

**Biomarkers for Air Pollutants:  
Development of Hemoglobin Adduct Methodology  
for Exposure Assessment**

Harrell E. Hurst, Ph.D.

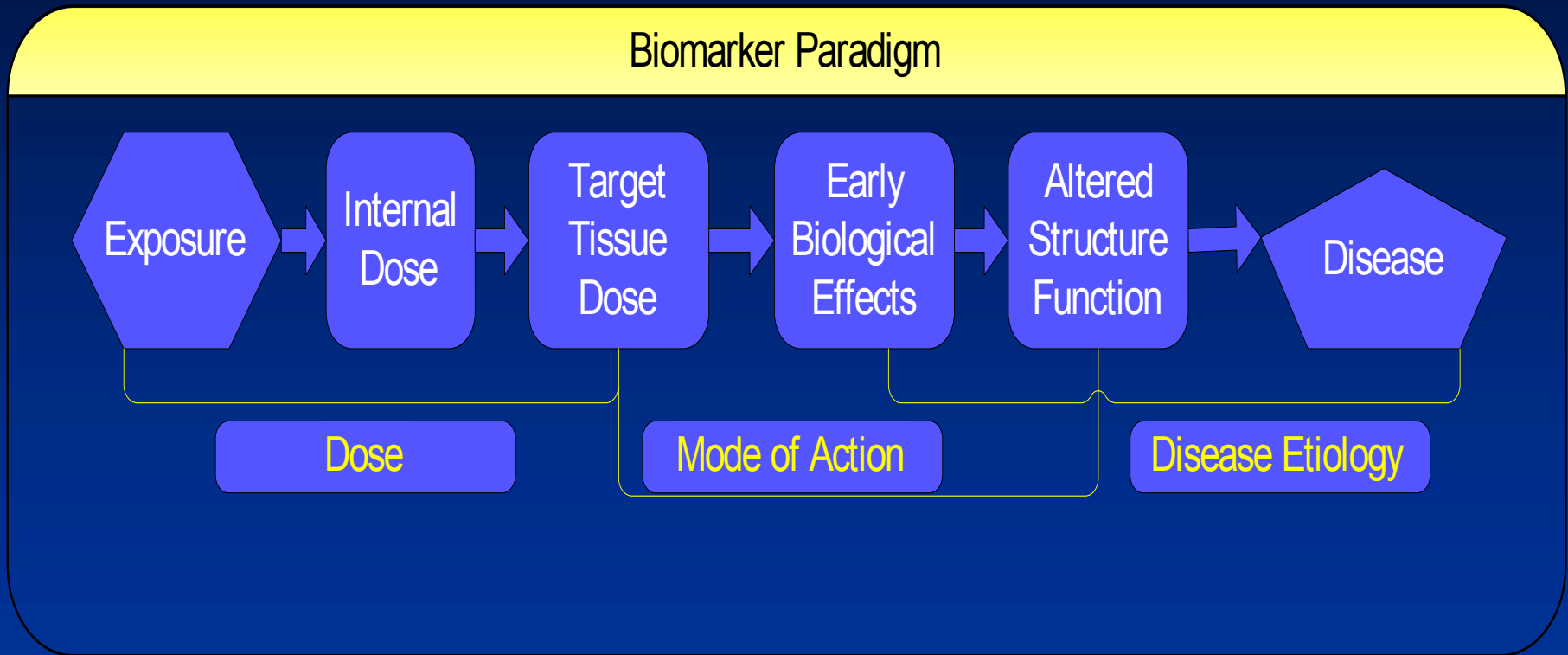
Dept. Pharmacology & Toxicology

University of Louisville School of Medicine

# Biomarker Definition:

- Measurable internal indicator of change at molecular or cellular level to detect key event(s) linking specific exposure to health outcome

# Biomarkers in Environmentally-Induced Disease



# Hemoglobin

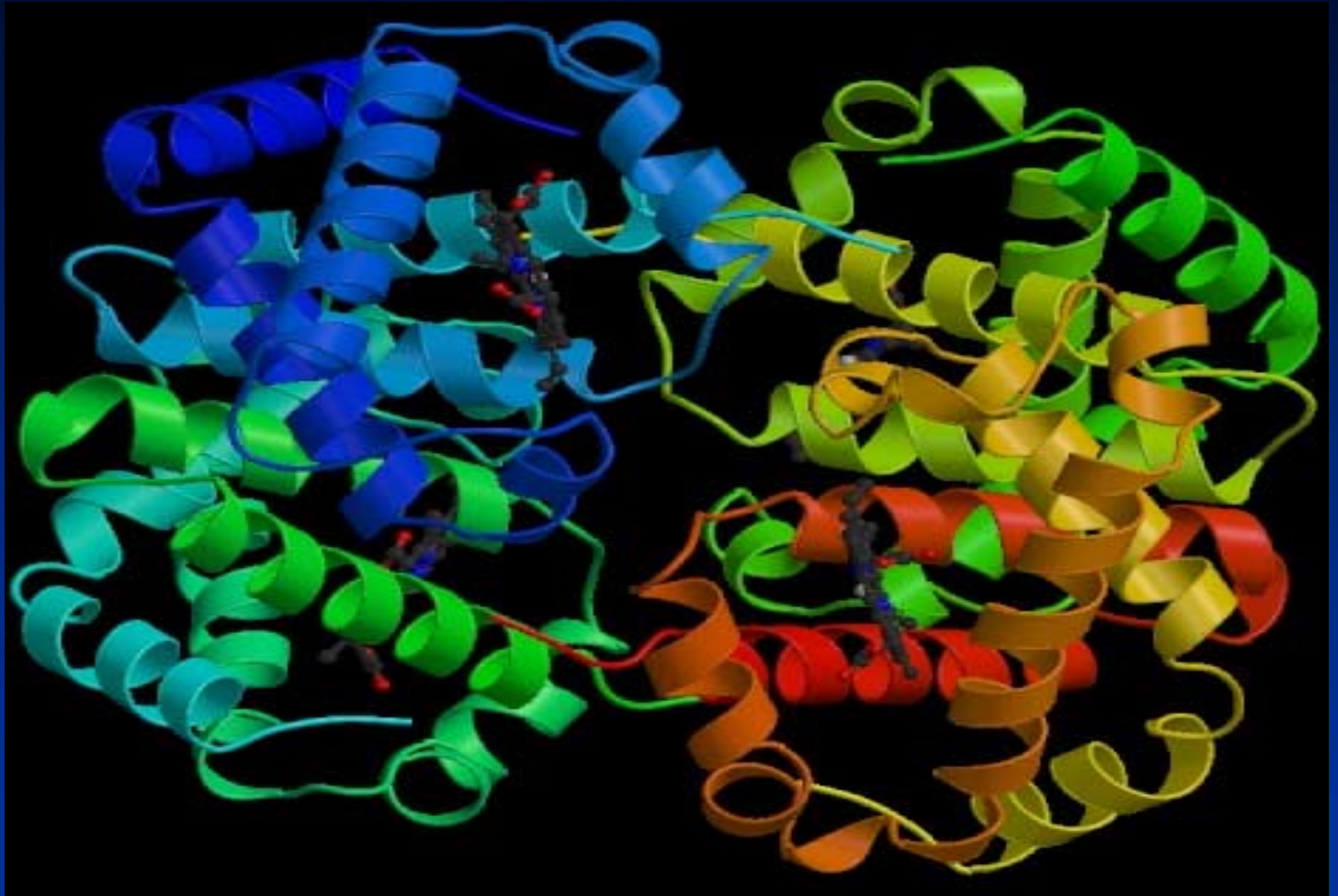
4 Protein  
Chains:

2  $\alpha$  Chains  
141 AA

MW=15100  
daltons

2  $\beta$  Chains  
146 AA

MW=15851  
daltons



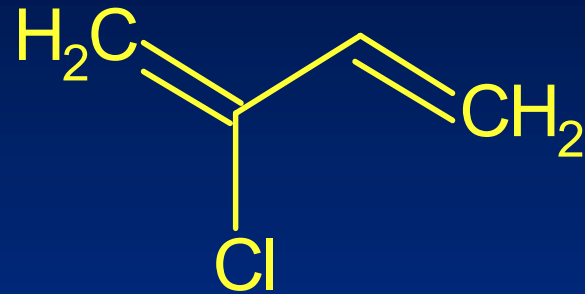
# Hemoglobin Adduct as Biomarker

## Hb:

- Abundant accessible protein for biomarker
- Monitors recent past exposure over red blood cell lifetime:
  - 120 days in human
- Exposure biomarker related to mechanism
  - Most cancer-causing chemicals are electrophilic
- Traps electrophilic chemicals and reactive metabolites at nucleophilic sites:
  - N-terminal amino acids on  $\alpha$  and  $\beta$  chains (**Valine**)
- Surrogate monitor for mutagenic DNA adducts

# Chloroprene

- CAS 126-99-8
- M.W. = 88.54
- B.P. = 59.4°C
- Vapor pressure = 174 mm Hg at 20°C
- Monomer for production of:
  - Polychloroprene
  - Neoprene
- Autopolymerizes !
- Stored at < 0 °C under N<sub>2</sub> with polymerization inhibitors
- Present in Jefferson Co. air from fugitive emissions

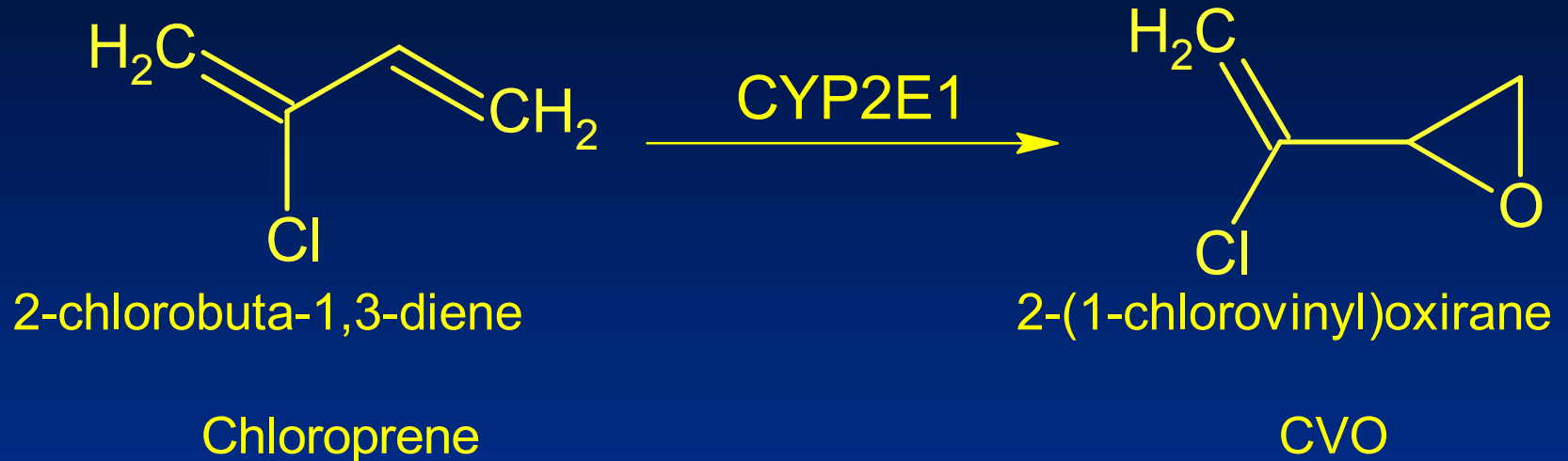


2-chlorobuta-1,3-diene

# Chloroprene Toxicity

- Inhalation subchronic lethal concentrations:
  - ~ 500 ppm for rats,  $\leq$  200 ppm mice
- Toxic effects:
  - Narcosis and hypoactivity, Weight loss
  - Nasal epithelial degeneration
  - Hepatocellular centrolobular necrosis
  - Forestomach squamous epithelial hyperplasia
  - Thymic necrosis, Myocardial hypertrophy
  - Lung & liver non-protein sulfhydryl content (glutathione) decrease

# Chloroprene Bioactivation



Chloroprene is metabolized by cytochrome P<sub>450</sub> oxidative enzymes to a reactive electrophilic epoxide, chlorovinyl-oxirane, also known as chloroethenyloxirane.



# Chloroprene Metabolism

- Toxic activation by CYP2E1 to CVO
  - Chlorovinylloxirane (CVO) is a relatively long-lived, reactive electrophile
  - CVO reacts with nucleophilic sites in DNA and proteins
- Detoxification of CVO occurs through:
  - Conjugation with glutathione
  - Hydrolysis by epoxide hydrolase

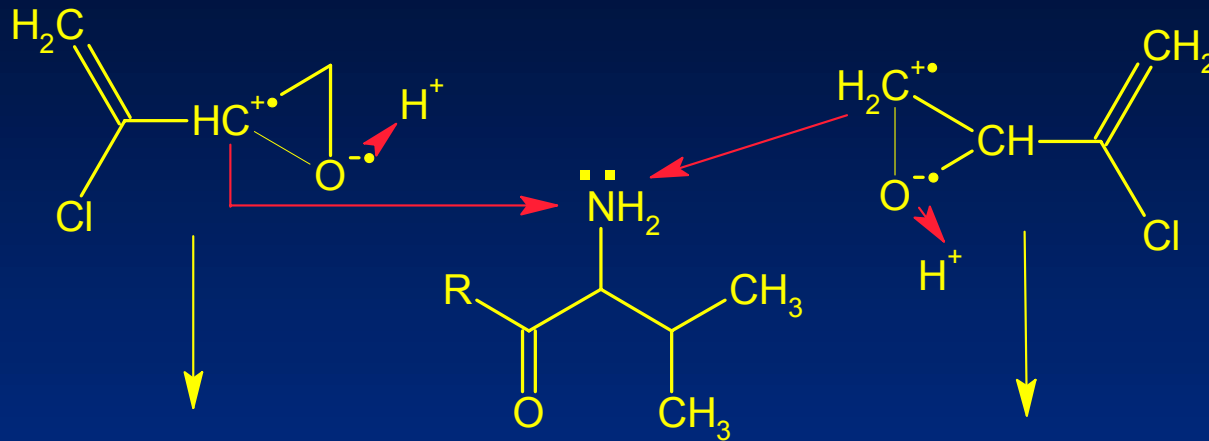
# Chloroprene Biomarker Strategies

- Use Hb N-terminal valine adduct as:
  - Biomarker of chloroprene exposure with activation to epoxide (CVO)
- Biomarker analytical approach
  - Isolate globin from blood exposed to CVO
  - Use Edman reaction for Hb N-terminal valine cleavage and derivatization for gas chromatographic separation
  - Use mass spectrometry for detection
  - Synthesize standards from valine peptides

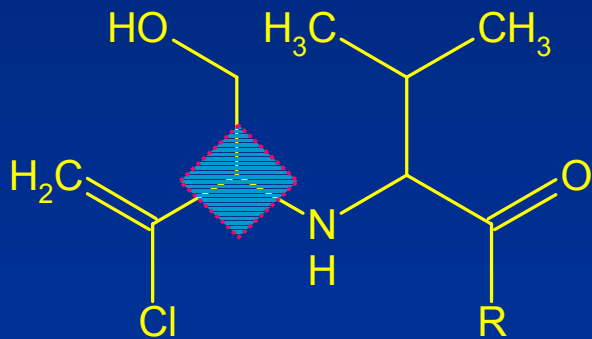
# Biomarker Basis: Epoxide Reactions

## Adducts Depend on Epoxide Ring Opening

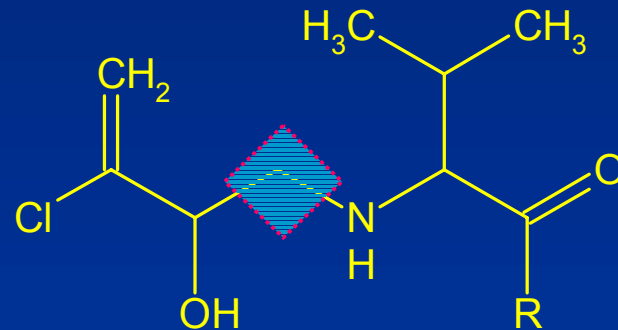
2-(1-chlorovinyl)oxirane



N-terminal Hb valine



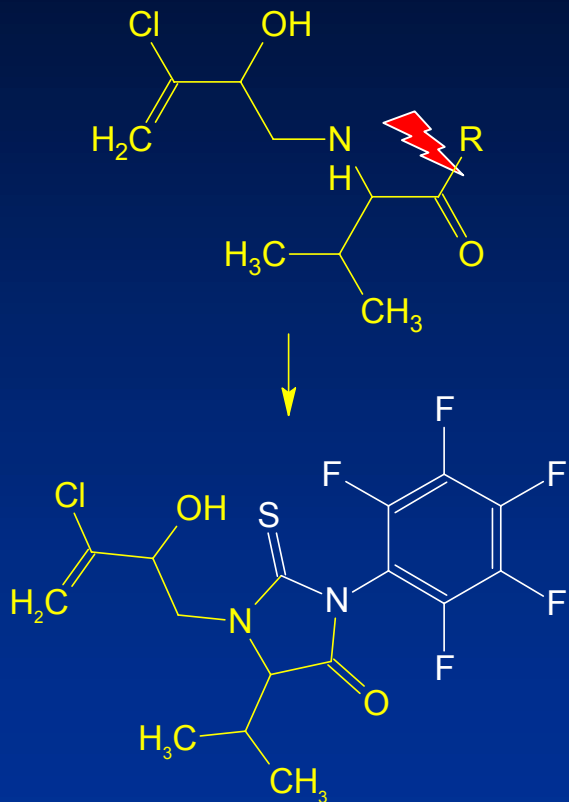
1-hydroxychlorobutene adduct



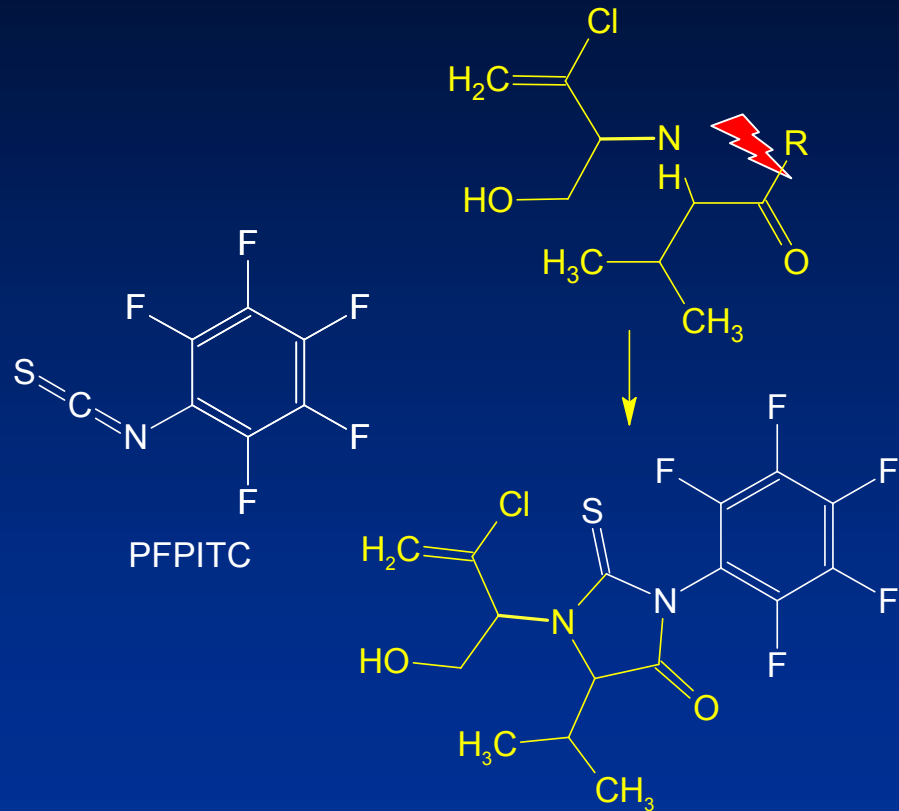
2-hydroxychlorobutene adduct

# Edman Reaction:

1. Cleave N-Terminal Amino Acid
2. Form Stable Cyclic Derivative

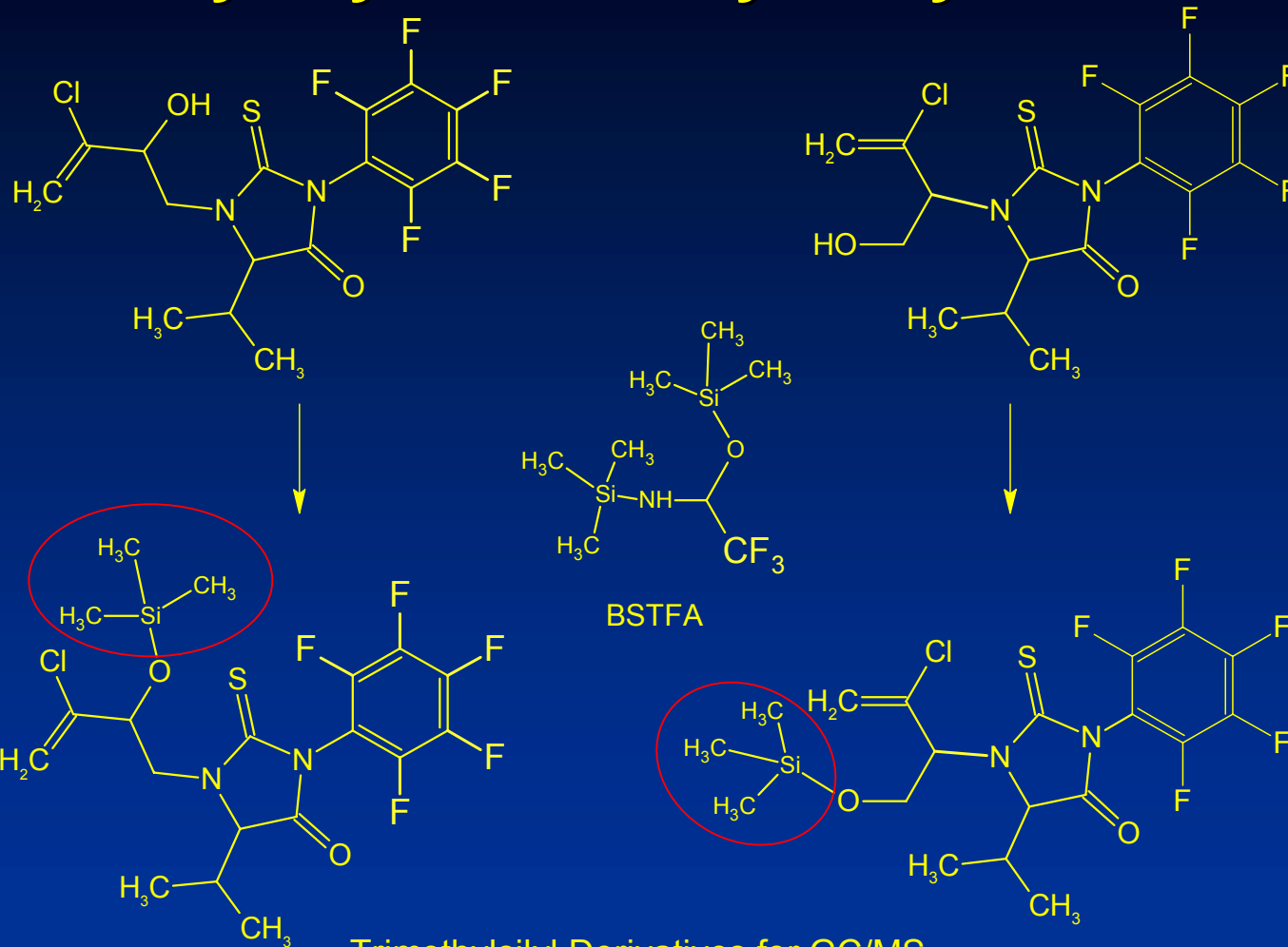


1-(3-chloro-2-hydroxybut-3-en-1-yl)-5-isopropyl-3-(pentafluorophenyl)-2-thioxoimidazolidin-4-one



1-[2-chloro-1-(hydroxymethyl)prop-2-en-1-yl]-5-isopropyl-3-(pentafluorophenyl)-2-thioxoimidazolidin-4-one

# Additional Reaction Required for GC/MS: Trimethylsilylation of Hydroxyl for Volatility



Trimethylsilyl Derivatives for GC/MS  
Nominal Mass = 500 Da

# Biomarker Reaction Review

## 1. Edman degradation

- Cleaves N-terminal adduct-valine
- Produces stable cyclic derivative

## 2. Hydroxyl group derivatization

- Converts  $-OH$  to  $-OTMS$  for volatility

## 3. Detect and measure by GC/MS

(gas chromatography / mass spectrometry)  
using selected ion monitoring (SIM)

# Analytical Biomarker Standards

- Reference standard material
  - Tri-peptide – Valine-Tyrosine-Valine (VYV)
  - Reacted with CVO to give VYV-CVO adduct standard
  - Purified to obtain gravimetric weight
- Internal standard (added to each sample)
  - Valine enriched with deuterium (atomic mass = 2) in place of hydrogen (mass = 1)
  - Used 99% enriched  $d_8$ -valine
  - Reacted with CVO for  $d_8$ -valine-CVO

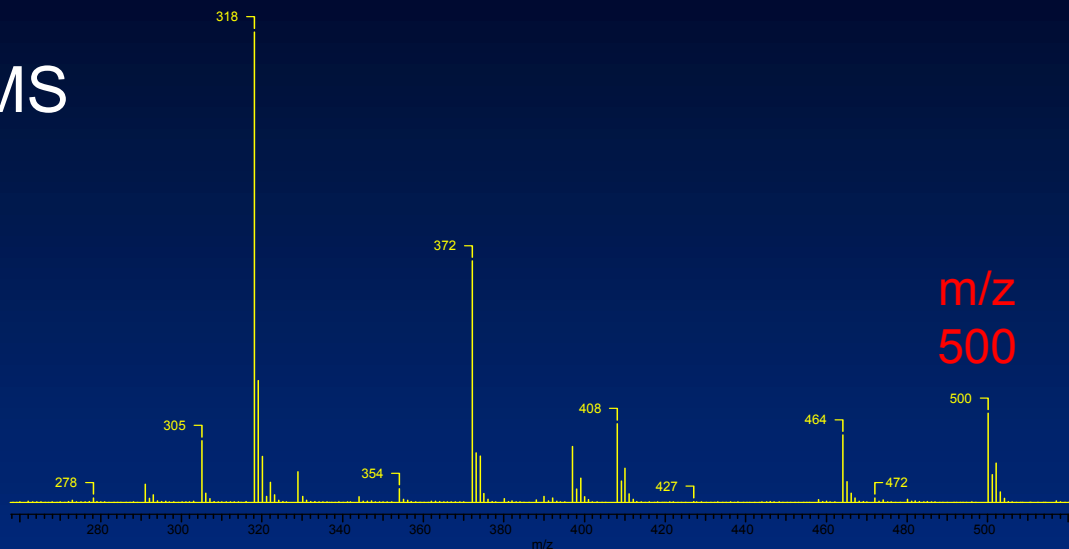
# Mass Spectra of CVO-Valine Derivatives

Negative  $\text{NH}_3$  CI GC/MS

Peak 1

$R_t = 12.82$  min

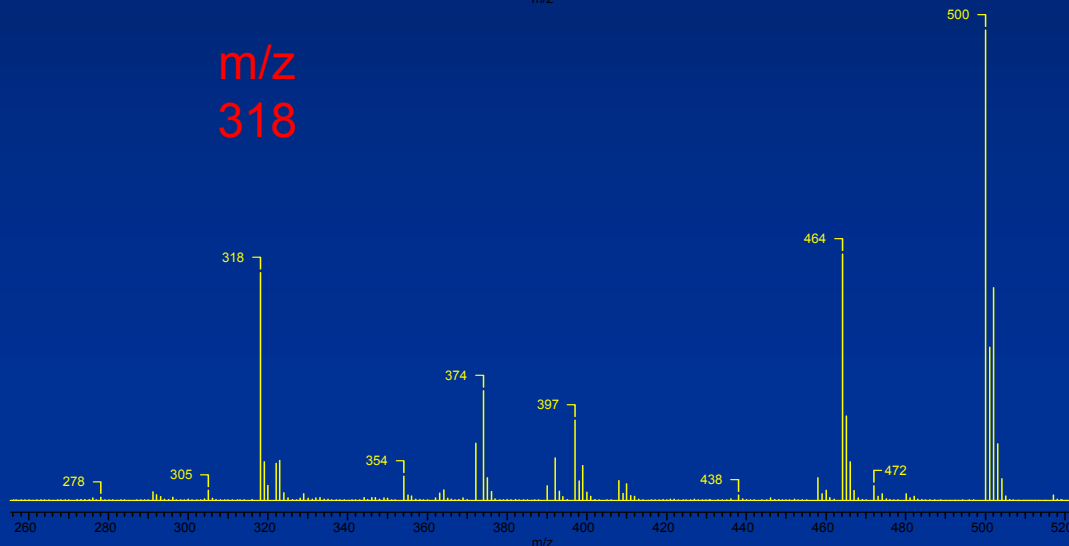
$m/z$  318, 372, 500  
(in order of intensity)



Peak 2

$R_t = 12.96$  min

$m/z$  500, 464, 318  
(in order of intensity)



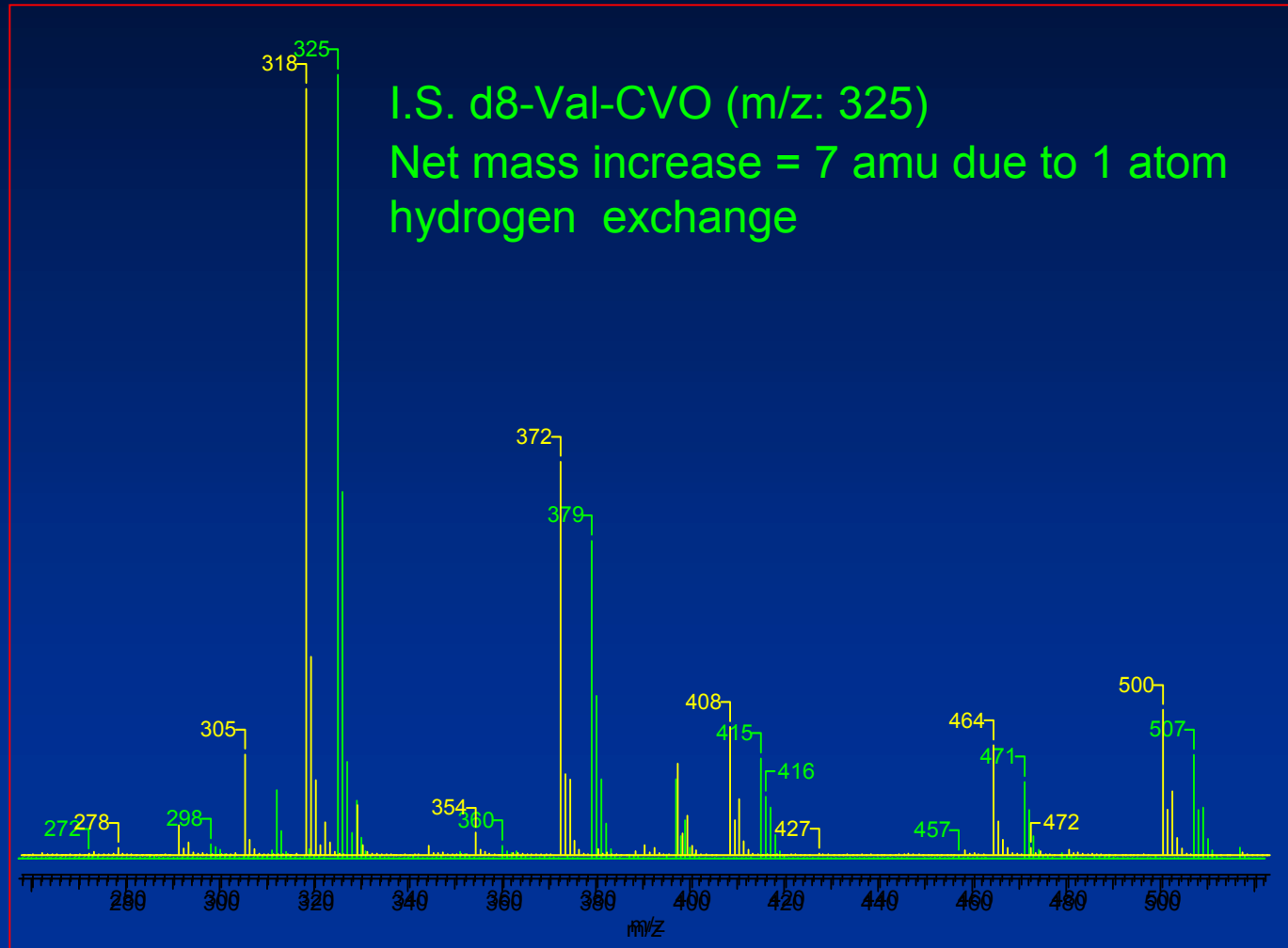


# Stable Isotopic Standardization

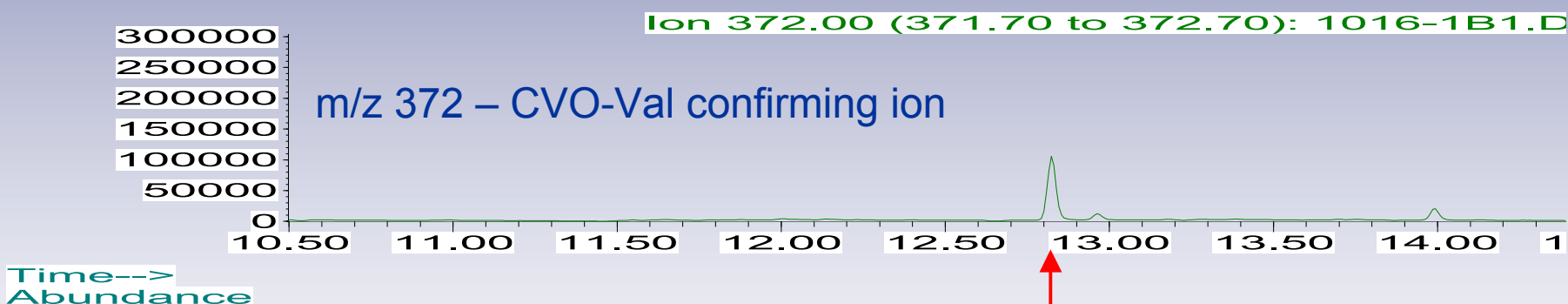
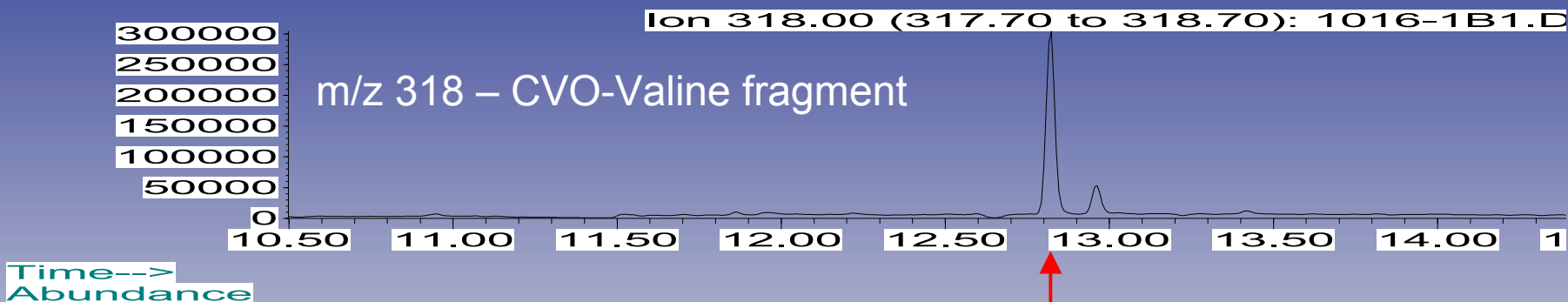
## Possible only with Mass Spectrometry

Peak 1: Val-CVO  
m/z: 318

Use peak area  
ratio (318 / 325)  
for SIM  
quantitation

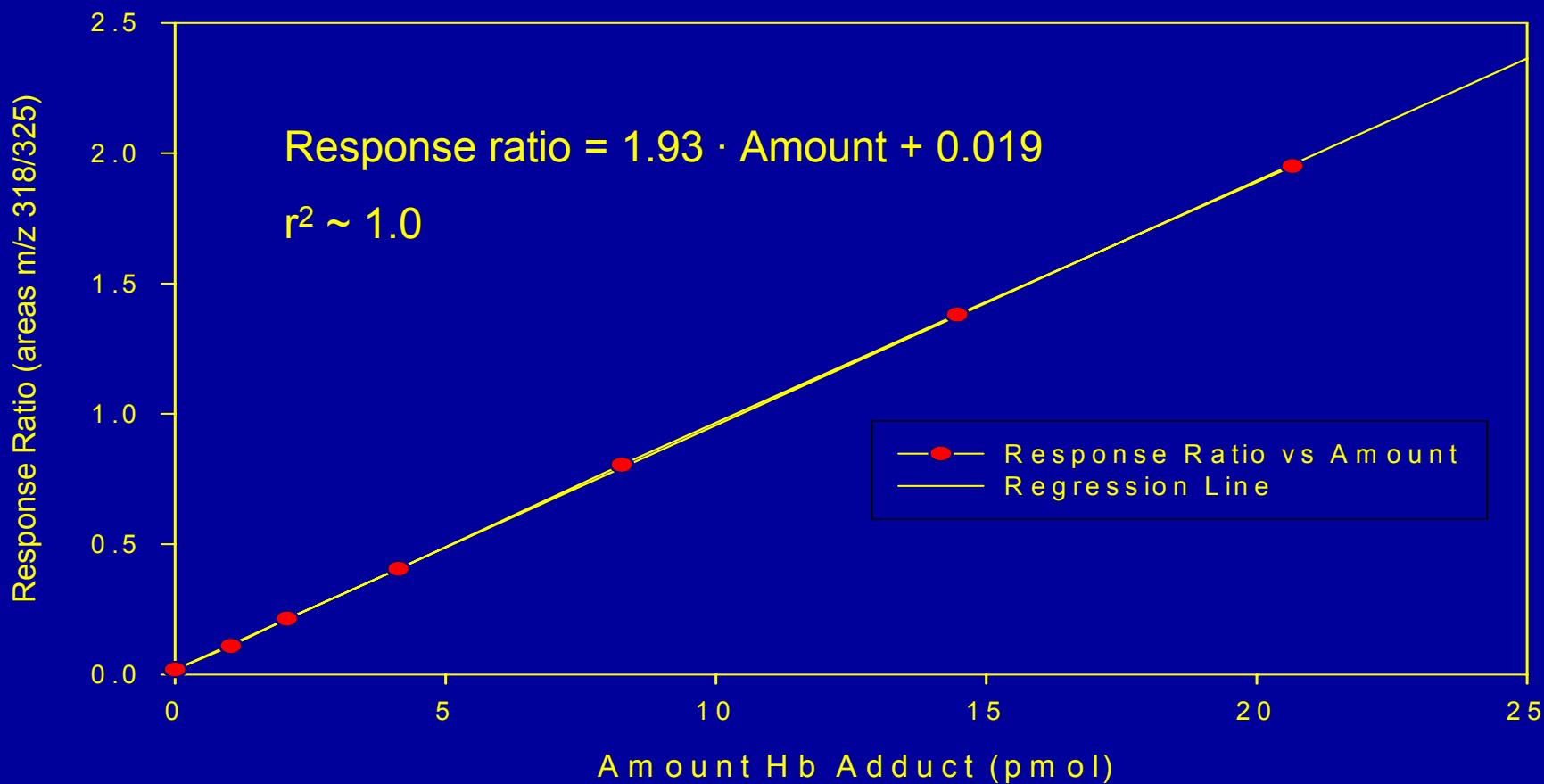


# Selected Ion Monitoring GC/MS



# CVO-Adduct Assay Standard Response

Negative NH<sub>3</sub> Ionization SIM GC/MS  
CVO-Valine Adduct using VYV-CVO as Standard



# Status of Project

- Prototype Val-CVO assay successful
  - Detects 1 pmole of Val-CVO adduct reliably
  - Should enable detection from ~ 5 mg Hb, depending on level of Hb-adduct formed
- Refinements are necessary for practical sample processing
- Utility still to be demonstrated
  - Have measured 25 pmoles Val-CVO adduct from in vitro treatment of mouse hemoglobin

# Acknowledgements

- Jian Cai, Ph.D.
- Matthew Himmelstein, Ph.D.
- KY EPA EPSCoR Program
- EPA EPSCoR Grant Number R-82941091-0
  - Although the research described in this article has been funded wholly or in part by the United States Environmental Protection Agency through grant/cooperative agreement R-82941091-0, it has not been subjected to the Agency's required peer and policy review and therefore does not necessarily reflect the views of the Agency and no official endorsement should be inferred.
- Synthetic Rubber Producers
  - The (1-chloroethenyl)oxirane was provided by the International Institute of Synthetic Rubber Producers' Chloroprene Scientific Oversight Committee through Matthew Himmelstein of DuPont Haskell Laboratory.
- UofL Office of the Vice President for Research

