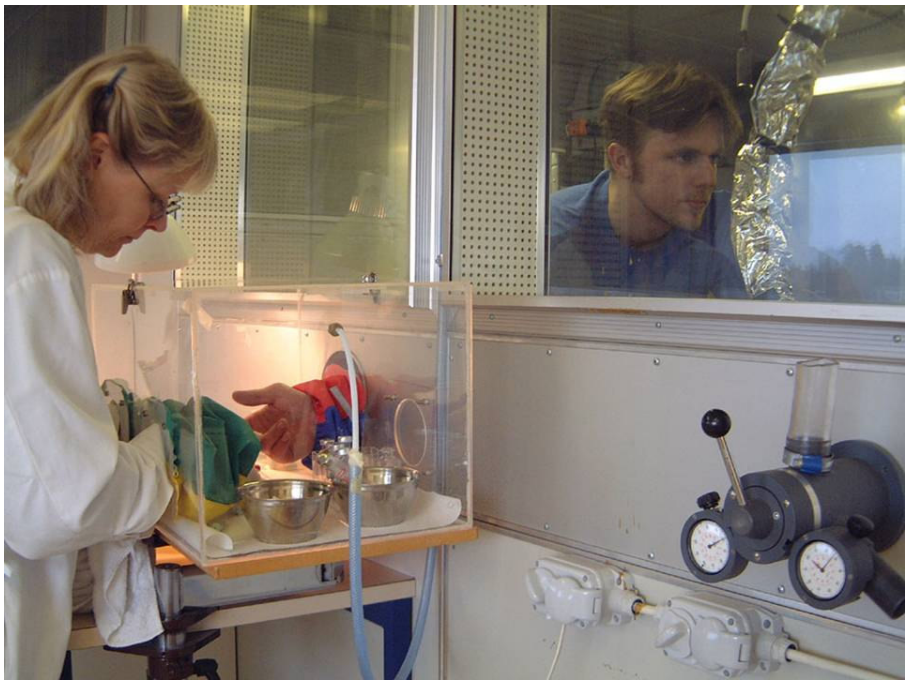


Figure 6. Sampling of capillary blood from the finger tips during exposure. Sampling took place within a glove box flushed with clean air which was situated outside the exposure chamber.

7.3 PHYSIOLOGICALLY-BASED TOXICOKINETIC MODEL

A physiologically-based toxicokinetic model was developed to describe the time courses of HFC-134a and HFC-143a in blood and exhaled air in each individual volunteer (Figure 1). Six compartments were used; lungs and arterial blood, rapidly perfused tissues, fat, working and resting muscles, and liver. Volumes of the organs and blood flows were calculated from the body weight and length of each individual volunteer, as described by Nihlén and Johanson [90]. Also, blood flows and, thus, cardiac output was scaled to the 50 W workload performed by the volunteers in this experiment. The blood:air partion coefficients for HFC-134a and HFC-134a were obtained from Eger [34] and Beliveau at al. [12].

To establish how much changes in the different model parameters (i.e. height, body weight, partition coefficients for liver:blood, vessel-rich group:blood, fat:blood, muscle:blood and blood:air, intrinsic metabolic clearance and ventilation-perfusion ratio) would influence the concentration of HFC-134a or HFC-143a in blood and exhaled air, a sensitivity analysis was performed. The analysis was based on 2 hours of exposure to either HFC-134a or HFC-143a, which was followed by 5 hours of no exposure. According to the sensitivity analysis, the blood:air partition coefficient was the only parameter with significant influence on the outcome of the model.

7.4 EFFECTS

Effects of exposure to HFC were monitored in Study I and II. The volunteers acted as their own controls when ratings and inflammatory markers were compared.

The electrocardiograms (ECG) of the volunteers were monitored with telemetry during the 2-h exposures (Figure 8). This was mainly a safety precaution, but also gave the opportunity to discover changes in heart activity.

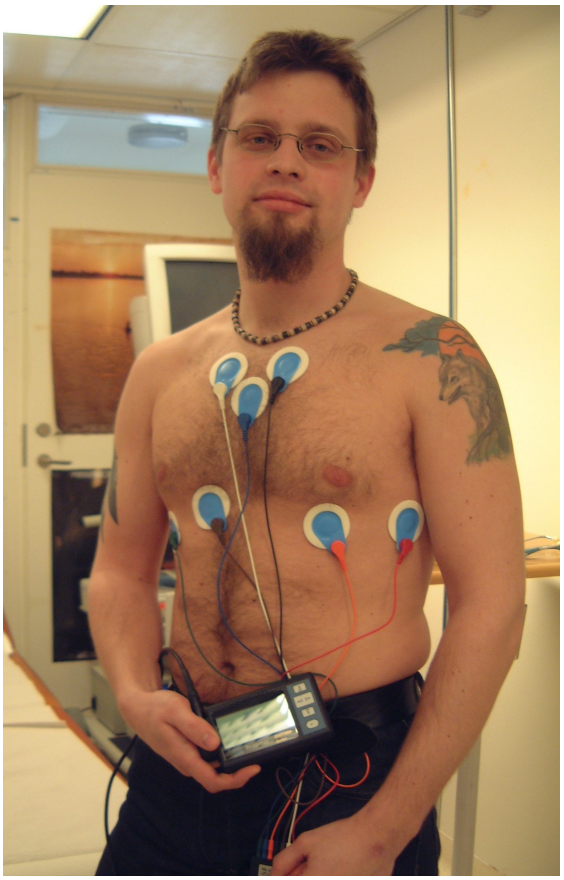


Figure 8. The electrocardiograms of the volunteers were monitored with telemetry during the 2-h exposures to HFC-134a or HFC-143a.

The volunteers rated perceived discomfort related to irritation and the central nervous system (CNS) on a 0-100 mm visual analogue scale (VAS) (Figure 9) in a questionnaire before they entered the exposure chamber, twice during exposure and after exposure. Originally, the scale was developed to assess the annoyance of noise in the work environment [70], but it has later been used to in several inhalation studies of solvent vapors (see e.g. [41-43, 65, 91]. The 10 items in the questionnaire were (as translated from Swedish): “Discomfort in the eyes: burning, irritated, or running eyes”, “Discomfort in the nose: burning, irritated or runny nose”, “Discomfort in the throat or airways”, “Breathing difficulty”, “Solvent smell”, “Headache”, “Fatigue”, “Nausea”, “Dizziness”, “Feeling of intoxication”.

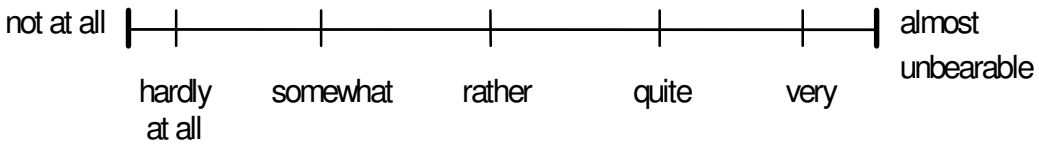


Figure 9. A visual analogue scale (VAS) used for rating of perceived discomfort related to irritation and CNS-symptoms.

Markers of inflammation
Venous blood was collected from the brachial vein before exposure and 21 after exposure for preparation of plasma. The plasma was analysed for markers of inflammation (C-reactive protein, serum amyloid A protein, D-dimer, fibrinogen) and uric acid.

7.5 AUTOIMMUNITY (STUDY III)

7.5.1 Study groups

Swedish, male, refrigeration mechanics (n=44), occupationally exposed to halogenated hydrocarbons, where examined for antibodies against CYP2E1. They were selected from a cohort of 280 refrigeration mechanics belonging to the Swedish Building Workers´ Union who had been interviewed about symptoms related to asthma, influenza-like symptoms, problems with joints, and occupational exposure conditions [73]. Two groups of workers were selected from the material; (1) individuals with symptoms of inflammation and autoimmunity (n=30) and (2) asymptomatic individuals (n=14). Furthermore, the participants were asked about exposure and health conditions since the interview by Lillienberg et al. [73] to confirm they were still classified correctly.

Thirty-five unexposed, healthy, Swedish men (students, researchers, and office workers) constituted control group. Their health status, any medication, and that they were no previously exposure to refrigerants were confirmed with a questionnaire.

The exposed workers and the unexposed Swedish controls were further compared to a group of 26 healthy, Italian controls that were recruited from hospital and university staffs. No detailed health status (anamneses) or information of sex, age, or smoking habits is available for this group (data not presented in the original study (III)).

7.5.2 Sampling and analyses

Venous blood (15 ml) for sera preparation was collected from the refrigeration mechanics and controls. The serum samples were screened for auto-antibodies against CYP2E1 with enzyme-linked immunosorbent assay (ELISA).

7.6 STATISTICAL ANALYSES

Data was tested for normality with Shapiro-Wilkes W test (JMP Statistical Discovery Software, v. 4.0.2, SAS Institute Inc., Cary, NC) (Paper I-III). Since most data were not normally distributed or were referring to ordinal scales, non-parametric statistics was mainly used. The significance level was set to 0.05 in all statistical analyses.

The symptom ratings were statistically analysed with the Friedman test (StatView, v. 5.0, SAS Institute Inc., Cary, NC) (Study I and II).

In Study I and II, the differences between pre- and post exposure levels in markers of inflammation were normally distributed except for serum amyloid A protein. Accordingly, Students´s paired t-test was used to test for biomarker changes during exposure (JMP v. 4.0.2, SAS Institute Inc., Cary, NC).

In order to compare the anti-CYP2E1 autoantibody titers in different groups (Study III), Kruskal Wallis test and Wilcoxon rank-sum test was used (JMP v. 4.0.2, SAS Institute Inc., Cary, NC). Spearman´s Rho, also in JMP, was used to test for correlations between the anti-CYP2E1 autoantibody titers and parameters such as age, years in occupation and symptom score.

8 RESULTS AND DISCUSSION

8.1 TOXICOKINETICS (STUDY I AND II)

8.1.1 Results

The blood concentrations of HFC-134a and HFC-143a increased rapidly when exposure started (Figure 10). The AUC (calculated by the trapezoidal rule) as well as the apparent steady-state (average of concentration from 30-120 min) were twice as high at exposure to HFC-134a compared to HFC-143a. Minor amounts (1-2 %) of HFC were exhaled after exposure or excreted via the urine.

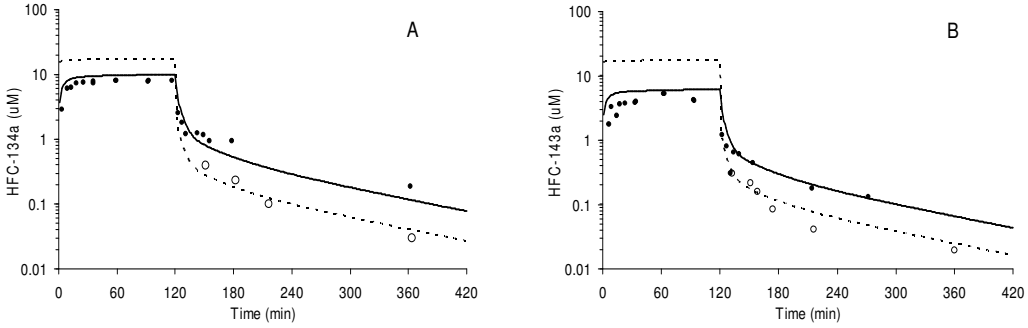


Figure 10. Experimentally observed (dots) and PBTK simulated (lines) time courses of HFC-134a and HFC-143a in blood (●, ——) and exhaled air (○, -----) in one of the volunteers (2 h exposure, 50 W work load).

The time courses of HFC-134a and HFC-143a could be described effectively with a PBTK model that assumed zero metabolism. As seen in Figure 10, the predictions from the PBTK model agree well with the observed time courses. The relative uptake of HFC-134a was about twice as high as that of HFC-143a (3.7 % and 1.6 %, respectively), which corresponds to the higher partition coefficient of HFC-134a compared to that of HFC-143a. Four phases of elimination were seen which are presented in Table 3.

Table 3. Half-times calculated with a PBTK model based on data from 2-h exposure to HFC-134a and HFC-143a performed at different occasions in an exposure chamber.

	Half-time (min)	
	HFC-134a	HFC-143a
Vessel-rich group	0.44	0.64
Liver	0.60	0.87
Working muscle	3.4 ± 0.3	4.9 ± 0.5
Resting muscle	23	33
Fat tissue	114 ± 21	166 ± 31

No increased concentrations of fluoride ions were seen after exposure to HFC-134a or HFC-143a. The concentrations after exposure to HFC-134a and HFC-143a were 18-64 µM and 14-70 µM fluoride, respectively. Fluorides could not be detected in plasma except in one volunteer in the HFC-134a study and two volunteers in the HFC-143a study.

8.1.2 Discussion

Study I and II are the first studies that simultaneously describes the human inhalation toxicokinetics of HFC-134a and HFC-143a in blood, exhaled air and urine.

The toxicokinetics of HFC-134a and HFC-143a were rather similar with low relative uptakes of a few percent. Low uptakes were expected since theses substances have a low solubility in blood due to physical characteristics (non-polar and highly volatile). However, it is higher in HFC-134a than in HFC-143a. The same pattern was seen as in the concentration at steady-state and the AUCs. This is in accordance with the lower solubility in blood of HFC-143a compared to that of HFC-134a (partition coefficients of 0.56 and 0.34, respectively).

The experimental data suited well within a PBTK framework. The model assumes no metabolism, which makes the curves fit well to experimental data and which is also indicated by the lack of fluoride ions in the urine and plasma samples. However, if zero metabolism was true, no uptake would be possible at steady-state. Even if there may seem to be no uptake at the end of exposure, it is still a few percent after 2-h exposure according to the model. A low metabolic clearance of HFC-134a is supported by experimental human [87, 98, 123] and animal [36] data. No such data for HFC-143a were found in the literature.

Small proportions of the inhaled HFC was retained in the urine or exhaled after exposure. Since less HFC-143a was taken up, less was excreted compared to that of HFC-134a. The elimination of HFC-134a was faster than that of HFC-143a as described by the model (half times).

According to the PBTK model as well as experimental studies in animals and humans, the metabolism of HFCs is limited. The fluoride concentrations in urine from the exposed volunteers are similar to previously reported background levels of fluoride (<10-137 µM [109] and 16-45 µM [50]). Fluoride is formed *in vitro* during oxidative defluorination of HFC-134a in liver microsomes from rat, rabbit and man [93-95]. However, negative results with respect to urinary fluoride have been reported after human exposure to HFC-134a [87].

Fluoride ions were the only metabolites analysed within this project. For the completeness of the toxicokinetic studies, data on trifluoroacetic acid concentrations in urine would have been desired. However, so far the gas chromatographic analyses of the urine samples, performed with flame ionizing detection after trifluoroacetic acid had been derivatized with dimethyl sulphate to the volatile methyl trifluoroacetate [127], has not been successful.

8.2 EFFECTS (STUDY I AND II)

8.2.1 Results

No remarkable findings were made in the electrocardiogram recordings during exposure to either HFC-134a or HFC-143a. Significant increases in ratings during exposure to HFC-134a were observed for “breathing difficulty”, “solvent smell”, “dizziness”, and “feeling of intoxication”. No changes in ratings were observed neither during, nor after exposure to HFC-143a compared to pre-exposure ratings. The fibrinogen concentration had increased by 5% after exposure to HFC-134a and by 11% after exposure to HFC-143a. None of the other inflammatory markers or uric acid had changed significantly after exposure (Table 4).

Table 4. Markers of inflammation and uric acid in plasma. Plasma was collected before and one day after a 2-h experimental exposure to HFC-134a (n=10) or HFC-143a (n=9). Average values and standard deviations are presented.

	HFC-134a		HFC-143a	
	Before	After	Before	After
CRP (mg/l)	1.69 ± 2.11	2.01 ± 1.50	1.21 ± 1.64	1.50 ± 1.47
SAA (mg/l)	2.14 ± 1.76	3.54 ± 2.03	1.96 ± 1.48	2.64 ± 1.96
Fibrinogen (g/l)	2.46 ± 0.38	2.57 ± 0.38*	2.45 ± 0.26	2.72 ± 0.27*
D-dimer (µg/l)	81.0 ± 73.4	84.7 ± 77.3	92.9 ± 88.3	94.9 ± 91.8
Uric acid (µM)	326 ± 63.5	336 ± 53.9	328 ± 59.0	326 ± 46.2

* Significantly different from the pre-exposure value (Student´s paired t-test)

8.2.2 Discussion

Some effects of exposure to HFC-134 and HFC-143a were evaluated in these studies. There were slight increases of the faster markers of inflammation (CRP and SAA) but these changes were not statistically significant. There were small but significant increases of plasma fibrinogen which may be an indication of an inflammatory process. Since both substances are inert and not prone to be metabolised, which would at least in theory be necessary to cause an inflammation, no plausible mechanism can be found. The increased levels after exposure to both HFC-134a and HFC-143a makes a chance finding less probable. Unfortunately, and since these studies were initially designed as toxicokinetic studies, no control exposure with clean air was included. However, fibrinogen levels in plasma collected prior to the two different exposures did not differ significantly. Also, there were unchanged fibrinogen levels one day after a former (unpublished) exposure study, performed in the same exposure chamber with clean air.

Plasma fibrinogen vary in a circadian pattern [17], and the plasma concentration of fibrinogen may increase as a result of physical exercise [37] and mental stress [77]. The circadian fluctuations should have no influence on the results since pre- and post exposure samples were taken at the same time of the day. Even though acute exercise has been shown to increase coagulant activity, there have been conflicting results with

respect to plasma fibrinogen [37]. Thus, induction due to the short, light exercise performed in these studies seems to be unlikely. The possible mental stress owing to the experimental situation is contradicted by the non-elevated fibrinogen levels seen in the unpublished exposure study.

The half time of fibrinogen in plasma is 100 h [60]. CRP and SAA rise an decline much faster than fibrinogen and it may be so that we did not catch this reaction due to the study design with blood sampling as long as 24 hours after the beginning of the exposure.

If these finding represents a true effect, the magnitude is hardly of any clinical relevance. However, some of the refrigeration mechanics in the studies by Ahlborg et al. [4] and Lillienberg et al. [73] had symptoms consistent with inflammation. In addition, the occupational exposures during e.g. refrigeration repair work may be much higher than the exposure level in the chamber studies, and peaks of up to 1400 ppm HFC-134a has been reported [52]. In a meta analysis of associations between inflammatory factors and coronary heart disease, a risk ratio of 1.8 was found when individuals in the top third (3.5 g/l) were compared with the bottom third (2.5 g/l) with respect to plasma fibrinogen [27]. Consequently, occupational exposures as refrigeration repair work may represent a slightly increased risk of coronary heart disease if our finding represents a true effect.

8.3 AUTOIMMUNITY (STUDY III)

8.3.1 Results

There was no difference in anti-CYP2E1 concentration between exposed workers (symptomatic and asymptomatic) and unexposed controls. No increase in CYP2E1 antibody titer was detected among refrigeration mechanics with symptoms, neither compared with healthy Swedish controls, nor with asymptomatic workers (Figure 11). Further, no relation between antibody titer and smoking, previous exposure to anesthetics, severity of symptoms, age, or year in occupation was detected (Figure 11).

Inclusion of Italian controls into the control group did not change the significance of the result, i.e. no increase in anti-CYP2E1 could still be seen among the exposed workers (Wilcoxon rank-sum test, p=0.40). The median values for exposed and unexposed groups were then 0.128 and 0.126, respectively.

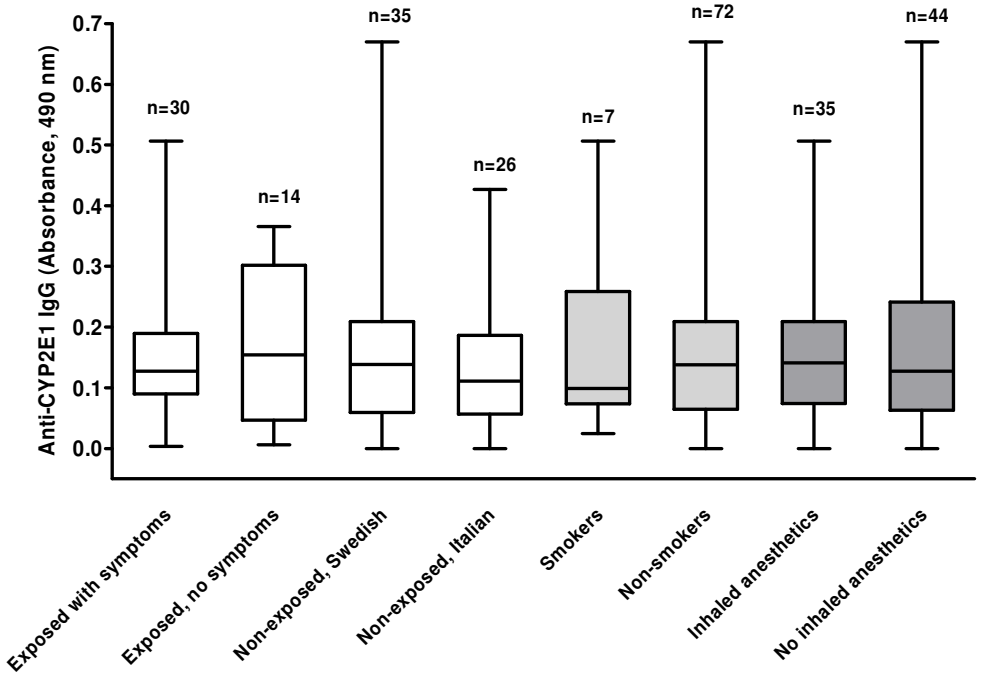


Figure 11. Titers of autoantibodies reacting with CYP2E1 (absorbance at 490 nm) in serum as analyzed with enzyme-linked immunosorbent assay (ELISA) after subtraction of the background reactivity for each serum. Smokers and those exposed to anesthetics all belong to the groups of Swedish workers and controls.

8.3.2 Discussion

No increase in antibodies against CYP2E1 was seen in exposed individuals compared to unexposed controls. The results contradicts some previous studies of effects in humans exposed to similar substances, e.g. liver diseases after accidentally exposure to 1,1-dichloro-2,2,2-trifluoroethane (HCFC 123) and 1-chloro- 1,2,2,2-tetrafluoroethane (HCFC 124) [61], patients with alcoholic liver disease (fibrosis/cirrhosis) [124], and among halothane hepatitis patients [15, 35]. However, exposure as well as liver damages were severe in those studies. On the other hand, high auto-antibody levels are not necessary for clinical symptoms of autoimmune diseases as seen in studies of anesthesiology personnel [15, 92], and consequently, elevated anti-CYP2E1 titers would have been quite possible among the refrigerations mechanics. Thus, the occupational exposure of the refrigeration mechanics [52] should be sufficient to generate autoimmune responses.

Autoimmune reactivity increases with age [55]. Still, no correlation between age and anti-CYP2E1 titer was seen among the individuals (exposed as unexposed persons) in Study III. Neither did number of years in the refrigeration mechanics´ occupation correlate with the antibody titer. Since the use of refrigerant type has changed during the years due to the environmental consideration and technical development [20], the

workers who has been working for a longer period of time will inevitably have been exposed to other kinds of refrigerants than the younger individuals whose exposure pattern may be less diverse. Despite that, no correlation between working years and antibody titer was seen.

It is not clear how long antibodies against CYP2E1 will remain in the body after exposure to halogenated hydrocarbons. About one months duration has been suggested after halothane exposure, but halothane hepatitis has been reported as long as 28 years after the primary exposure [80]. To exclude influence of time since exposure, currently exposed workers were compared to previously exposed ones. However, current exposure did not generate increased antibody titer levels compared to previous exposure.

Exposure to inhalation anesthetics did not change the anti-CYP2E1 antibody titer. Theoretically, cross-reactivity can occur between halogenated anesthetics and halogenated hydrocarbons used as refrigerants since reactive halogenated intermediates (acetyls and aldehydes) can be formed during the metabolism in both cases. These may bind to proteins like CYP2E1, resulting in trifluoroacetyl-CYP2E1. Antibodies against this neoantigen, or against native CYP2E1 due to decreased tolerance caused by occurrence of the trifluoroacetyl-CYP2E1 neoantigen, may then form. Cross-reactivity between different anesthetics (e.g. halothane and enflurane) has been reported previously [106, 108], but exposure to halogenated anesthetics did not influence the antibody titer in exposed workers either.

Smokers did not have increased titers of anti-CYP2E1 antibodies compared to non-smokers. However, as only 7 smokers were participating in the study, it is difficult to draw any firm conclusions. Cigarette smoke can suppress the immune system [110] as well as trigger immunological responses in individuals predisposed for autoimmune diseases [51]. Smoking may increase the risk for autoimmune diseases, e.g. rheumatoid arthritis in genetically predisposed individuals [96] and systemic lupus erythematosus (SLE) [25].

Addition of the Italian controls did not change the results considerably. Since their anamneses and information of sex, age, or smoking habits were missing, they could not be included except when exposed individuals were compared to unexposed ones. Accordingly, the results were disqualified from Study III, but are presented here to further support that there was no difference between exposed and non-exposed individuals.

There are a number of plausible explanations to the non-positive results of Study III:

(1) Exposure to HFCs, HCFCs, and CFCs may not cause autoantibody responses against CYP2E, i.e. the key hypothesis is wrong. However, antibodies against CYP2E1 has been detected after exposure to HCFC-123 and HCFC-124 [61] and for that reason, it is likely that they would be formed after exposure to other halogenated hydrocarbons as well.

(2) The occupational exposure is not sufficient to trigger auto-antibody formation. Considering that reactivity against CYP2E1 was demonstrated among anesthesiology personnel who had been exposure to low concentrations of anesthetics [15], the low but recurring occupational exposure levels of the refrigeration mechanics should presumably be sufficient to trigger autoimmune responses.

(3) Autoreactivity to CYP2E1 is too rare to be detected in a group of this size. Halothane hepatitis only occurs in about one of 6 000 to 35 000 patients [100]. This exceptionality may also apply to antibody formation caused by exposure to the CFC substitutes. In that case, inclusion of all the 700 Swedish refrigeration mechanics would not have been sufficient to detect such an effect.

In summary, associations between exposure to refrigerants and autoimmune effects can not be completely rejected by this study. However, the relation is less likely at the occupational exposure levels of the refrigeration mechanics.

8.4 DECOMPOSED CHLOROFLUOROCARBONS AND MYOCARDIAL INFARCTIONS

Possible relations between exposure to decomposed HCFC-22 and a subsequent myocardial infarction are discussed in Study IV. A 65-year-old man experienced respiratory symptoms (cough, blood-stained sputum, and increasing dyspnea) when he had been exposed to decomposed HCFC-22. He had been replacing a tank containing HCFC-22, an operation that required soldering. He was forced to go outside for some fresh air immediately after these activities. The following weeks his physical capacity decreased, and after about 3 weeks he was prescribed V-penicillin by his family physician to cure infectious bronchitis. Five days later he was admitted to hospital because of increasing dyspnea. Traces of a myocardial infarction could be seen on the electrocardiogram, and decreased ventricular function and a minor defect in the ventricular septum were seen in an echocardiogram. Three days later he died owing to a myocardial infarction. He did not have any pain in his chest neither the weeks after he had been exposed, or when he was admitted to hospital.

The man who had the myocardial infarction was in good health prior to the welding incident when he was exposed to decomposed HCFC-22. He was blood donor, which is an implication for his good health status, and a never-smoker. Also, he had no remarkable coronary sclerosis. In his job, he repaired and exchanged broken processors. Usually he did not use a respirator, i.e. his respiratory problems at work were probably due to exposure to decomposed HFCF-22.

Welding, which is a common activity of refrigeration mechanics although only performed during short periods of time [52, 73], causes decomposition of refrigerants [52]. Irritants as phosgene ($COCl_2$), chlorinated hydrocarbons, aldehydes, chlorine, and hydrochloric acid are being formed during welding in an environment containing the refrigerant HCFC-22 (chlorodifluoromethane) [89]. However, no phosgene was detected in the work environment of Norwegian refrigeration mechanics performing welding in the presence of HCFC-22 (detection limit: 0.02 ppm) [52]. Absence of phosgene may be due to low concentrations of HCFC-22 during the short welding periods, since presence of hydrogen fluoride and hydrochloric acid indicates that there actually is degradation of refrigerants during welding.

The man who had the myocardial infarction had most likely inhaled decomposed HCFC-22, and an inflammatory process was subsequently initiated. The weeks after exposure he had a cough and blood-stained sputum and his condition was interpreted as an infectious bronchitis. Dyspnea and chest pain has been reported when performing a similar operation [126]. Acute respiratory-tract infections is a risk factor for acute myocardial infarctions [84]. An increased relative risk for an acute myocardial infarction was related to acute respiratory-tract infection occurring within 10 days before the infarction. Chronic bronchitis has been associated with coronary heart disease. Several other chronic inflammatory diseases such as periodontal disease [67], rheumatoid arthritis, systemic lupus erythematosus [2] and psoriasis [79] have been linked to the occurrence of coronary heart disease.

Thus, in this case an inflammatory process was caused by the inhalation of decomposed freon and this inflammation may have been associated with the developing myocardial infarction.

With this case report, the importance of using protection equipment when soldering or welding in the presence fluorinated hydrocarbons is stressed. It also emphasizes the importance of an early medical treatment of inflammations.

9 FURTHER DISCUSSION

In this thesis, the toxicokinetics of two common HFCs used as refrigerants, HFC-134a and HFC-143a, were studied. The experimental data were described by a PBTK model. In addition, effects of short term experimental exposure as well as occupational exposure were assessed. The original reason for the project was scarce human data for this new group of chemicals which would potentially be used in large quantities since refrigeration and freezer systems are common everywhere.

The refrigeration mechanics experienced symptoms like asthma, influenza-like symptoms and joint problems after the introduction of the "new" refrigerants in the 1990s. The results from the present thesis would rather indicate that the new refrigerants as such could not have caused these symptoms. Only small amounts of HFC-134a and HFC-143a were taken up in the body, and even smaller amounts were metabolised, i.e. there is no prerequisites for large quantities of reactive intermediates to be formed. No effects have been found in Study I-III except for a slightly elevated fibrinogen plasma concentration (Study I and II).

The focus of this thesis has been the refrigerants as such. Overall, the substitution from CFCs to HCFCs and HFCs should not have posed any health problem. However, there are other factors in the work environment of refrigeration mechanics that could influence their health situation:

1. The substitution process also includes replacement of compressor oils from mineral oils used in systems with CFCs and HCFC to ester oils used in the HFC systems. During the 1990s when there was a great demand for substitution of the old CFCs, many refrigeration mechanics had to work very hard both with exchange of refrigerants and ordinary maintenance and repair. This work situation would also result in higher mean exposure of refrigerants, mineral oils, ester oils as well as smoke from welding and soldering tasks. More welding and soldering and poor ventilation would result in irritant smoke and gases, which might explain some of the respiratory symptoms reported [4, 73]. Lillienberg and co-workers found a correlation between the risk for swollen joints among refrigeration mechanics and high exposure of mineral oils on the hands [73]. Exposure to mineral oils has been shown to be related to rheumatoid arthritis [116]. No increased risk for swollen joints and exposure to ester oils was found.

2. Exposure to thermally decomposed refrigerants may initiate inflammation. The exposure may have initiated some of the symptoms of inflammation reported by Ahlborg et al. [4] and Lillienberg et al. [73] as well as the inflammatory process of the man who had the myocardial infarction (Study IV).

3. Refrigeration mechanics work in many different places, and often there is not much space around the refrigeration installations. Moreover, the mechanics sometimes have to carry heavy equipment in those narrow environments. The ergonomic situation in these places can be difficult and joint problems may also be related to bad ergonomic situations among these workers [32, 56].

10 CONCLUSIONS

Toxicokinetics

- The toxicokinetic characteristics of HFC-134a and HFC-143a are low respiratory uptakes, fast attainment of an apparent steady-state, and a fast decrease post-exposure. Experimental data can be described with a physiologically-based toxicokinetic (PBTK) model.

Effects

- The fibrinogen concentration in plasma was increased one day after the 2 h exposure to HFC-134a or HFC-143a. This effect could not be seen for other inflammatory markers (C-reactive protein, serum amyloid A protein, D-dimer, fibrinogen) or for uric acid.

Autoimmunity

- Exposure to halogenated hydrocarbons does not generate auto-antibodies against CYP2E1. An association between the occupational exposure levels of these compounds and autoimmune responses is less likely, although it can not be completely excluded.

Exposure and myocardial infarction

- It is important to use protective equipment and good ventilation when welding or soldering in the presence of HCFCs and to be aware of the possible interactions of exposure to decomposed halogenated hydrocarbons, a subsequent inflammation and the risk of developing a myocardial infarction.

11 SAMMANFATTNING

År 1974 kom den första varningen om att freoner (CFC, chlorofluorocarbons) påverkar ozonskiktet. Elva år senare observerades ett ozonhål över Antarktis och 1987 gjordes en internationell överenskommelse om att avveckla användningen av ozonnedbrytande ämnen. Som ersättare för CFC introducerades bl. a fluorkolväten (HFC, hydrofluorocarbons) och klorfluorkolväten (HCFC, hydrochlorofluorocarbons).

I samband med att CFC byttes ut mot HFC och HCFC rapporterade en del kylmontörer att de fått luftvägsbesvär, problem med lederna och influensaliknande symptom vilket de kopplade ihop med de nya köldmedierna. Vissa av köldmedierna är välstuderade i djurförsök, men data från människor förekommer mer sparsamt. Syftet med denna avhandling är att undersöka hur två vanliga fluorkolväten, HFC-134a (1,1,1,2-tetrafluoretan) och HFC-143a (1,1,1-trifluoretan), tas upp och omsätts i kroppen. Immunologiska reaktioner på grund av exponering för köldmedier har också undersökts och ett fall av hjärtinfarkt har diskuterats.

Upptag, omsättning och effekter: Frivilliga, manliga försökspersoner exponerades för 500 ppm HFC-134a (n=10) och HFC-143a (n=9) vid två olika tillfällen i en exponeringskammare. Alla försökspersoner var eller hade varit exponerade för HFC eller andra köldmedier i arbetslivet. Före, under och till och med 19 h efter exponering samlades blod, urin och utandningsluft in för analys av HFC med hjälp av gaskromatografi med flamjonisationsdetektor. I urin analyserades också förekomsten av fluoridjoner med jonselektiv elektrod. I blodplasma insamlad före och 20 minuter efter exponering analyserades förekomsten av fluorider.

Under försöket följdes försökspersonernas hjärtaktivitet med elektrokardiogram (EKG). Det var huvudsakligen en säkerhetsåtgärd eftersom tidigare exponeringsstudier av HFC-134a givit upphov till cirkulationsproblem hos människor och störd hjärtrytm hos hundar som fått adrenalin. Före, under och efter exponeringarna svarade försökspersonerna på frågor som var relaterade till irritation och CNS-besvär. Svaren skattades på en visuell analog skala (VAS). I venöst blod insamlat före och 21 h efter exponering undersöktes förekomst av inflammationsmarkörerna C-reaktivt protein, serum amyloid A-protein, fibrinogen och D-dimer. Även förekomst av urinsyra analyserades i dessa prover.

Både HFC-134a och HFC-143a tas upp snabbt i blodet och en platå uppnås inom en halvtimme. Utsöndringen sker snabbt efter det att exponeringen upphört. Förloppet beskrevs med en fysiologiskt baserad toxikokinetisk (PBTK) modell. Sammantaget tyder data på mycket låg metabolism vilket stöds av simuleringarna och ämnenas inerta egenskaper. Något upptag av HFC kunde inte påvisas experimentellt men beräknades till 4% med hjälp av PBTK-modellen. Att upptaget är lågt förklaras av den låga lösligheten av HFC i blodet. Att upptaget ändå är högre än noll förklaras av upplagring i fettväv.

Ingen ökad koncentration av fluorider kunde ses i urin efter exponering för HFC-134a eller HFC-143a. Fluorider kunde inte detekteras i plasma förutom i en individ vid exponering för HFC-134a och 2 individer vid exponering för HFC-143a.

Inga effekter på hjärtats aktivitet kunde ses under exponering för vare sig HFC-134a eller HFC-143a. Fibrinogenhalten i blod ökade efter båda exponeringarna. En sådan signifikant ökning kunde inte ses för de övriga inflammationsmarkörerna eller för urinsyra. Det är märkligt att just fibrinogen förekom i en högre koncentration efter exponering, medan de inflammationsmarkörer som ger ett snabbare inflammatoriskt svar (C-reaktivt protein och serum amyloid A-protein) inte gav något sådant utslag. Om resultatet är sant innebär ökningen av fibrinogen att en nivå uppnås som skulle kunna leda till en ökad risk för hjärtinfarkt bland kylmontörer.

Generellt skattade försökspersonerna i dessa studier högt jämfört med försökspersoner som exponerats för lösningsmedel i samma exponeringskammare. Under exponering för HFC-134a skattade försökspersonerna högre på frågorna ”svårighet att andas”, ”lösningsmedelslukt”, ”yrsel”, och ”berusningskänsla”. Under exponering för HFC-143a fanns ingen ökad skattning under eller efter exponering.

Autoimmuna reaktioner: Venöst blod för analys av antikroppar mot CYP2E1 samlades in från 44 manliga försökspersoner som var eller tidigare hade varit exponerade för köldmedier i arbetslivet. Urvalet skedde utifrån de svar som givits i en tidigare intervjustudie av kylmontörer. Av de utvalda individerna hade 30 astma, influensa-liknande symptom och/eller ledbesvär. De övriga 14 individerna var symptomfria. De exponerade individerna jämfördes sedan med friska och icke-exponerade manliga svenska (n=35) och italienska (n=26) kontroller. Förekomst av antikroppar mot CYP2E1 analyserades i blodserum med enzyme-linked immunosorbent assay (ELISA).

Ingen skillnad i koncentration av antikroppar mot CYP2E1 kunde ses mellan exponerade och icke-exponerade personer. Mängden antikroppar mot CYP2E1 hade inget samband med rökning, tidigare exponering för anestesigaser, ålder, antal år inom kylmontörsyrket eller hur allvarliga symptomen på inflammation var. De negativa resultaten kan bero på att den yrkesmässiga exponeringen för köldmedier är för låg för att ge en mätbar reaktion. Det kan också vara så att hypotesen är felaktig, dvs. att de halogenerade kolväten som kylmontörer utsätts för inte orsakar autoimmuna reaktioner mot CYP2E1.

Fallstudien: En 65-årig man fick luftvägsbesvär omedelbart efter att ha exponerats för sönderdelningsprodukter från köldmediet HCFC-22 (klorodifluorometan) vid reparation av ett kylsystem. Tre veckor senare fick han diagnosen infektiös bronkit av sin läkare och efter ytterligare en vecka avled han av en hjärtinfarkt. I rapporten diskuteras hur inflammation orsakad av att man inhalerar sönderdelad HCFC-22 kan ha samband med hjärtinfarkt.

Sammanfattningsvis är upptaget av HFC-134a och HFC-143a litet. En inflammatorisk reaktion kan förekomma vid det svenska hygieniska gränsvärdet på 500 ppm HFC-134a, men ytterligare studier behövs för att bekräfta detta. Exponering för

halogenerade kolväten som används som köldmedier kunde inte kopplas samman med antikroppar mot det kroppsegna enzymet CYP2E1.

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