MINOR HEMOGLOBIN ADDUCTS FOR ENVIRONMENTAL MONITORING

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National Center for Environmental Health
Division of Laboratory Sciences

Outline

- Background on protein adducts
- How to measure them
- Applications to environmental monitoring

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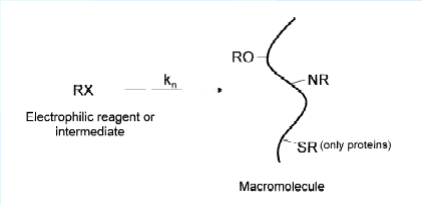
Protein Adducts

- A Protein adduct is the compound formed when a chemical binds irreversibly to a protein



Tornqvist, M. et al J Chromatography B, 778 (2002) 279-308

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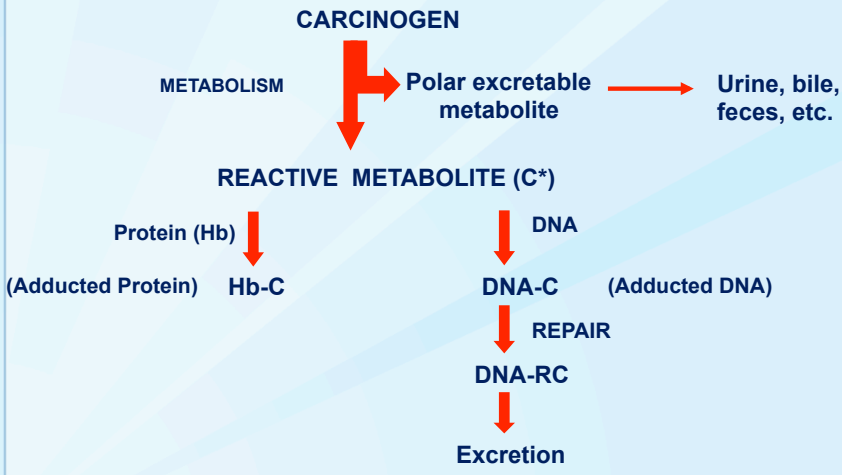
Metabolism of Genotoxic Carcinogens Leading to Protein and DNA Adducts



Adapted from Skipper and Tannenbaum; Carcinogenesis, 1990, 11, 507-518

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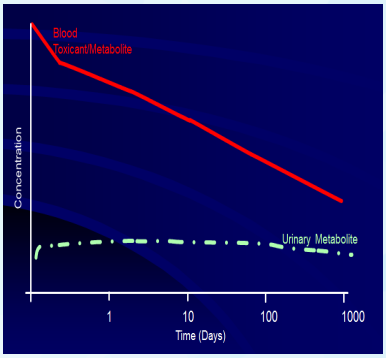
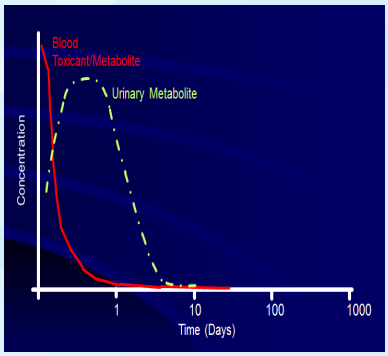
Post-exposure Fate of a Toxicant in Blood and Urine

Persistent



Non-Persistent



Needham and Sexton, JEAEE 10:611-629 (2000)

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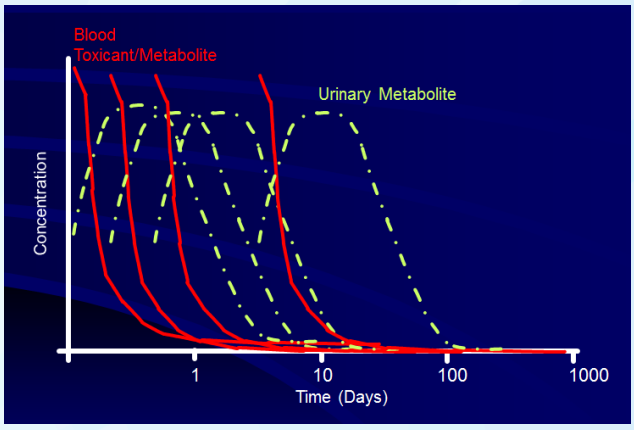
Post-exposure Fate of a Non-persistent Toxicant in Blood and Urine – Chronic Exposure



Needham and Sexton, JEAEE 10:611-629 (2000)

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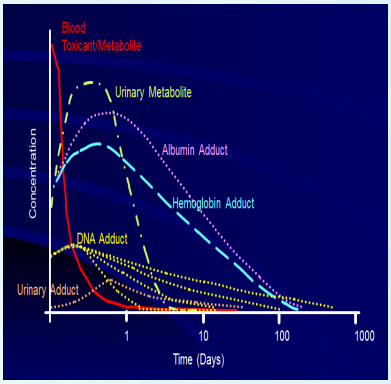
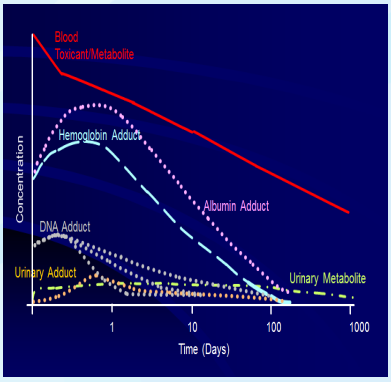
Post-exposure Fate of a Toxicant in Blood and Urine

Non-Persistent



Persistent



Needham and Sexton, JEAEE 10:611-629 (2000)

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Uses of Protein Adducts

- **Markers of exposure**
  - assess and estimate dose associated with exposure
- **Surrogate markers for DNA adduct formation**
  - Compounds that bind covalently to DNA most likely will form protein adducts in target tissues
- **Indicators of potential disease risk and diagnostic tools**
  - HBA1c
  - Carbamylated Hb-isocyanic acid adduct derived from dissociation of urea
- **Information about metabolism**
  - Determine inter-individual differences in metabolism
  - presence of adduct may suggest the formation of a reactive intermediate

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Advantages of Blood Proteins

- **Accessibility of material for chemical, biological or epidemiological studies**
- **Chemical stability under biological conditions**
  - Not subjected to repair mechanisms as DNA
- **Abundance of material - greater sensitivity**
  - In 10 ml of blood: g amounts of Hemoglobin and Albumin vs.

  1 mg of leukocyte DNA
- **Known kinetics and turnover rates:**
  - Age distribution of Hb is uniform
  - lifespan of erythrocyte equivalent to lifespan if Hb (~120 days)-Hb is used as dose monitor

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Procedures for Measuring Protein Adducts

- Isolation of the protein
- Detachment of the adduct, the adduct-amino acid or the adduct-peptide complex
- Isolation of the detached adduct
- Derivatization
- Analysis

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Detachment of the adduct from the protein

- **Mild Hydrolysis (0.1M HCl or NaOH)**
  - Esters to carboxyl groups (PAH, BaP, NNK)
  - Adducts from organic anhydrides
  - Sulfinamides to cysteines (nitroso arenes formed from N-hydroxylated aromatic amine and isocyanates)

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Detachment of the modified aminoacid adducts by cleavage of peptide bonds

- **Total acid hydrolysis of proteins (6M HCl, 110˚C, overnight)**
  - Stable adducts
    - His, Cys: ethylene oxide-hydroxyethylated his
    - Adducts of a heterocyclic amine (pmol/g)

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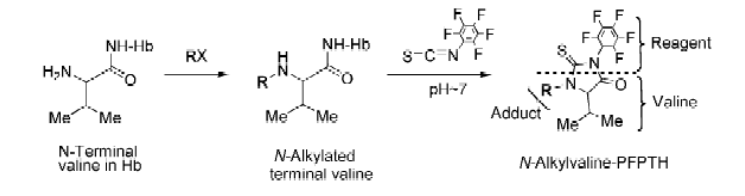
Detachment of the modified aminoacid adducts by cleavage of peptide bonds

- **N-alkyl Edman method for detachment of modified N-terminal valine residues of hemoglobin**



Epoxides, acrylonitrile, acrylamide

Tornqvist, M. et al J Chromatography B, 778 (2002) 279-308

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Detachment of the modified aminoacid adducts by cleavage of peptide bonds

- **Enzymatic hydrolysis of proteins into peptides**
  - Identification of adducts binding sites:
    - diepoxybutane
    - methyl bromide
    - epichlorohydrin
    - styrene oxide
  - Human exposure analysis of BaP

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What is Biomonitoring?

Assessment of internal dose by measuring a toxicant (or its metabolite or reaction product) in human blood, urine, saliva, adipose, or other tissue.

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Exposure and Health Effects Assessment

Proximity to source of toxicant

External Dose: levels in air, water, food, soil

Inhalation
Ingestion
skin absorption

Internal Dose: levels in blood, serum, urine, tissue

Biologically Effective Dose: levels in affected tissues

Health effect

Exposure Assessment

Health Effects Assessment

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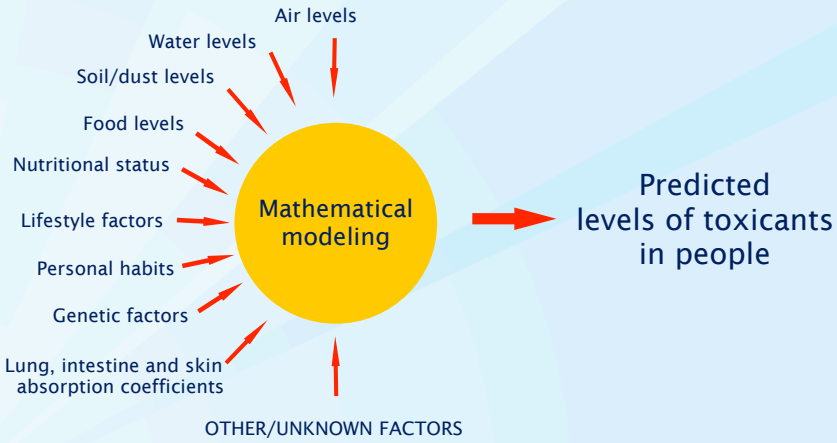
Predicting Levels of Toxicants



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Goals of Biomonitoring

- Rather than trying to predict the levels of toxicants, biomonitoring allows us to actually measure human exposure to toxicants.
- To allow health research to determine what toxicants and what internal dose levels cause disease or death
- Identify long-term trends in the population
- Identify geographic locations, age brackets, personal habits and other factors where people have much different exposures than the general population

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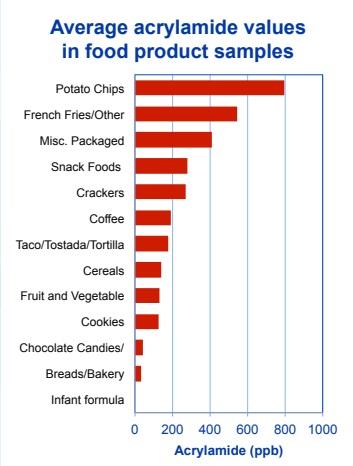
Acrylamide-Biomonitoring

- Acrylamide is a suspected carcinogen
- The general population is exposed to acrylamide
- Main exposure sources are food and tobacco smoke
- Acrylamide arises in food when asparagine, an amino acid, is heated with sugars such as glucose
- Extend of exposure in the population is unknown



Adapted from: FDA-Survey Data on Acrylamide in Food –data through Oct 2004

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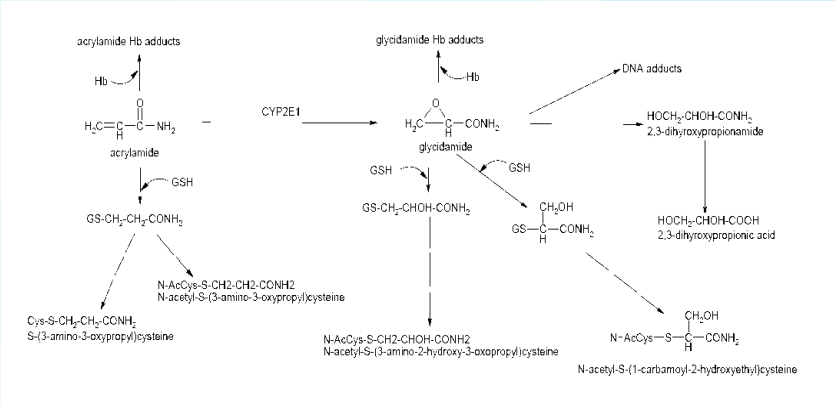
Acrylamide Metabolism



Adopted from EPA/IRIS 2007, Fennell 2005, Calleman1992

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Acrylamide-Laboratory Activities

- **Determine Human Exposure**
  - in the U.S. population (NHANES) collaboration with NCHS
  - in the European population (EPIC) collaboration with WHO/IARC
- **Investigate the Impact of Different Exposure Sources**
  - Smoking: Collaboration with DLS/ERAT
  - Food: DLS "Potato Chips" Study
  - Occupation: Collaboration with NIOSH
- **Identify Parameters Affecting Acrylamide Metabolism** (and Toxicity)
  - Lifestyle (smoking/alcohol): Collaboration with WHO/IARC, NCHS
  - Genetics: Collaboration with NIOSH
- **Assess the Potential Cancer Risk of Acrylamide Exposure**
  - Collaboration with Harvard School of Public Health (Nurses Health Study II)
  - Collaboration with WHO/IARC (EPIC)
  - Uema University, Sweden & Danish Cancer Society

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IARC/EPIC Study - Introduction

Aim

- determine the acrylamide exposure in individuals from different European countries
- obtain information on factors affecting acrylamide exposure and metabolism

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Study Population

- 510 individuals from EPIC study population from 9 countries (Sweden, Denmark, United Kingdom, The Netherlands, France, Germany, Italy, Greece, Spain)
- 60 individuals per country:
  - 30 men (15 smokers, 15 non-smokers),
  - 30 women (15 smokers, 15 non-smokers),
  - France: 30 women (15 smokers, 15 non-smokers).
- The age range was 41–60 years in men and 43-60 years in women

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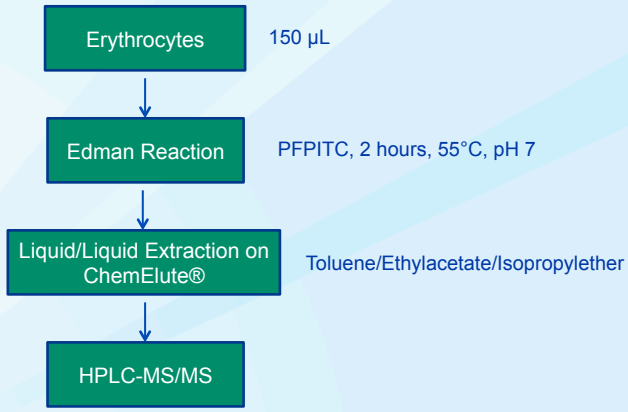
Procedure for analyzing HB adducts of Acrylamide and Glycidamide



Vesper et al, *Rapid Commun. Mass Spectrom* **2006**: 20:959-64
Tornqvist, M., *Methods in Enzymol.* **1994**;231:650

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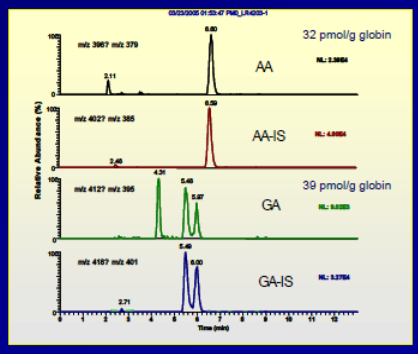
Chromatographic Conditions:

HPLC: Surveyor (ThermoFinnigan)
Column: Luna C18(2) column, (100 x 2 mm, 3 µm, Phenomenex)
Column Temp.: 50°C
Eluent: methanol/water (63/37 v/v)
Flow Rate: 300 µL/min
Inj. Vol.: 50 µL

MS Conditions:

MS: TSQ Quantum Ultra (ThermoFinnigan)
Ionisation: APCI (pos. ion mode)
Discharge Current: 4.5 uA
Vaporizer Temp.: 375°C
Sheath Gas Pressure: 35
Auxiliary Gas Pressure: 5 au
Capillary Temp.: 250°C



Chromatograms Low QC Sample

Vesper et al. RCM 2006;20:959

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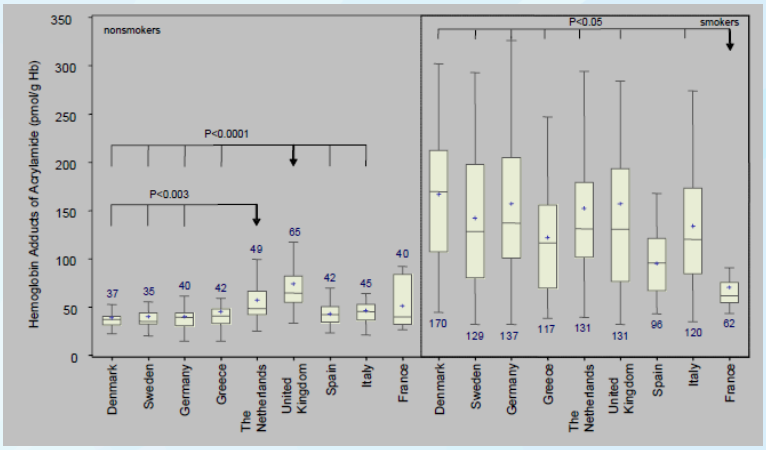
Acrylamide Adducts



Vesper, et al J. Agric. Food Chem. **2008,** *56,* 6046–6053

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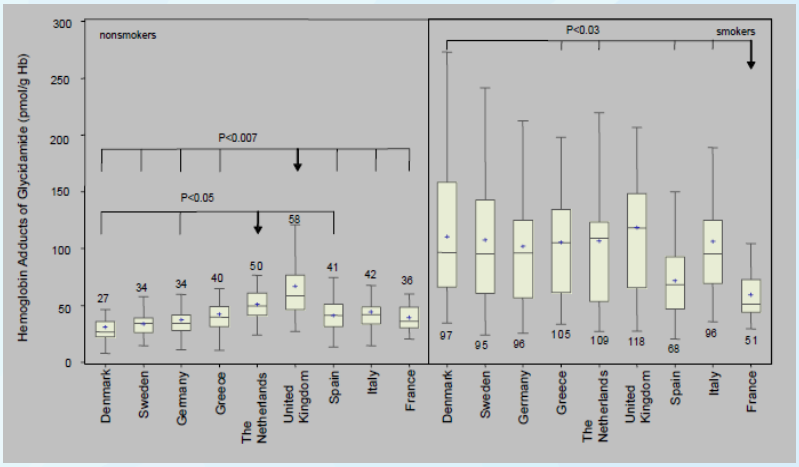
Glycidamide Adducts



Vesper, et al J. Agric. Food Chem. **2008,** *56,* 6046–6053

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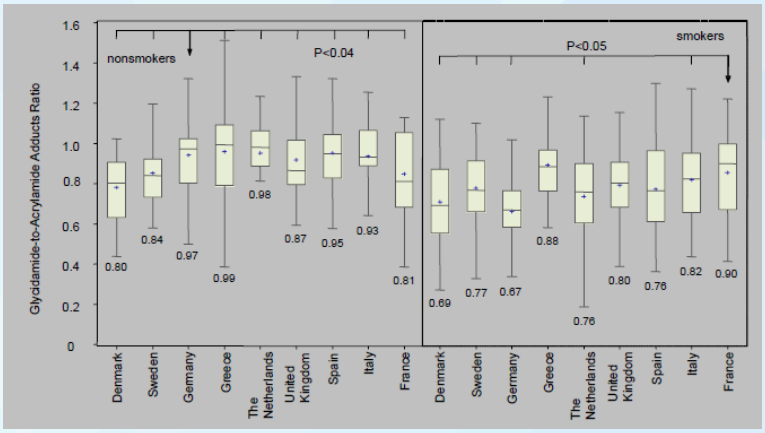
Glycidamide to Acrylamide Adduct Ratio



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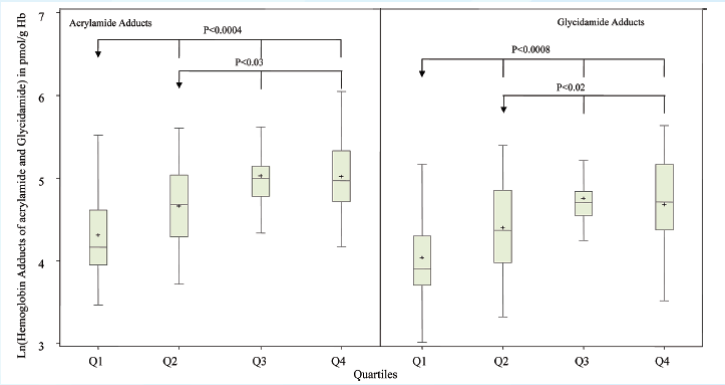
Glycidamide-to-acrylamide adduct ratios in smokers and nonsmokers versus quartiles of cigarette consumption



Q1, <7 cigarettes/day; Q2, 7-15 cigarettes/day; Q3, 16-20 cigarettes/day; Q4, >20 cigarettes/day.

Vesper, et al J. Agric. Food Chem. **2008,** *56,* 6046–6053

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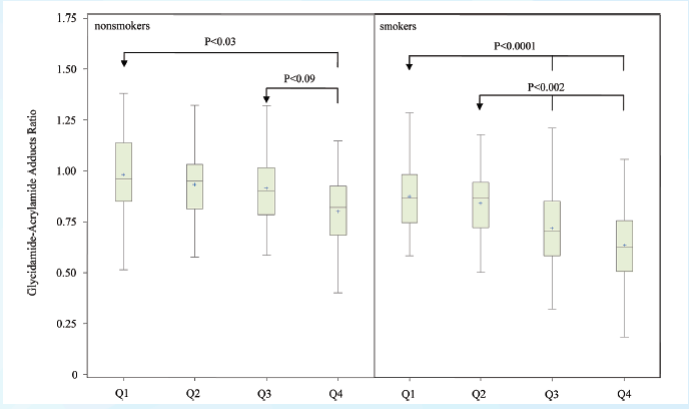
Glycidamide-to-acrylamide adduct ratios in smokers and nonsmokers versus quartiles of alcohol consumption



Nonsmokers: Q1, <1.18 g of ethanol; Q2, 1.19-5.45 g of ethanol; Q3, 4.56-14.43 g of ethanol; Q4, >14.43 g of ethanol.
Smokers: Q1, >1.7 g of ethanol; Q2, 1.8-9.64 g of ethanol; Q3, 9.65-29.96 g of ethanol; Q4, >29.96 g of ethanol)
Vesper, et al J. Agric. Food Chem. **2008,** *56,* 6046–6053

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Conclusions

- Protein adducts can provide comprehensive information about internal dose, compound metabolism and enzymatic pathways
- Long time biomonitoring studies may link biomarkers of exposure to effects
- Knowing whether adduct formation can be moderated by diet changes, lifestyle changes, or even biological interventions would be a major advance in understanding the progression of some diseases and the toxicity of certain compounds

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Acknowledgements

The Acrylamide/Glycidamide Hemoglobin Adducts Group members especially:

- *Hubert W. Vesper*
- *Tunde Meyers*
- *Deanna Scott*
- *Antoinette Smith*
- *Magaly Mendez*
- *Leah Ingham*

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Thanks!

For more information please contact Centers for Disease Control and Prevention
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