

# IN-VITRO SCREENING OF A SERIES OF FLUOROPROPENES AND FLUOROCYCLOPROPANES FOR HEPATIC S-GLUTATHIONE CONJUGATION AND NADPH DEPENDENT OXIDATION

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## Abstract

We have studied a series of fluorohydrocarbons to determine the extent of GSH conjugation and NADPH dependent oxidation using hexafluoropropene (HFP) as a positive control. HFP produced 4 very large GSH conjugates, one by addition and 3 by substitution. 1,1,3,3,3-pentafluoropropene (R1225zc) and 1, 1, 2, 3, 3-pentafluoropropene (R-1225yc) both produced 3 very large GSH conjugates, 1 by addition and 2 by substitution. 1,2,3,3,3-pentafluoropropene (R1225ye Z isomer) produced 1 very small GSH conjugate by substitution. 1,2,3,3,3-pentafluoropropene (R1225ye E isomer) produced 4 very small conjugates, 3 by addition and 1 by substitution. 2,3,3,3-tetrafluoropropene (R1234yf) produced one small substitution conjugate. Trifluoropropene, tetrafluorocyclopropane and difluorocyclopropane 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. No NADPH dependent oxidative metabolites were detected. These data suggest that of the compounds examined only HFP, R1225zc and R1225yc could potentially elicit nephrotoxicity via a GSH conjugate/cysteine conjugate beta-lyase mechanism.

## Introduction

This study was aimed at screening a series of gaseous fluoropropenes and fluorocyclopropanes for hepatic S-glutathione conjugation and NADPH dependent oxidation. The compounds examined were as follows:-

- 1, 1, 2, 3, 3, 3-hexafluoropropene
- 1, 2, 3, 3, 3-pentafluoropropene (R-1225ye Z isomer)
- 1, 2, 3, 3, 3-pentafluoropropene (R-1225ye Z isomer) purified
- 1, 2, 3, 3, 3-pentafluoropropene (R-1225ye E isomer)
- 1, 2, 3, 3, 3-pentafluoropropene (R-1225ye E isomer) purified
- 1, 1, 3, 3, 3-pentafluoropropene (R-1225zc)
- 1, 1, 2, 3, 3-pentafluoropropene (R-1225yc)
- 2, 3, 3, 3-tetrafluoropropene (R-1234yf)
- 3, 3, 3-trifluoropropene
- Tetrafluorocyclopropane
- Trifluorocyclopropane
- Difluorocyclopropane

## Methods

### Incubation conditions

Incubations of each compound were performed at 37°C in Tris-HCl buffer pH 7.4 containing 10mM GSH or an NADPH regenerating system and 1mg protein/mL of either rat or human liver microsomes or cytosol. Each gas was bubbled through the incubation mixtures at approximately 1 bubble/second. Samples were removed at 0, 5, 10, 20 and 30 minutes and added to an equal volume of acetonitrile, centrifuged and the supernatant removed and frozen at -70°C until analysis. Air was simultaneously bubbled through the mixtures for NADPH dependent incubations.

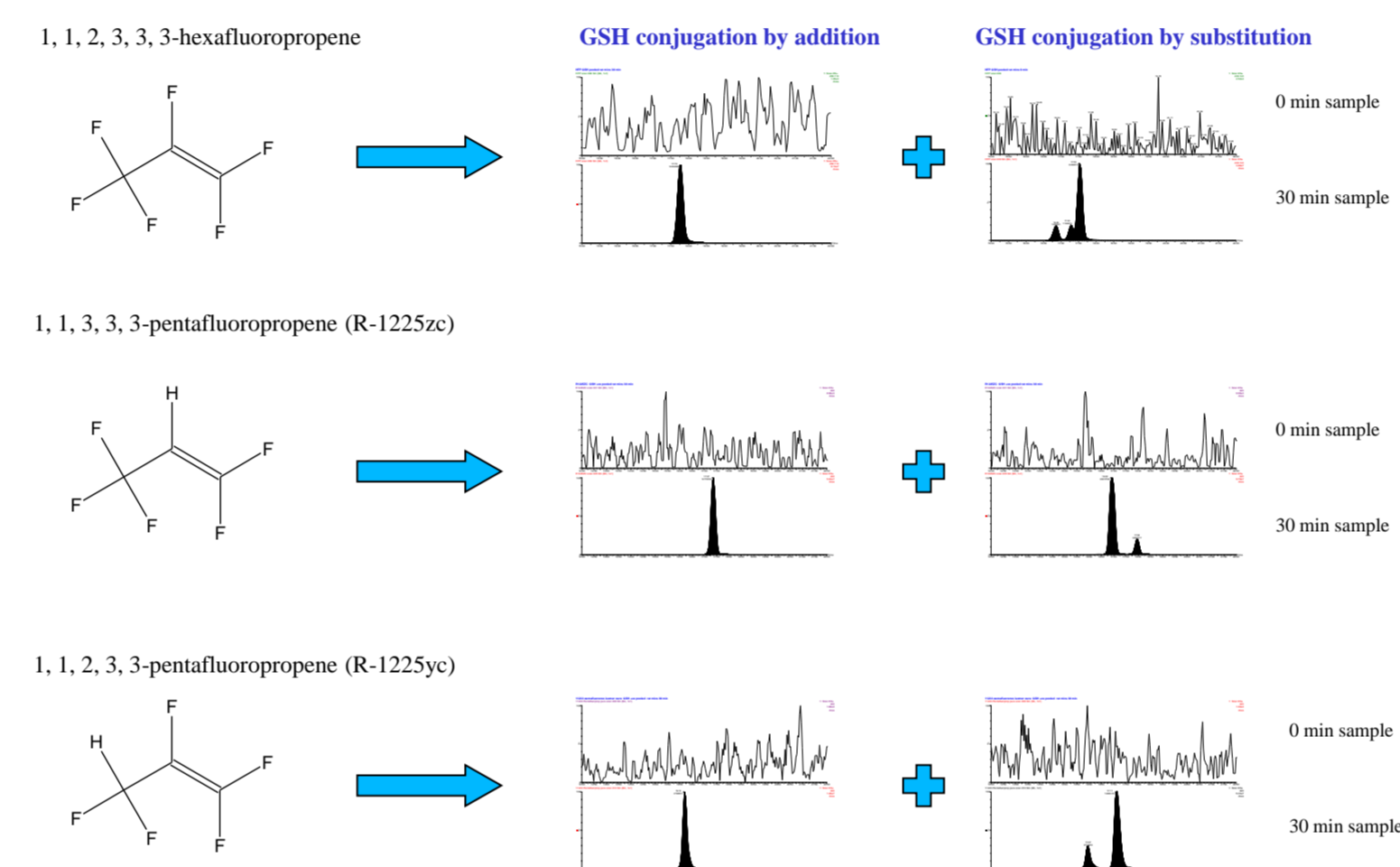
### Analysis

LC-MS analysis was performed on the samples scanning in both +ve and -ve ion modes between 50-700m/z using a Waters Quattro Micro mass spectrometer. LC-MS/MS analysis was performed on the samples to determine the rates of depletion of the S-glutathione from the incubations.

## RESULTS

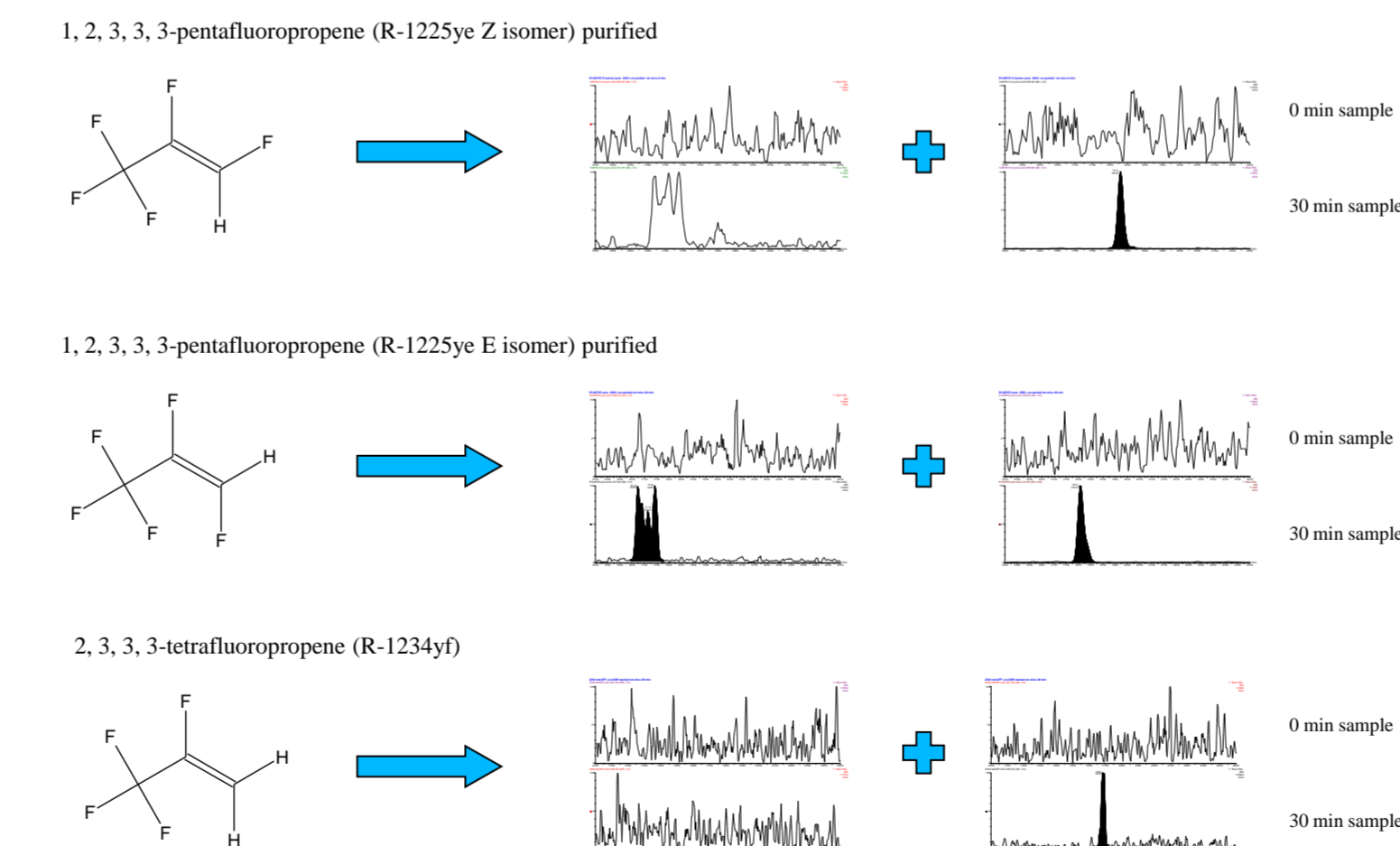
### Major S-glutathione conjugate peaks detected

(Chromatograms of rat microsomal samples shown as examples)



### Minor S-glutathione conjugate peaks detected

(Peak areas are approximately 0.1% or less compared to major conjugates above)



No S-glutathione conjugates were detected in the incubated samples of 3, 3, 3-trifluoropropene, tetrafluorocyclopropane, trifluorocyclopropane or difluorocyclopropane.

### Rates of S-glutathione depletion

The rates of depletion (nmoles/min/mg protein) of the S-glutathione from the incubations with the rat microsomes, rat cytosol, human microsomes and human cytosol, respectively, were as follows:-

- 1, 1, 2, 3, 3, 3-hexafluoropropene = 92, 106, 77 and 98
- 1, 1, 3, 3, 3-pentafluoropropene (R-1225zc) = 159, 69, 65 and 20
- 1, 1, 2, 3, 3-pentafluoropropene (R-1225yc) = 109, 70, 159 and 10

There was insufficient depletion of S-glutathione from the incubations with the remaining compounds to calculate the rates.

### NADPH dependent oxidation

No NADPH dependent oxidative metabolites could be detected in the incubations of any of the compounds tested.

## Conclusions

From the results it can be concluded that 1, 1, 2, 3, 3, 3-hexafluoropropene, 1, 1, 3, 3, 3-pentafluoropropene (R-1225zc) and 1, 1, 2, 3, 3-pentafluoropropene (R-1225yc) have all produced major S-glutathione conjugates by both addition of the S-glutathione to the molecules and by substitution of one of the fluorines on the molecule with the S-glutathione. The S-glutathione conjugation has also occurred in both rat and human liver microsomes and rat and human liver cytosol.

If it is assumed that the ion response peaks of the S-glutathione conjugates are similar across the range of compounds tested, then the S-glutathione conjugates detected with the incubations of both pure and impure 1, 2, 3, 3, 3-pentafluoropropene (R-1225ye Z isomer), 1, 2, 3, 3, 3-pentafluoropropene (R-1225ye E isomer) and 2, 3, 3, 3, 3-tetrafluoropropene are all very minor. These conjugates detected have peak areas approximately 0.1% or below when compared with the peak areas of the conjugates of 1, 1, 2, 3, 3, 3-hexafluoropropene, 1, 1, 3, 3, 3-pentafluoropropene (R-1225zc) and 1, 1, 2, 3, 3-pentafluoropropene (R-1225yc).

There was insufficient depletion of the S-glutathione from the incubations with 1, 2, 3, 3, 3-pentafluoropropene (R-1225ye Z isomer), 1, 2, 3, 3, 3-pentafluoropropene (R-1225ye E isomer), 2, 3, 3, 3, 3-tetrafluoropropene, 3, 3, 3-trifluoropropene, tetrafluorocyclopropane, trifluorocyclopropane and difluorocyclopropane to calculate the rates of depletion.

No S-glutathione conjugates could be detected in the incubations with 3, 3, 3-trifluoropropene, tetrafluorocyclopropane, trifluorocyclopropane or difluorocyclopropane.

These results suggest that of the compounds examined only HFP, R1225zc and R1225yc could potentially elicit nephrotoxicity via a GSH conjugate/cysteine conjugate beta-lyase mechanism.