FDA U.S. FOOD & DRUG ADMINISTRATION
NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH

Division of Biochemical Toxicology

Frederick A. Beland, Director

DIVISION OF BIOCHEMICAL TOXICOLOGY STAFF

- Government Positions - # full time employees (FTE)
  - Research Scientists, Staff Fellows & Visiting Scientists: 23
  - Support Scientists: 21
  - Administrative: 2
  - FDA Commissioner Fellows: 0
- ORISE Post Docs and Graduate Students: 10
- Total = 56

DIVISION OF BIOCHEMICAL TOXICOLOGY OUTREACH

- **Collaborations with:**
  Divisions of Bioinformatics and Biostatistics, Genetic and Molecular Toxicology, Microbiology, Neurotoxicology, and Systems Biology, and the Office of Scientific Coordination.
  CBER, CDER, CDRH, CFSAN, CTP, and CVM.
  NIEHS/NTP, NCI, EPA, CDC, and various universities.
- **Global leadership/outreach:**
  IARC, WHO, EFSA, OECD

DIVISION OF BIOCHEMICAL TOXICOLOGY MISSION

- **Mission:** To conduct fundamental and applied research designed to define the biological mechanisms of action underlying the toxicity of products regulated by FDA.

- **Goals:** To characterize the toxicities and carcinogenic risks associated with chemicals, specifically those of interest to FDA.

- **Strategies:** Bioassays, mechanistic studies,and computational modeling.

MAJOR ACCOMPLISHMENTS DURING THE LAST 5 YEARS

- Bioassays and mechanistic studies on:
  - Acrylamide/glycidamide (CFSAN)
  - *Aloe vera* (CFSAN)
  - Furan (CFSAN)
  - Bisphenol A (CFSAN and CDRH)
  - Melamine/cyanuric acid (CFSAN and CVM)

REPRESENTATIVE CURRENT PROJECT #1
ARSENIC (As)

- Inorganic As (As^{i} = As^{III} and As^{V}) is a naturally occurring contaminant in the earth's crust.
- EPA and WHO drinking water guidelines: 10 ppb.
- Estimated mean daily exposure to inorganic arsenic in the U.S. 0.08 – 0.20 µg/kg bw/day.
- As^{i} is acutely toxic and chronic exposures are linked to many disease states (cancer, neurotoxicity, cardiovascular, metabolic).

HYPOTHESIS BEING TESTED

- Exposure to carcinogens during perinatal lifestages can confer additional susceptibility, relative to adults, based on either:

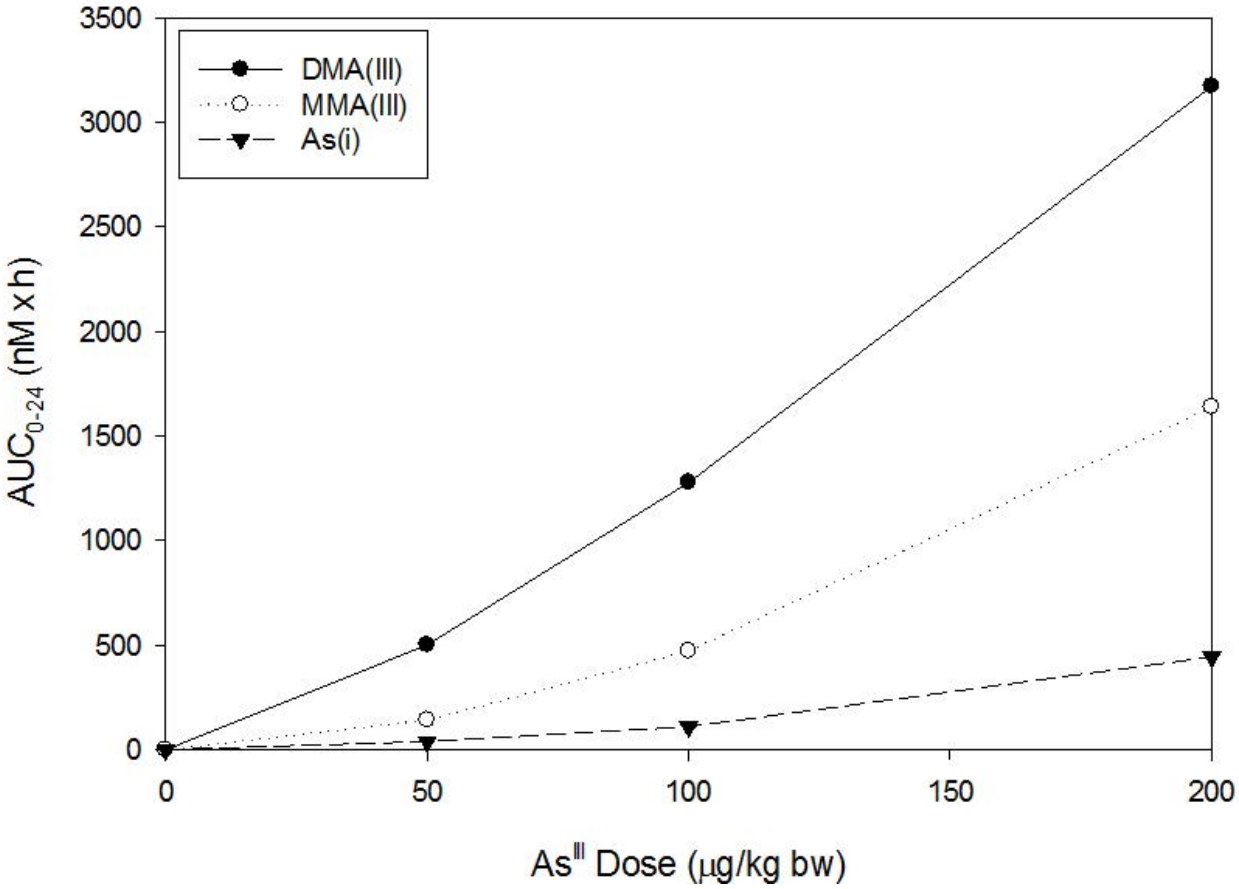
    1) metabolic/physiological immaturity that leads to elevated internal exposure of toxic species.
    2) developmental programming that presents unique molecular targets for toxicant action (e.g., stem cells).

- Such additional susceptibility can be manifested as increased incidences or decreased latencies of cancer later in life.

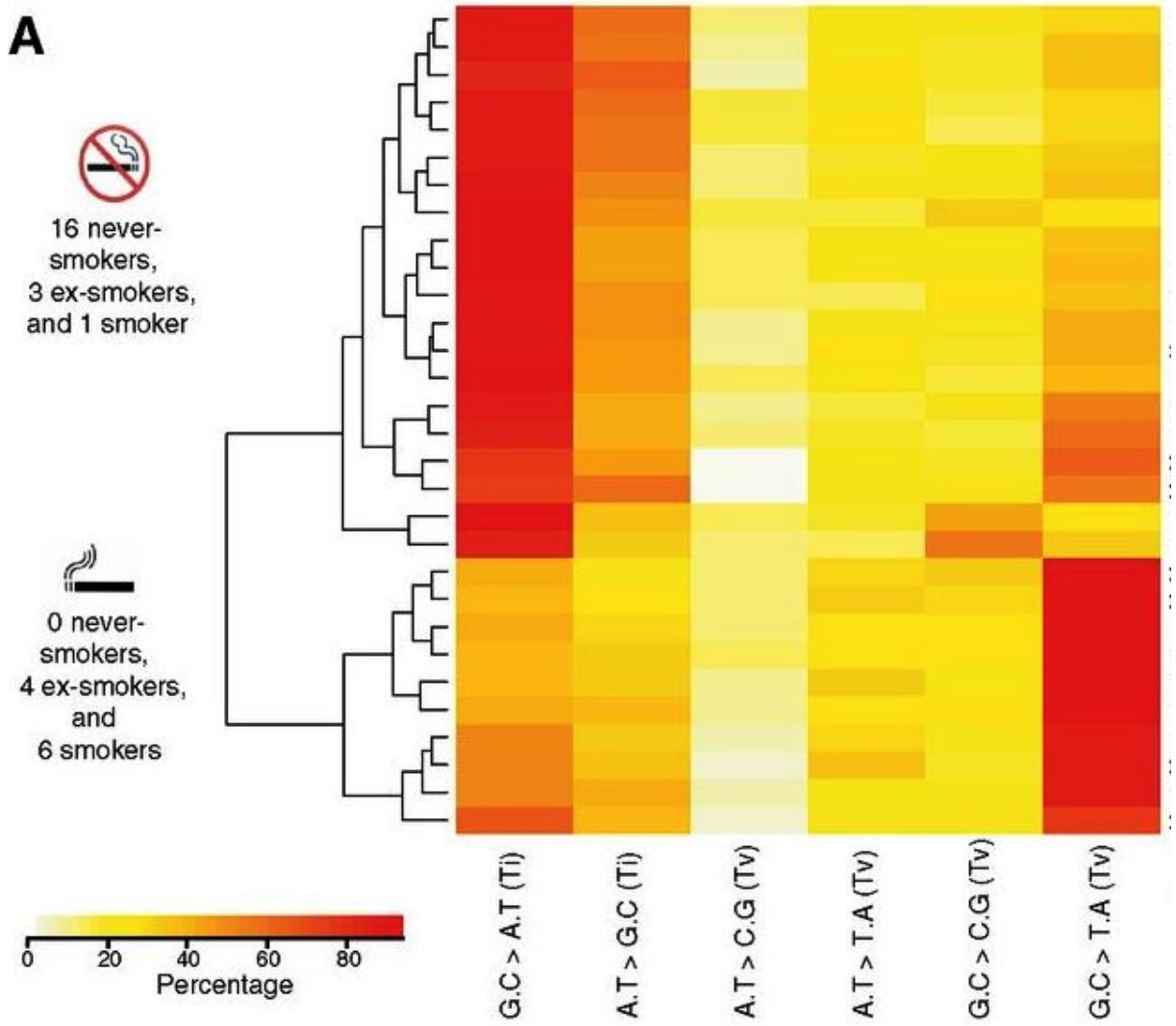
PHARMACOKINETIC STUDY ELEMENTS

- Adult CD-1 mice
- Single gavage dosing
- 50, 100, and 200 µg As^{III}/kg bw.
- Collect plasma, RBCs, tissues (liver/lung), urine, and feces; 15 min - 48 hr after dosing
- LC-ICP/MS measurement of speciated arsenic

AUC OF BOUND ARSENIC SPECIES IN ERYTHROCYTES

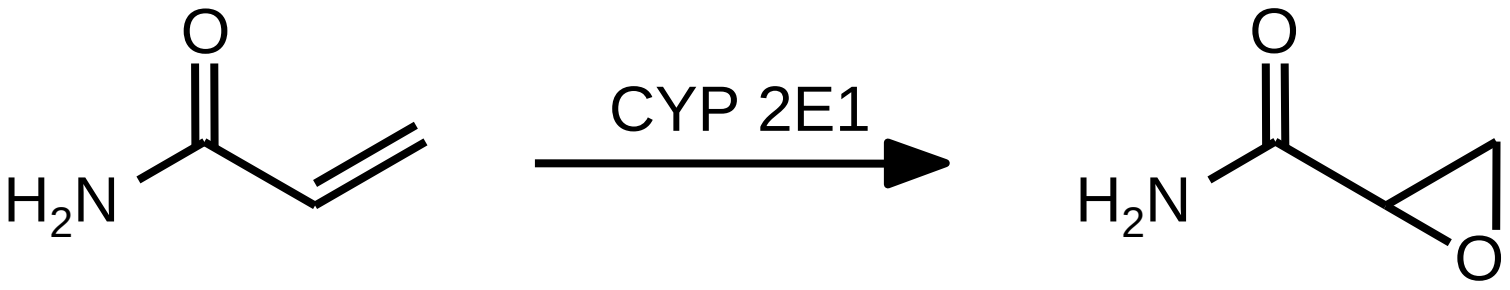


REPRESENTATIVE CURRENT PROJECT #2
MUTATIONAL SIGNATURES



Krishnan *et al.*, *Cancer Res*. 74, 6071, 2014

METABOLISM OF ACRYLAMIDE



Acrylamide

Glycidamide

DNA

DNA adducts

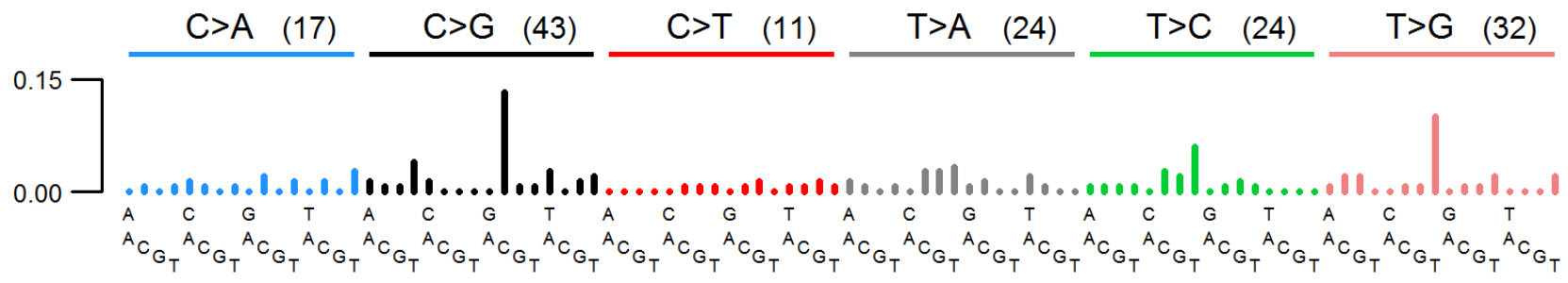
MUTATIONAL SIGNATURES PROTOCOL

Specific Aim 1: To determine the mutational signatures of tumors induced in experimental animals by acrylamide and glycidamide.

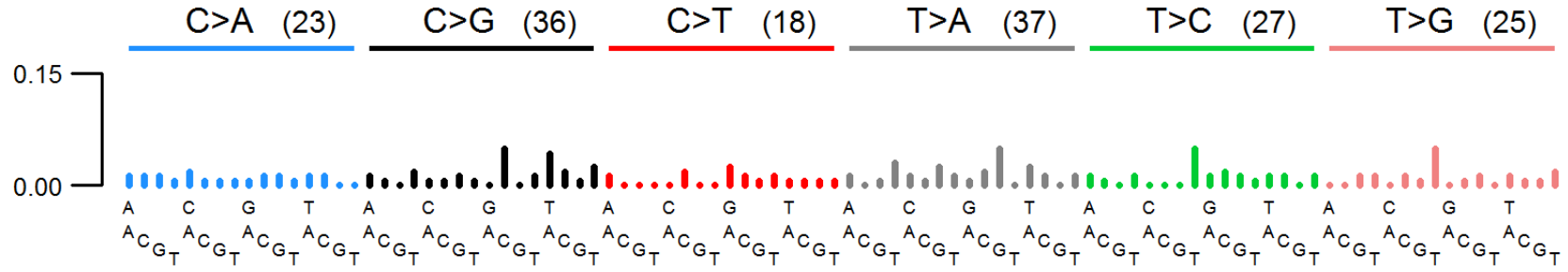
- Male and female $B6C3F_1$ mice
- Male and female F344/N rats
- Tumor DNA will be assessed by whole exome next-generation sequencing.

Specific Aim 2: To compare the mutational signatures obtained from acrylamide and glycidamide in experimental animals with mutational signatures of human tumors in published databases.

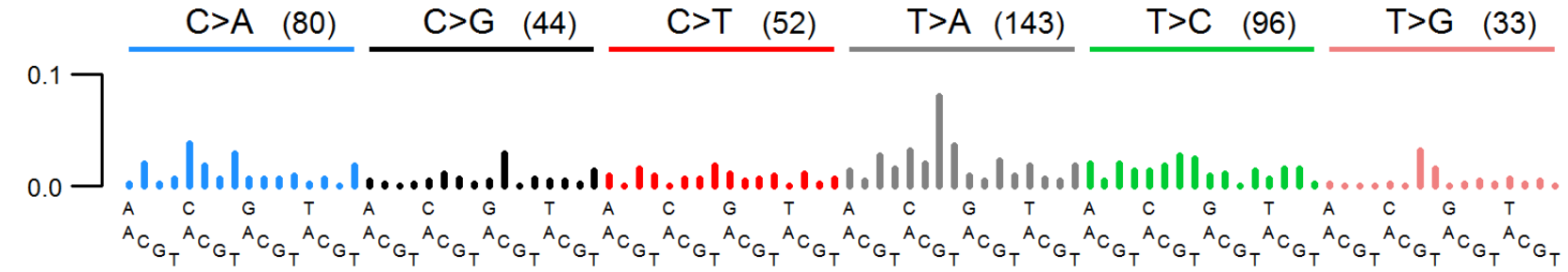
MUTATIONS IN MOUSE FIBROBLASTS



Spontaneous



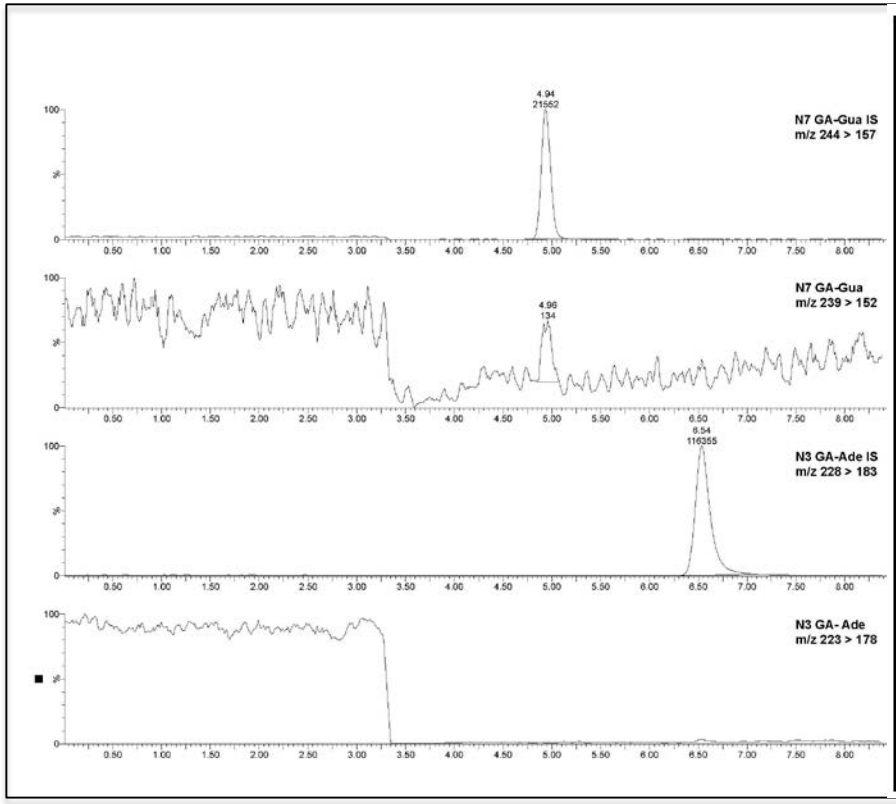
Acrylamide-treated



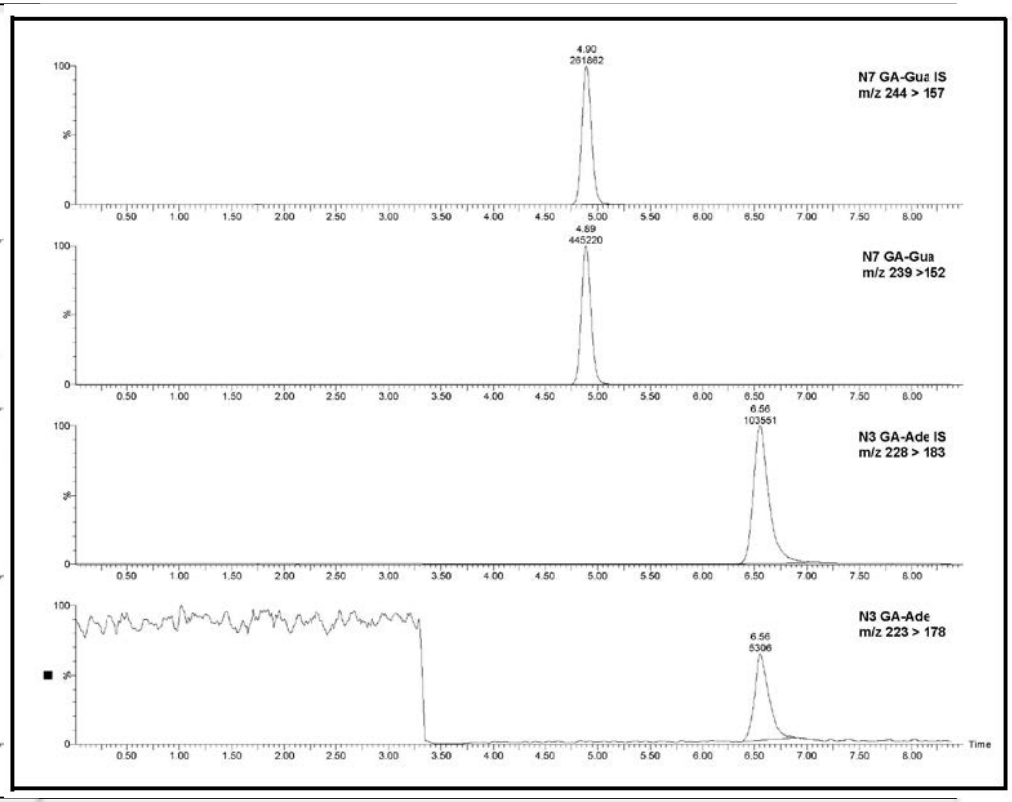
Glycidamide-treated

DNA ADDUCTS IN MOUSE FIBROBLASTS

Acrylamide-treated



Glycidamide-treated

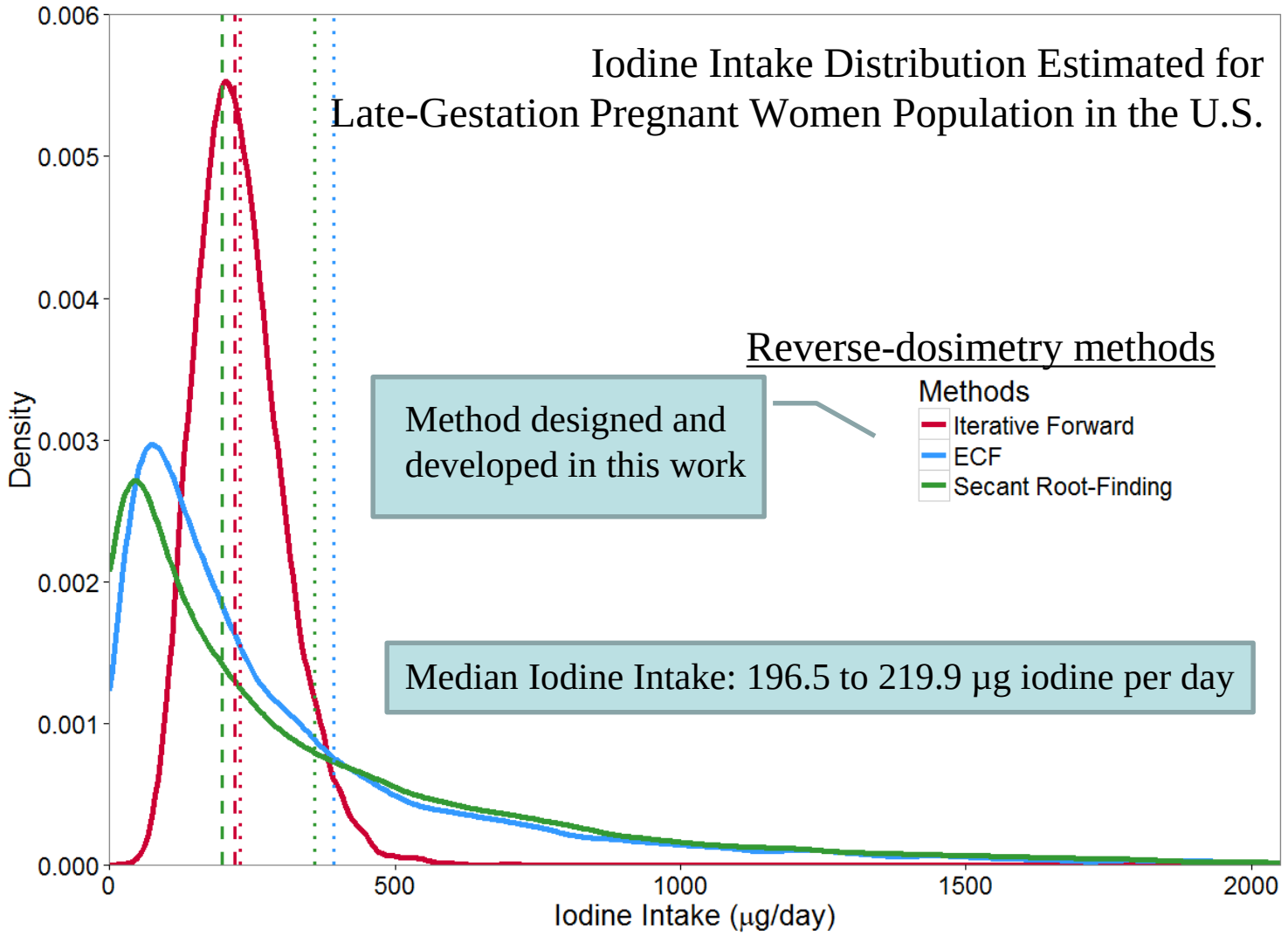


REPRESENTATIVE CURRENT PROJECT #3 POPULATION-BASED PBPK AND DOSE-RESPONSE MODELING

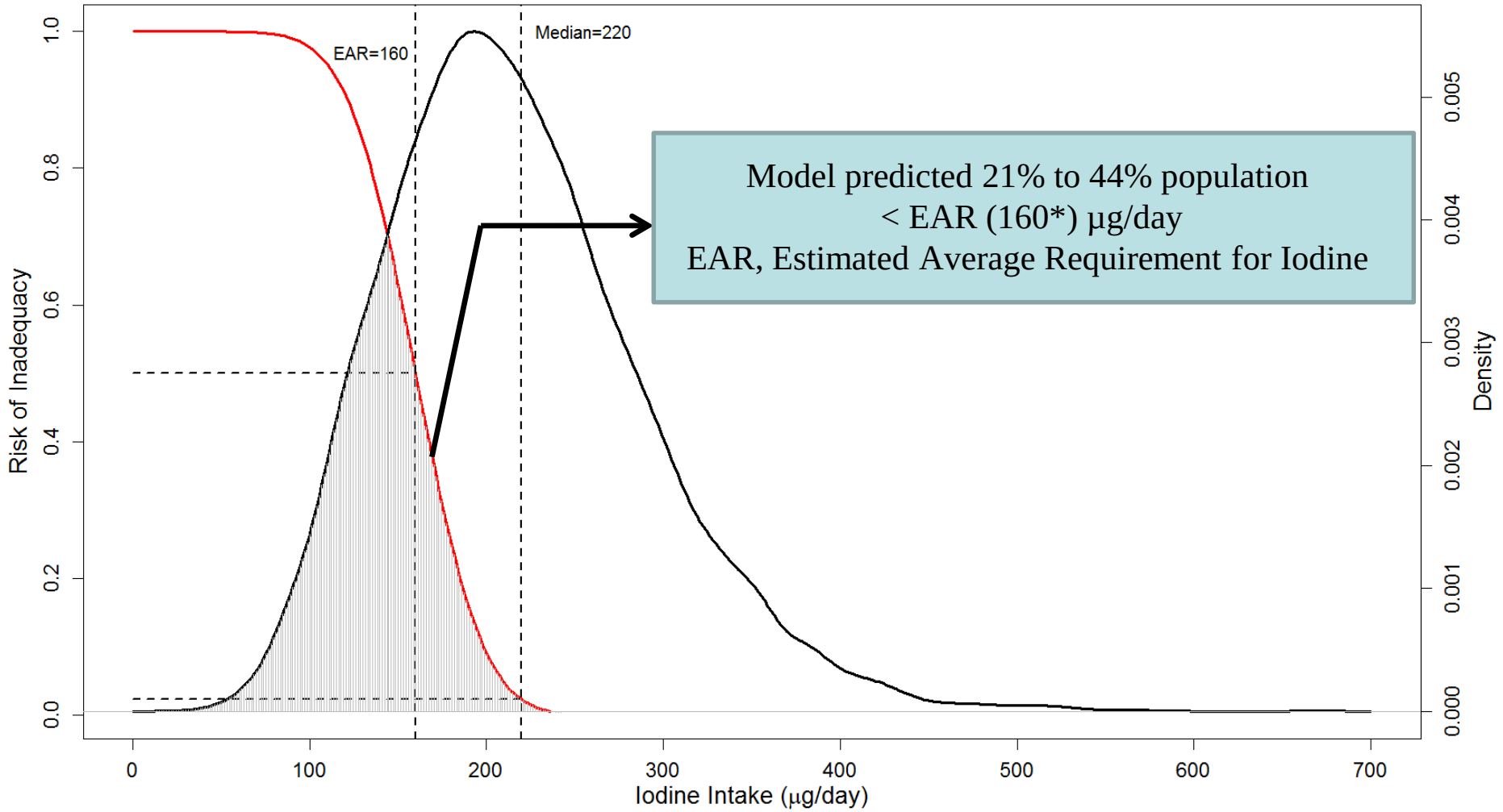
Development of a computational framework for assessing quantitatively the risk of exposure to thyroid active-chemicals during pregnancy to protect women's health.

- At an average individual level: deterministic modeling.
- At a population level: probabilistic modeling.

POPULATION-BASED PBPK AND DOSE-RESPONSE MODELING



POPULATION-BASED PBPK AND DOSE-RESPONSE MODELING



NEW INITIATIVE #1 PEGYLATED BIOPHARMACEUTICALS (in collaboration with CDER/CBER)

PEGylation is the process of both covalently and non-covalently binding a PEG polymer to another molecule, normally a drug or therapeutic protein/peptide.

- ✓ Improved drug solubility.
- ✓ Reduced dosage frequency, without diminished efficacy and with potentially reduced toxicity.
- ✓ Extended circulating half-life.
- ✓ Increased drug stability.
- ✓ Enhanced protection from proteolytic degradation.

PEGYLATED BIOPHARMACEUTICALS CONCERNS AND DATA NEEDED

- Several PEGylated biopharmaceuticals have caused PEG accumulation and cellular vacuolization in various tissues, including the choroid plexus, in pre-clinical studies.
- There is concern that PEG accumulation and the formation of these vacuoles may lead to adverse outcomes for PEGylated biopharmaceuticals used chronically and/or in pediatric populations.
  - ✓ The tissue levels of PEG over time.
  - ✓ Long-term effects of PEG on some tissues, especially the choroid plexus.

PEGYLATED BIOPHARMACEUTICALS EXPERIMENTAL DESIGN

- Evaluate the toxicokinetic profile of high-molecular-weight polyethylene glycols (20, 40, and 60 kDa) in Sprague-Dawley rats given a single dose of the test articles via subcutaneous injection.
- Evaluate the bioaccumulation of high-molecular-weight polyethylene glycols in organs/tissues of Sprague-Dawley rats upon repeat subcutaneous injection of the test articles for 24 weeks (~ 6 months).
- Assess the toxicities resulting from the bioaccumulation of the test articles.

NEW INITIATIVE #2 NATTOKINASE/LUMBROKINASE (in collaboration with CFSAN)

Nattokinase

- Serine protease
- Produced by *Bacillus subtilis;* "natto" (fermented soybeans); first characterized in1987
- Taken as a dietary supplement for its claimed support of cardiovascular and circulation health.

Lumbrokinase

- Group of serine proteases
- Extract of earthworms, mainly *Lumbricus rubellus* and *Eisenia fetida;* first characterized in 1991
- Taken as a dietary supplement for claimed benefits similar to nattokinase.

NATTOKINASE/LUMBROKINASE AIMS AND STUDY DESIGN

- To assess the effects of nattokinase and lumbrokinase on the risk of bleeding.
    - Individually
    - In combination with aspirin

- Sprague-Dawley rats; 28 day exposure by gavage.
    - Nattokinase (1000 mg/kg bw)
    - Lumbrokinase (1000 mg/kg bw)
    - Aspirin (10 and 50 mg/kg bw)

NATTOKINASE/LUMBROKINASE ENDPOINTS TO BE MEASURED

- Bleeding time
- Body weight, food and water consumption
- Blood parameters
  - Clinical chemistry
  - Hematology
  - Platelet aggregation
  - Whole blood hemostasis
  - Coagulation assays
  - Thrombin time
  - Fibrinolysis assays
- Histopathology
- Motor coordination and grip strength

NEW INITIATIVE #3
THA PHOTO-MUTAGENESIS ASSAY
(in collaboration with CFSAN)

- One-year photo-co-carcinogenesis bioassay
  - Designed to evaluate whether or not exposure to ultraviolet radiation (UVR) + test agent alters the risk of skin cancer induced by UVR alone.
  - The bioassay is labor- and time-intensive and needs a relatively large number of animals for statistical power.

- THA photo-mutagenesis assay
  - Developed a SKH-1 (hr-/hr-) gpt delta transgenic hairless albino (THA) mouse model.
  - Advantages: fewer animals and resources and decreased time.

THA PHOTOMUTAGENESIS ASSAY STUDY OBJECTIVES

- To establish the correlation between UVR dose and duration with the mutation frequency and mutation spectral patterns.
  - Mutation frequency should increase with increasing dose and duration of UVR, and the spectral patterns of the mutations should reflect signature mutations of UVR-induced DNA damage.
- To evaluate whether or not differences in mutation frequencies and mutation spectra can be detected between mice exposed to UVR and those exposed to UVR in the presence of a photo-co-carcinogen (retinyl palmitate).

THA PHOTO-MUTAGENESIS ASSAY PARAMETERS TO BE MEASURED

- Mutation frequencies and spectral patterns
  - Mutation frequencies will be measured at 1, 2, and 4 weeks to determine the effect of exposure duration on mutation response.
  - Mutation frequencies will be measured upon exposure to 0.000, 0.685, and 1.370 standard erythema dose units per day to determine whether or not the responses correlate to a dose-response for light exposure.
  - DNA from mutant colonies will be evaluated for mutations synonymous to solar-UV-induced signature mutations (C→T at Py-mCpG sites), UV signature (C →T, CC→TT).

QUESTIONS, COMMENTS, AND/OR SUGGESTIONS