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Consideration of Hydrofluoroolefins (HFOs) as potential candidate medical propellants[1]

Andrew A Lindley –Mexichem Fluor
Timothy J Noakes – Mexichem Fluor

Summary

Moves within the fluorochemicals industry to find alternative partially fluorinated molecules with low Global Warming Potentials (GWP)s) to perform as air-conditioning and refrigeration media are reviewed. In particular, candidates from the group of *Hydrofluoroolefins* (HFOs) are discussed in some detail, in terms of selection criteria, physical properties, and toxicology. HFOs are defined as containing hydrogen, fluorine, and a carbon backbone containing one *double bond.*

Whilst the public domain information reviewed here regarding this group is not yet complete, some points can be discussed regarding the possible suitability of members of the group as candidate lower GWP pharmaceutical propellants. Toxicological information so far available can be contrasted with the toxicology of the 'gold standard' of HFC 134a.

In particular, the question is addressed as to whether the information so far available on HFOs provides confidence in the way PAFT-1 information did regarding HFC-134a; sufficient to justify a costly 'IPACT-3' type respiratory toxicology programme on one or more of the HFOs, as a first step to developing them as lower GWP medical propellants.

The toxicology of the HFOs is found to be quite variable, but in all cases reviewed here, some level of activity was reported, sometimes quite significant. Furthermore, there are major gaps in the data, such as the absence of 2-year chronic exposure studies. Calculations based upon published results for some of these HFOs indicated that (in the absence of chronic data) the daily maximum safe exposure could be the equivalent of less than 1 MDI shot per day.

Conclusions

1. At present, there is no clear evidence of any molecule emerging with a toxicological 'clean slate' close to that of HFC 134a.

2. Therefore, there is at present no candidate molecule to put forward for respiratory toxicology testing in an "IPACT-3" type toxicology programme.

[1] Revised and updated April 2010

3. Given the frequent trajectory that as more toxicology results are unveiled exposure limits often go down, it is Mexichem Fluor's current belief that it is unlikely that any HFO will become acceptable as a respiratory propellant.

4. The situation should be kept under review, as further information regarding the HFOs, or indeed other future candidates, emerges.

5. It must be emphasised that a substance that may not suitable for use as a medical propellant may be suitable for safe use as an industrial refrigerant.

1 Introduction

As part of compliance with the Montreal Protocol for the protection of the ozone layer, work has been in hand since the late 1980's to convert Metered Dose Inhalers (MDIs) from Chlorofluorocarbon (CFC) to Hydrofluoroakane (HFC) propellants. The fact that this task is still unfinished (indeed there is a process for providing CFC Essential Use Allowances under the Protocol to cover MDI needs over the period 2010 to 2015 in certain developing countries) is evidence of the many issues and difficulties that are attendant on making such changes in the technically demanding and highly regulated world of the MDI.

The Montreal Protocol was put in place with the objective of phasing out CFCs, which had become implicated in stratospheric ozone depletion. The Montreal Protocol has also contributed to a significant reduction in global warming emissions. Replacing high quantities of higher GWP (Global Warming Potential) CFCs by lower quantities of lower GWP HFCs, contributed dramatically to reducing Climate Impact - about 3-4 times the objective of the Kyoto Protocol.

Since then, the non-ozone depleting replacement HFCs (the term HFC, covers industrial uses of these gases, but also encompasses HFA, which is reserved for grades used in the high purity MDI use) that have replaced CFCs in many applications, are in turn being considered as part of the increasing efforts to control emissions of greenhouse gases (GHGs). The conclusion of the IPCC/TEAP Special Report, Safeguarding the Ozone Layer and the Global Climate System[2], puts the use of HFCs into perspective. HFC radiative forcing (cumulative contribution to global warming) will remain below 1% of the estimated radiative forcing of all greenhouse gases by 2015, while, in terms of yearly emissions, HFCs will account for 2% of greenhouse gas emissions. Furthermore, according to the Special Report, the use of HFCs in MDI applications is estimated to be about 2 to 3% of total HFC demand in 2015.

Even so, and though much less powerful greenhouse gases than the CFCs they have replaced, HFCs are now becoming subject to regulation in some industrial applications[3], with some discussions about HFC regulation at Montreal Protocol meetings. At present there is no declared regulatory intention to seek to control the use of HFCs in MDI applications. In fact, it has been shown[2] that hypothetically replacing the small amount of HFCs in the MDIs with DPIs are one of the least cost-effective GWG replacement options.

Nevertheless, regulators and many companies believe that each sector that uses HFCs should ensure that it is taking the appropriate steps to assess the options for reductions in HFC emissions. However it is accepted that for asthma and COPD, the health and safety of the patient is of paramount importance in treatment decisions and policymaking that might impact those decisions.

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[2] The IPCC/TEAP Special Report, Safeguarding the Ozone Layer and the Global Climate System, Issues related to hydrofluorocarbons and perfluorocarbons, 2005.

[3] EC Regulation 842/2006 on certain fluorinated greenhouse gases & EC Directive 2006/40/EC relating to emissions from air-conditioning systems in motor vehicles and amending Council Directive 70/156/EEC

Even allowing for the long lead times, at least 10-15 years, due to the development timescales involved, it is prudent to subject any potential alternatives that may appear in the industrial landscape from time to time to critical review, as opportunities to reduce HFC emissions should be carefully evaluated, and there is a developing consensus that GHG emissions globally need to be reduced by at least 50% by 2050. In this way, any promising candidate molecule can be at least considered, for the long and complex respiratory toxicology (and subsequent product development programmes), in a timely manner. Equally those molecules that do not make the grade can be weeded out to ensure that limited and costly resources are not expended on studies that would be ultimately doomed, and which could potentially mislead regulators about the possibility of replacing the currently used HFCs.

1.1 **Sources of possible alternatives**

At present, the regulatory focus is on some of the refrigeration applications of HFCs, such as replacement refrigerants for automotive air-conditioning, and the chemical industry endeavour to meet these requirements is therefore also one possible source of compounds that might be suitable as alternate MDI propellants. As time proceeds there may be others.

1.2 **Candidate Selection Criteria**

A candidate alternative medical propellant must be tested against a number of criteria. These can be briefly listed as:

Very low toxicity
Non-flammable
Boiling range (-10 to –30ºC)
Acceptable solvent behaviour
Liquid Density (>1.0 gm/cm^3)
Best deliverable environmental gain over current propellants
Chemically stable
Acceptable to patients in terms of taste and smell

These criteria guided the selection process for the current HFC propellants. Whilst there may be the potential for some compromise with some of these criteria, there is no room for compromise on the toxicological profile. This is hardly surprising when its use in this application is considered. Not only is most or all of the propellant inhaled, it can be done several times a day, for life, into an organ that is usually already compromised and which can represent an extremely effective pathway for introducing chemical species into the human body.

Very low toxicity, in the context of a medical propellant, means toxicology performance suitable for use in pulmonary medications recognising that substances deliberately inhaled by this route may be rapidly absorbed into the blood stream. This is in contrast to an industrial refrigerant where the objective is not to inhale the substance, but also to make sure that safe working limits are identified and adhered to.

It must be emphasised that a substance that may not suitable for use as a medical propellant may be suitable for safe use as an industrial refrigerant.

It is only to be expected that medical regulatory authorities look for comprehensive and convincing evidence of the safety of any component in a respiratory medication that is to be regularly inhaled, with the propellant first and foremost, as it often makes up nearly all the inhaled dose. This was clearly illustrated during the qualification process for HFCs 134a and 227ea, which are also considered as pharmacologically inert.

2 Hydrofluoroolefins (HFOs)

In order to identify a non-flammable refrigerant gas molecule, with at the same time a low Global Warming Potential (GWP), some compromise with some of the selection criteria will be needed.

Whilst refrigerant gas selection criteria are similar to those for medical propellants, there are some differences both in criteria and their priority. At a simple level, these would be:

Non flammable where necessary
Non corrosive- compatible with metals, polymers and lubricants
Chemically stable in use at elevated temperatures
Boiling range suitable for refrigeration or air-conditioning eg –20 to –30ºC
Low Toxicity appropriate for an industrial chemical
Acceptable thermodynamic behaviour leading to energy efficiency

Non-flammability is usually achieved by inclusion of sufficient fluorine in the molecular structure, but fluorinated molecules typically absorb IR energy in the range 8 to 12 μm, which is a region transparent in the atmosphere. If a fluorinated molecule also has a relatively long atmospheric lifetime, then it becomes a greenhouse gas with a high GWP. Therefore one possibility is to develop molecules, which are sufficiently fluorinated to keep flammability under sufficient control, but have short or very short atmospheric lifetimes.

Based on this principle, most of the potential alternatives to HFC 134a that are actively being looked at as potential industrial refrigerants belong to the class of hydrofluoroolefins (HFOs). Typically, they are 3-carbon molecules containing one double bond, with fluorine and hydrogen atoms attached to this backbone. Due to the fact that they are 3 carbon (or more), can contain a varying ratio of F to H, and also contain a double bond, there is a significant number of different molecules and isomers possible – more so than with the simpler HFCs.

Most of the molecules in this group have short atmospheric lifetimes, due to the fact that they are rather reactive once released into the environment. The primary mechanism for reaction in the atmosphere is with hydroxyl radical, which proceeds via an addition mechanism at the double bond. The reaction of hydroxyl radical with HFO is about two orders of magnitude faster than reaction of hydroxyl radical with HFC 134a (which is via hydrogen abstraction).

Of course, as is so often the case, the other side of the reactivity coin can be biological activity, toxicological activity, or chemical instability. This is where the trade off may be starting to be made in the industrial context – acceptance of a possibly lower, but still suitable toxicological performance in order to retain other, more essential attributes.

Indeed, with regard to chemical stability, the initial screening[4] to identify CFC replacement medical propellants that identified HFAs 134a and 227ea, ruled out any compounds identified as unacceptably "reactive" including those containing functional groups such as double bonds. The concern was the possibility of reaction with canister materials or with the other components of the formulation including the drug substance. This is still a valid concern.

Nevertheless, some of these gases are attracting a lot of attention especially for automotive air-conditioning, as they are expected to meet enough of the critical industrial criteria.

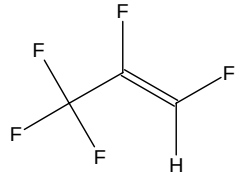
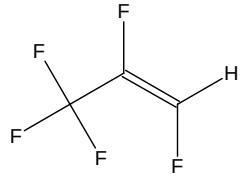
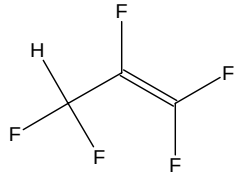
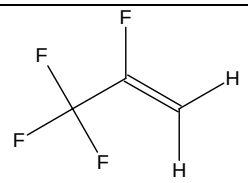
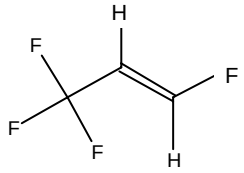
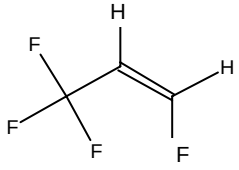
A significant number of molecules and isomers are possible for C_3 HFOs. The limits are set by propene and hexafluoropropene, which are well characterised. Both have low acute toxicity, both are metabolised to some extent but by different primary metabolic pathways (See Appendix I).

Name	**Propene**	**Hexafluoropropene**
Halocarbon number	1270	1216
Formula	C3H6	C3F6
Structure	CH3CHCH2	CF3CFCF2
Boiling Pt C	-47.7	-29.6
Flammability	Highly flammable	Non- flammable
Flammable limits	2-11 % vol/vol	
Toxicity	Anaesthetic	Kidney; CNS; respiratory tract
Exposure limit	TLV 500 ppm	TLV 0.1 ppm
Atmospheric Lifetime	~11 hours	~6 days

Within the limits of propene and hexafluoropropene, extensive work has identified more promising candidates, which were screened based on boiling point, flammability, and known toxicology for some of the molecules or structural alerts. These candidates have been further reduced through more extensive toxicology studies, including metabolic studies, taking into account the known potential metabolic pathways.

[4] Ensuring Patient Care, The role of the HFC MDI, IPAC 1997

The HFOs examined are shown in the table

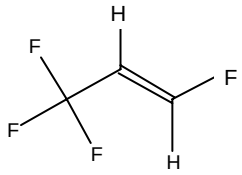
HFO 216 HFP	Hexafluoropropene	$CF_3C=CF_2$
HFO-1225yeZ	1, 2, 3, 3, 3-pentafluoropropene Z isomer	
HFO-1225yeE	1, 2, 3, 3, 3-pentafluoropropene E isomer	
HFO-1225zc	1, 1, 3, 3, 3-pentafluoropropene	$CF_3CH=CF_2$
HFO-1225yc	1, 1, 2, 3, 3-pentafluoropropene	
HFO-1234yf	2, 3, 3, 3-tetrafluoropropene	
HFO-1234zeE	Trans-1,3,3,3-tetrafluoropropene	
HFO-1234zeZ	Cis-1,3,3,3-tetrafluoropropene	
HFO-1243zf	3, 3, 3-trifluoropropene	$CF_3CH=CH_2$

The HFOs have a range of boiling points, which depends more on structure than the number of fluorine atoms.

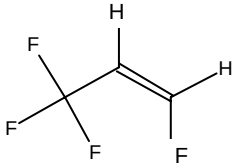
Selected HFOs	**Boiling Point ºC**
HFO-216	-29.6
HFO-1225yeZ	-21
HFO-1225yeE	-16
HFO-1234yf	-29.4
HFO-1234zeE	-19
HFO-1234zeZ	-6
HFO-1243zf	-26

Even stereo-isomers can have significantly different boiling points, for example the difference between the stereo-isomers of HFO-1234zeE and HFO-1234zeZ.

HFO-1234zeE (-19 ºC)



HFO-1234zeZ (-6 ºC)



2.1 **Flammability of HFOs**

Apart from hexafluoropropene, the only clearly non-flammable HFOs are the pentafluoropropenes. The tetrafluoropropenes are typically very close to the boundary of flammability/non-flammability with for example HFO 1234yf being very marginally flammable. The trifluoropropenes appear to have moderate flammability. There will be some variation with structure but this provides a reasonable basis for further selection.

In the context of industrial refrigeration there are very few promising non-flammable candidate substances outside the HFO family. One that was proposed as a means of designing non-flammable mixtures with flammable HFOs is CF_3I (iodotrifluoromethane). This is a very good fire extinguishant but is relatively unstable, and also has some toxicological issues.

Therefore this led to an initial focus on pentafluoropropenes (HFO 1225ye) and tetrafluoropropenes (HFO-1234 series) for non flammable or marginally flammable refrigerants. Current work across the industry for industrial refrigerant candidates is now focused on the HFO-1234 isomers such as HFO-1234yf and HFO-1234zeE.

2.2 **Toxicology**

The limited information available suggests that HFP is metabolised in the rat leading to nephrotoxicity. This may be a potential pathway for some HFOs. An initial screening method for selection is to compare reactivity, in vitro, using hexafluoropropene as a benchmark for a series of HFOs. Such a work programme has been carried out by CXR Biosciences Ltd and presented at ICT in 2007[5]. The results are summarised in Appendix II.

The correlation of high reactivity with toxicity (albeit not nephrotoxicity) is shown for HFO-1225zc, which has a rat 4-hr, ALC of 851 ppm, is Ames positive, and mouse micronucleus positive. HFO-1225zc also has the $=CF_2$ structural alert.

2.2.1 **Pentafluoropropenes HFO-1225yeE & Z**

The acute toxicity of HFO-1225yeZ was encouraging, but sub-chronic and chronic inhalation studies demonstrated that HFO-1225yeZ toxicity prevented its use as an industrial refrigerant. Similar conclusions were reported for HFO-1225yeE. The acute toxicity data for HFO-1225yeZ is in Appendix III.

2.2.2 **Tetrafluoropropenes HFO-1234 series**

The HFO-1234 series of isomers has a range of boiling points some of which are appropriate for use as a medical propellant. One of the isomers, HFO-1234yf, is proving to be of particular interest as an industrial refrigerant with a boiling point of approximately –30 ºC.

Considerable toxicology information[6] and a metabolic study[7] have been published for HFO-1234yf. See Appendix IV for more details.

The detailed toxicology information[6] for HFO-1234yf shows encouraging toxicology results for a wide range of tests. However, the authors reported a rabbit developmental toxicity study (exposures for 6 hours/day from days 6-28 of gestation) resulted in deaths at 5500 ppm (2 out of 24 rabbits). At 7500ppm it was reported that there were 6 deaths out of 24 rabbits. No deaths were reported at lower exposure levels (4000 ppm). The authors reported no significant developmental effects were seen in pup from any exposure group. The authors concluded that the No-observed-effect-level

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[5] "In-vitro Screening of a Series of Fluoropropenes and Fluorocyclopropanes for Hepatic S-glutathione Conjugation and NADPH Dependant Oxidation" poster presentation at the XIth International Congress of Toxicology July 15-19, 2007 in Montréal, Canada. R. H. Powrie, D. G. Farrar, A. K. Barton, C. R. Elcombe CXR Biosciences Ltd, Dundee, Scotland & Ineos Chlor Ltd, Runcorn, England. Sponsored by INEOS Fluor Limited.

[6] See ASHRAE Standard 34 Refrigerant Designation and Safety Classification for HFO-1234yf, submission by Honeywell and DuPont

[7] See Biotransformation of 2,3,3,3-tetrafluoropropene (HFO-1234yf) poster presentation at the Society of Toxicologists, 47th Annual Meeting, Seattle 2008, P Schuster, R Bertermann, G M Rusch and W Dekant, Department of Toxicology, University of Wuerzburg Germany and Honeywell, Morristown USA

for maternal and pup toxicity is 4000 ppm and 7500 ppm respectively for HFO-1234yf.

Mortality in dams in the absence of developmental toxicity is not uncommon. What is of importance with this result is the apparent high sensitivity of the rabbit or the pregnant rabbit to the effects of HFO 1234yf. It seems unlikely that the observation will be invalidated, although the species differences might be explained or understood, including its relevance, if any, to humans.

This result is different to the HFC 134a data, where no such anomalies were observed.

At this point there are no results from a 2 year combined carcinogenicity and inhalation study for the rat. A suitable 2-year study would be seen as an essential precursor to any attempt at executing a medical toxicology package.

Toxicology information for HFO-1234zeE is available on the EPA SNAP (Significant New Alternatives Policy Program)[8] website. From the published data the primary effect for 28 day and 90-day inhalation studies is cardiac inflammation (mononuclear cell infiltration) with effects seen at 15,000 ppm for the 28-day inhalation study.

2.3 **Toxicology and HFO candidate MDI propellants**

From the data reported for a range of C_3 HFOs it seems unlikely that they will be considered as inert as the current propellants HFCs 134a and 227ea.

As a group HFOs have always been treated with caution by the inhaled medications community due to generic toxicological concerns around the various structural alerts that exist within these compounds, and models related to them.

This was, and is, reflected in inhalation grade 134a specifications, most of which get their toxicological cover from the IPACT-1 (International Pharmaceutical Aerosol Consortium toxicology programme #1) which was sponsored by a consortium of pharmaceutical companies in the early 1990s.

When IPACT-1 was started HFC 134a was already looking very promising based upon a very good toxicological profile that had been assembled for industrial uses (PAFT-1 data). It was a substantial exercise, costing in the order of $10 millions at that time. The outcome of this programme was a submission to the regulatory authorities, which was intended to demonstrate the safety of this gas in pulmonary applications, and a specification justified by the results obtained[9].

[8] The Significant New Alternatives Policy (SNAP) Program is EPA's program to evaluate and regulate substitutes for the ozone-depleting chemicals that are being phased out under the stratospheric ozone protection provisions of the Clean Air Act (CAA).

[9] COMMITTEE FOR PROPRIETARY MEDICINAL PRODUCTS
Results of the Co-ordinated Review of 1, 1, 1,2-Tetrafluoroethane HFA-134a
Meeting of 12-13 July 1994

That specification is included below in Appendix V. It should be stressed that since then HFC 134a specifications have only tightened, this is the loosest toxicology driven cover specification.

Of particular interest to current discussions is the clause controlling 'total unsaturates' to no more than 5 ppm – the tightest control limit in the whole specification. This covers a group of unsaturated impurities, largely consisting of these HFOs.

This illustrates the wariness that still exists today in the MDI business when contemplating this type of entity as a potential propellant. Comprehensive evidence demonstrating very low toxicity, as with the case of HFC 134a, would be required before the industry could commit to the probably very extensive additional respiratory toxicology programmes, understanding that regulatory authorities would require a completely conclusive toxicological support package before approving such gases for respiratory use.

A good methodology for determining whether any of these candidate gases is well enough understood, and has a sufficiently clean record to be taken forward for a respiratory toxicology study, would be to compare the industrial toxicology results for the candidate gas with the benchmark toxicology results obtained for HFC 134a in the equivalent (PAFT-1) industrial toxicology programme. This is because it was partly on the basis of quality of these results that the decision to move forward to a medical tox programme for HFC 134a (IPACT-1) was taken.

Longer-term studies were conducted with HFC-134a for PAFT-1. No significant toxicological effects were observed in rats following inhalation exposure for up to one year at concentrations up to 50,000 ppm. Furthermore, the results from several genetic toxicity studies and data obtained from a two-year inhalation study for rats suggest that the increased incidence of benign tumours observed in rats in the two-year inhalation study is not due to an effect on genetic material. (The consequences of this finding for humans are considered to be biologically and toxicologically irrelevant. [10] Further information about the PAFT-1 results is contained in Appendix VI.)
On the basis of this and other information UK H&SE [8b] stated in 1995 that an OEL (8 hour time weighted Occupational Exposure limit) of 2000-5000 ppm would be justified on health grounds, before assigning the normal 'maximum assignable' limit of 1000ppm.

In contrast, No Effect Levels in some of the public domain information on the tests of some of the HFOs can be much lower. For example, as previously described HFO-1234yf, which has been determined to be safe for mobile air conditioning[11], has a No Effect Level of 4000ppm for dam mortality in rabbit developmental studies.

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[10] See ECETOC JACC Report No 50 for HFC 134a pages 35-40
[8a] US EPA methodology as described in Patty's Toxicology pp181, 196
[8b] 1,1,1,2 Tetrafluoroethane, Criteria Document for an Occupational Exposure limit. UK H&SE, 1995
[8c] T J Noakes Unpublished calculations
[11] SAE International Industry Evaluation of low global warming potential refrigerant HFO-1234yf, Phase 3 Final Report completed October 2009

To compare with the HFC 134a limit above, this result needs to be divided by appropriate safety factors, to cover inter-species, intra-species and study duration amongst others[8a]. These can be adjusted based on available data and other relevant factors such as toxicokinetics. Depending on the factors used a daily exposure limit for HFO 1234yf could be 50-125 times less than that of HFC 134a. Calculations show that this exposure would be the equivalent of less than 1 MDI shot per day [8c].

Mexichem Fluor UK Limited, The Heath Business and Technical Park,
Runcorn, Cheshire, WA7 4QX
Tel: +44 (0) 1928 511192, Fax: +44 (0) 1928 517592
e-mail: info@mexichem.com
www.mexichemfluor.com

Appendix I

Propene and Hexafluoropropene Metabolism

For propene a major route of metabolism is through the cytochrome P-450 system to propylene oxide, whereas for hexafluoropropene it is suggested that it is metabolised by conjugation with glutathione leading to kidney toxicity (nephrotoxicity).

Propene Metabolism Summary[12]
In rats and mice, most propylene inhaled into the lungs is exhaled again and does not reach the blood to become systemically available (Golka *et al.*, 1989; Svensson and Osterman-Golkar, 1984). Once absorbed, a major route of metabolism for propylene is through the cytochrome P-450 system to propylene oxide, a known carcinogen in experimental animals. Cytochrome P-450 enzymes in both the liver and nasal epithelium (Maples and Dahl, 1991) can convert propylene to its toxic metabolite. However, in rats, propylene metabolism becomes increasingly saturated at concentrations above 50 ppm (86 mg/m3) in the atmosphere (Golka *et al.*, 1989), which limits the amount of propylene oxide produced. Therefore, the amount of absorbed propylene may not reach high enough levels in classical long-term inhalation studies (Quest *et al.*, 1984) to show positive carcinogenic or serious chronic effects.

Hexafluoropropene Metabolism Summary[13]
The limited information available suggests that HFP is metabolised in the rat (*in vivo* and *in vitro*) by conjugation with glutathione (Figure 2). Two conjugates are formed, the first (PFPG) by displacement of a fluorine atom, the second (HFPG) by addition of glutathione without loss of fluorine. In the liver, PFPG appears to be the major product, both *in vitro* and *in vivo*; it is excreted in the bile. HFPG was the only metabolite formed in the kidney *in vitro*. The only metabolite identified in rat urine following exposure to HFP was N-acetyl-HFPC. The cysteine conjugates of HFP (PFPC and HFPC) are substrates for renal cysteine conjugate β-lyase.

[12] from Propylene Chronic Toxicity Summary at Office of Environmental Health Hazard Assessment California
[13] from ECETOC JACC No 48 Hexafluoropropylene

Appendix II

In-vitro Screening of a Series of Fluoropropenes

The glutathione metabolic pathway has the potential to lead to nephrotoxicity for HFOs. An initial screening method for selection is to compare reactivity, in vitro, for the glutathione metabolic pathway using hexafluoropropene as a benchmark for a series of HFOs. Such a work programme has been carried out by CXR Biosciences Ltd and presented at ICT in 2007[14].

This study was aimed at screening a series of HFOs for hepatic S-glutathione conjugation using hexafluoropropene (HFP) as a positive control in rat and human liver microsomes and cytosol.

The study determined the extent of hepatic S-glutathione (GSH) conjugation. HFO-216 (HFP) produced 4 very large GSH conjugates, one by addition and 3 by substitution. HFO-1225zc (1,1,3,3,3-pentafluoropropene) and HFO-1225yc (1, 1, 2, 3, 3-pentafluoropropene) both produced 3 very large GSH conjugates, 1 by addition and 2 by substitution. HFO-1225yeZ (1,2,3,3,3-pentafluoropropene Z isomer) produced 1 very small GSH conjugate by substitution. HFO-1225yeE (1,2,3,3,3-pentafluoropropene E isomer) produced 4 very small conjugates, 3 by addition and 1 by substitution. HFO-1234yf (2,3,3,3-tetrafluoropropene) produced one small substitution conjugate. HFO-1243zf (3,3,3-trifluoropropene) did not produce any detectable conjugates. The conjugates detected were produced in both microsomal and cytosolic samples with no significant differences seen between rat and human. These results suggested that of the compounds examined only HFO 216 (HFP), HFO-1225zc and HFO-1225yc could potentially elicit nephrotoxicity *via* a GSH/cysteine.

The correlation of high reactivity with toxicity (albeit not nephrotoxicity) is shown for HFO-1225zc, which has a rat 4-hr, ALC of 851 ppm, is Ames positive, and mouse micronucleus positive. HFO-1225zc also has the $=CF_2$ structural alert.

[14] "In-vitro Screening of a Series of Fluoropropenes and Fluorocyclopropanes for Hepatic S-glutathione Conjugation and NADPH Dependant Oxidation" poster presentation at the XIth International Congress of Toxicology July 15-19, 2007 in Montréal, Canada. R. H. Powrie, D. G. Farrar, A. K. Barton, C. R. Elcombe CXR Biosciences Ltd, Dundee, Scotland & Ineos Chlor Ltd, Runcorn, England. Sponsored by INEOS Fluor Limited.

Appendix III

Pentafluoropropenes HFO-1225yeZ Toxicity Testing Summary[15]

Acute Toxicity Summary	**HFO-1225yeZ**
Cardiac Sensitisation Risk	NOEL 75,000 ppm, LOAEL 100,000 ppm
Acute Toxicity	Inhalation rats LC50 4 hrs>50,000 ppm
	Inhalation rats 5 day, 50,000 ppm no deaths or significant clinical findings
AMES test (Mutagenicity test)	Negative
Chromosome Aberration	Slight effect at highest dose level similar to some other commercial fluorocarbons
Micronucleus	Rat - not active

The sub-chronic 28-day rat inhalation study for HFO-1225yeZ showed a range of toxicological effects at 10000, 25000 and 50000 ppm v/v.

The preliminary results suggested that the primary target for the toxicity of HFO-1225 yeZ is the myocardium. It cannot be dismissed that this result is due to fluoride. No NOEL for this effect was been established in the study. There are some indications of sex-specific differences in the toxicity of HFO-1225yeZ, although it is unclear whether these differences are a reflection of differences in sensitivity between males and females or some other more fundamental difference in their response.

It is noted that the urinary fluoride levels in both males and females exposed to similar concentrations HFO-1225yeZ were broadly similar, suggesting that it is metabolised to a similar extent in both sexes. Whilst some of the effects reported are likely to be the consequence of fluoride toxicity (e.g. the effects on the teeth), it is unlikely that all of the effects (e.g. cardiac toxicity) are related to fluoride.

The kidney and liver do not appear to be primary target organs for the toxicity of HFO-1225yeZ and, as such, the substance has a significantly different toxicity profile to the structurally related HFO 216 (HFP), which is consistent with the results from the glutathione metabolic pathway initial screening.

[15] Mexichem Fluor toxicity testing results for HFO-1225yeZ –not published

Appendix IV

Published Summary Toxicology Data for HFO1234yf

Considerable toxicology information[16] and a metabolic study[17] have been published for HFO-1234yf.

The *in vivo* metabolic study concluded that there was a low extent of biotransformation in rats and mice, based on <1% of dose being recovered as metabolites in urine. The study concluded that the major metabolic route is via epoxidation of HFO-1234yf leading to N-acetyl-S-(3,3,3-trifluoro-2-hydroxy-propyl)-L-cysteine. Minor urinary metabolites are 3,3,3-trifluoro-1,2-dihydroxy propane, 3,3,3-trifluoroacetone, trifluoroacetic acid, 3,3,3-trifluorolactic acid, inorganic fluoride, and 3,3,3-trifluoro-1-hydroxy acetone.

The detailed toxicology information[6] for HFO-1234yf shows encouraging toxicology results for a wide range of tests. The ASHRAE submission concludes that HFO-1234yf has a low acute toxicity with a 4 hour LC_{50} rat >405,800 ppm.. A 13 week exposure conclude a NOAEL of at least 50,000 ppm The mutagenicity studies (human lymphocytes, mouse and rat micronucleus) were negative except for the Ames test which showed activity for two bacteria strains.

However, the authors reported a rabbit developmental toxicity study (exposures for 6 hours/day from days 6-28 of gestation) resulted in deaths at 5500 ppm (2 out of 24 rabbits) At 7500ppm it was reported that there were 6 deaths out of 24 rabbits. No deaths were reported at lower exposure levels (4000 ppm). The authors reported no significant developmental effects were seen in pup from any exposure group. The authors concluded that the No-observed-effect-level for maternal and pup toxicity is 4000 ppm and 7500 ppm respectively for HFO-1234yf.

[16] See ASHRAE Standard 34 Refrigerant Designation and Safety Classification for HFO-1234yf, submission by Honeywell and DuPont

[17] See Biotransformation of 2,3,3,3-tetrafluoropropene (HFO-1234yf) poster presentation at the Society of Toxicologists, 47th Annual Meeting, Seattle 2008, P Schuster, R Bertermann, G M Rusch and W Dekant, Department of Toxicology, University of Wuerzburg Germany and Honeywell, Morristown USA

Appendix V

IPACT-1 134a specification.

TEST	SPECIFICATION
DESCRIPTION	HFC-134a is a colourless, odourless, non-flammable gas that exists as a liquid when under pressure
IDENTITY	
Test A	The main peak and elution time in both assay/purity methods is the same as that of the reference standard for HFC-134a
Test B (Test B is not done on a routine basis, but it is available should any further confirmation be required.)	IR spectrum of sample is concordant with the reference standard for HFC134a
WATER	Not more than 0.001% w/w
ACIDITY (as HCI)	Not more than 0.00001% w/w
TOTAL RESIDUE	Not more than 0.01% v/v.
NON-ABSORBABLE GASES	Not more than 1.5% v/v
ASSAY	Not less than 99.8% w/w of HFC-134a

NAMED ORGANIC IMPURITIES	
CFC-11 [1,1,1-trichloro-1-fluoromethane] CFC-12 [1,1-dichloro-1,1-difluoromethane] CFC-114 [1,2-dichloro-1,1,2,2-tetrafluoroethane] CFC-114a [1,1-dichloro-1,2,2,2-tetrafluoroethane] CFC-115 [1-chloro-1,1,2,2,2-pentafluoroethane] HCFC-22 [1-chloro-1,1-difluoromethane] HCFC-123 [1,1-dichloro-2,2,2-trifluoroethane] HCFC-123a [1,2-dichloro-1,2,2-trifluoroethane] HCFC-124 [1-chloro-1,2,2,2-tetrafluoroethane] HCFC-124a [2-chloro-1,1,2,2-tetrafluoroethane] HFC-125 [2-chloro-1,1,2,2-tetrafluoroethane] HFC-134 [1,1,2,2-tetrafluoroethane] HFC-143a [2,2,2-trifluoroethane] HFC-152a [1,1-difluoroethane] HFC-245cb [1,1,1,2,2-pentafluoropropane]	Not more than 0.1% w/w individually or in combination
NAMED ORGANIC IMPURITIES	
HCC-40 [Methyl Chloride]	Not more than 0.005% w/w.
NAMED ORGANIC IMPURITIES	
HFC-152 [1,2-difluoroethane]	Not more than 0.0005% w/w.
HCFC-132b [1,2-dichloro-2,2-difluoroethane]	Not more than 0.0005% w/w
HCFC 31 [1-chloro-1-fluoromethane]	Not more than 0.0005% w/w.
HCFC-133a [1-chloro-2,2,2-trifluoroethane]	Not more than 0.0005% w/w.
TOTAL UNSATURATES	Not more than 0.0005% w/w.

TOTAL OTHER ORGANIC IMPURITIES	Not more than 0.005% w/w.

NB 0.0001% = 1 p.p.m.

Appendix VI

Programme for Alternative Fluorocarbon Toxicity Testing (PAFT-1) Conclusions for HFC-134a[18]

HFC-134a has very low acute inhalation toxicity. The lowest concentration that causes mortality in rats -- the approximate lethal concentration (ALC) -- for a 4-hour exposure is greater than 500,000 ppm. Anaesthetic-like effects, such as lethargy and incoordination, are observed in rats at very high inhalation concentrations (greater than 200,000 ppm).

Longer-term studies have also been conducted with HFC-134a. No significant toxicological effects were observed in rats following inhalation exposure for up to one year at concentrations up to 50,000 ppm.

At the end of the two-year inhalation study, no effects were observed in body weights, in-life measurements, clinical observations or clinical chemistry, or haematology. Except for the testis of male rats, no grossly visible or microscopic changes were observed in the any of the HFC-134a exposed rats. At 50,000 ppm, an increased incidence of hyperplasia (cell growth) and benign tumours of Leydig cells was observed on microscopic examination of the testis. No malignant tumours attributable to exposure to HFC-134a were observed. An independent review of the pathology findings supported these conclusions. None of the benign tumours were life-threatening, and all occurred near the end of the study. No effects were observed at lower concentrations in this two-year study; the no-observed-effect level (NOEL) was 10,000 ppm.

Several genetic toxicity studies with HFC-134a have been completed. These included a bacterial reverse mutation (Ames) test, an in vitro chromosomal aberration study with human lymphocytes, and a cytogenetics assay with Chinese Hamster Lung Cell (CHL). In vivo studies included cytogenetics, mouse micronucleus, and a dominant lethal study in the mouse. Evidence from all in vitro and in vivo studies clearly indicates that HFC-134a is not genotoxic. Furthermore, these data and data obtained from the two-year inhalation study suggest that the increased incidence of benign tumours observed in the two-year inhalation study is not due to an effect on genetic material.

Results from inhalation developmental toxicity studies indicate that HFC-134a does not cause teratogenic effects in rats or rabbits. At inhalation concentrations of 300,000 ppm, slight maternal toxicity and embryotoxicity, evidenced by a decrease in fetal body weights, were observed in rats. Lower fetal body weights of rats and rabbits have been observed at 50,000 ppm with slight maternal toxicity; lower maternal body weights were also observed in rats at this concentration. In an additional study, no fetal effects were observed in rabbits at inhalation concentrations of up to 40,000 ppm.

[18] see www.paft.org

Although not metabolized to any significant extent in animals, HFC-134a is oxidatively metabolized following inhalation exposure, as suggested by a slight increase in urinary fluoride levels. However, the rate of metabolism of HFC-134a is very low, and about 99% of an inhaled dose is eliminated unchanged.

The testing results for HFC-134a under PAFT 1 are summarized below

Summary of PAFT Testing Results

- HFC-134a has very low acute and subchronic inhalation toxicity.
- HFC-134a caused an increased incidence of benign tumours in animals following long-term exposure to high concentrations.
- HFC-134a is not a developmental toxicant.
- HFC-134a is not genotoxic.