Division of Biochemical Toxicology

Presented by:

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DIVISION OF BIOCHEMICAL TOXICOLOGY STAFF

- **Government Positions - # full time employees**
  - ✓ Research Scientists, Staff Fellows, & Visiting Scientists: 31
  - ✓ Support Scientists: 9
  - ✓ Administrative: 2
- **ORISE Post Docs and Graduate Students: 6**
- **Total = 48 staff members**

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, and FSCJ

DIVISION OF BIOCHEMICAL TOXICOLOGY MISSION

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

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

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

TOP THREE ACCOMPLISHMENTS IN THE DIVISION OF BIOCHEMICAL TOXICOLOGY DURING 2019

- **BPA bioassay:**

  Camacho et al., Food Chem. Toxicol., 132, in press (2019).

- **Arsenic pharmacokinetics:**

  Twaddle et al., Food Chem. Toxicol., 123, 28-41 (2019).

  Twaddle et al., Food Chem. Toxicol., 130, 22-31 (2019).

  Twaddle et al., Food Chem. Toxicol., 133, in press (2019).

- **Acrylamide - epigenetic mechanisms:**

  de Conti et al., Chem. Res. Toxicol., 32, 869-877 (2019).

REPRESENTATIVE CURRENT PROJECT #1: TATTOO PIGMENTS

(in collaboration with CFSAN)

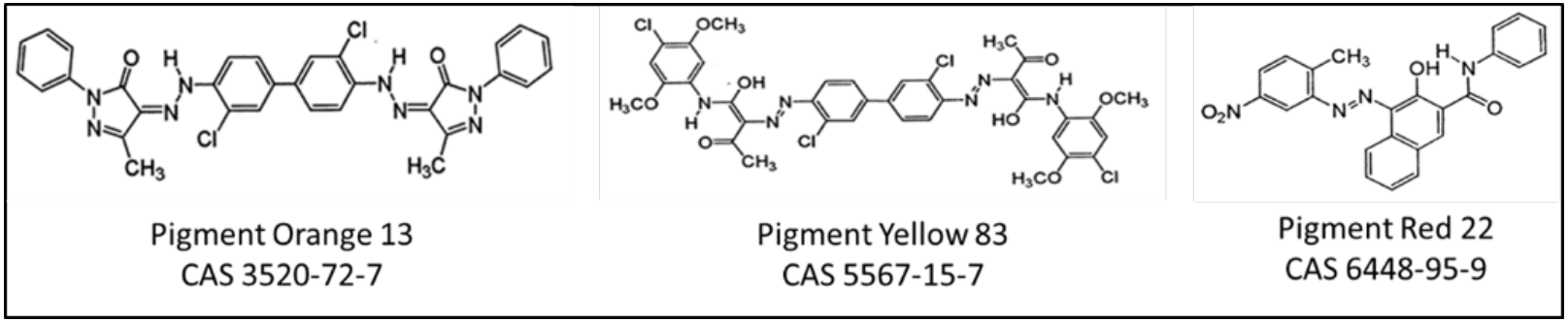
- Adults between the ages of 25 - 39 years have the highest tattoo prevalence rates (42-55%), with women of **child-bearing age** having higher rates than men.
- The **average** tattoo contains **250 mg** of tattoo pigment, with 30% of the U.S. population having ≥ 4 tattoos.
- Decreases of 87-99% of the pigment have been reported in skin after tattooing. **Where does the pigment go?**
- Since the highest rate of tattooing is found in women of child-bearing age, **exposure of the unborn fetus** to tattoo pigments may occur via placental transfer.

TATTOO PIGMENTS STUDY HYPOTHESIS

- The intradermal injection of tattoo pigments into the dorsal skin of pregnant mice will bio-distribute to organs of the dam and to the developing fetuses via placental transfer.

TATTOO PIGMENTS STUDY OBJECTIVES

- Assess the placental transfer and biodistribution of three commonly used azo tattoo pigments



Pigment Orange 13
CAS 3520-72-7

Pigment Yellow 83
CAS 5567-15-7

Pigment Red 22
CAS 6448-95-9

- Synthesize [^{14}C]-ring-labeled pigments to determine quantitatively mass distribution and deposition
- Tattoo pregnant SKH-1 mice (2.5 mg/cm^2; 10 mCi/mouse)
- Evaluate radioactivity in fetuses, organs, excrements for the deposition of the ^{14}C-label by scintillation counting

REPRESENTATIVE CURRENT PROJECT #2
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
  - ✓ Extended circulating half-life
  - ✓ Increased drug stability
  - ✓ Enhanced protection from proteolytic degradation
  - ✓ Reduced dosage frequency, without diminished efficacy and with potentially reduced toxicity

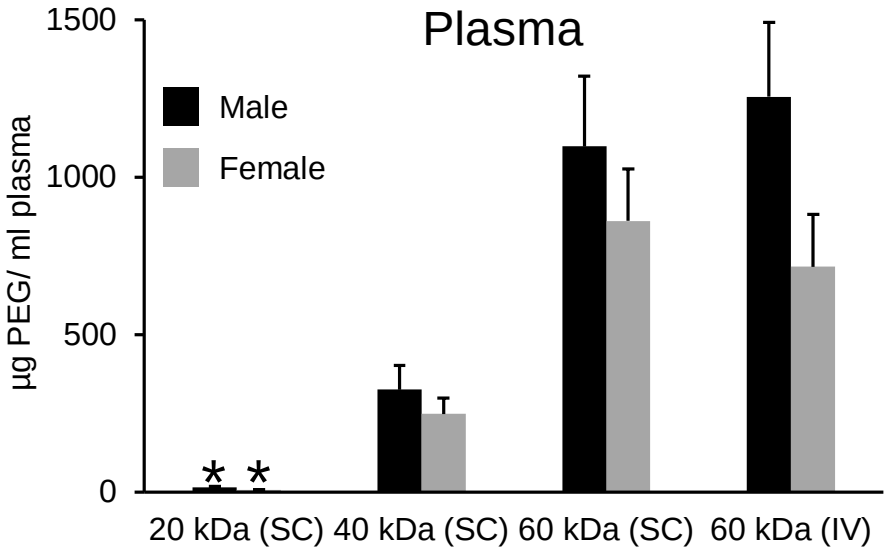
PEGYLATED BIOPHARMACEUTICALS CONCERNS AND DATA NEEDS

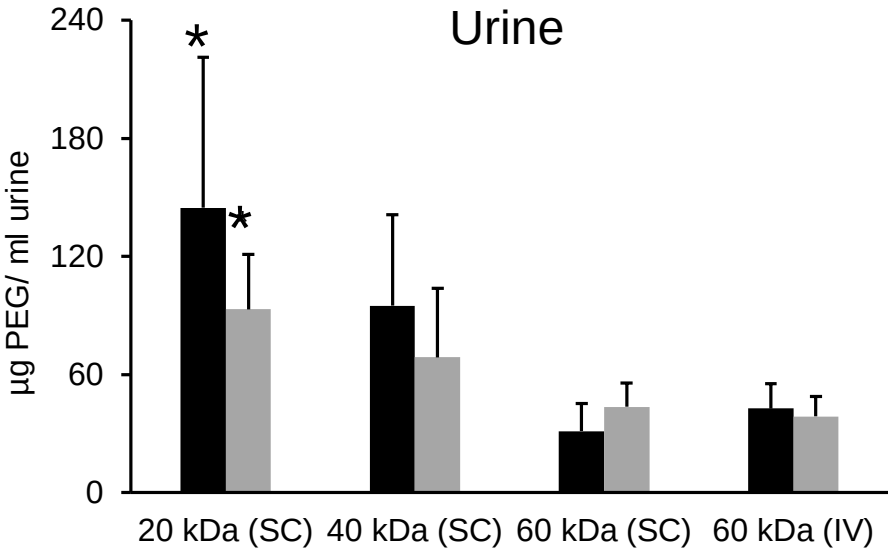
- Several PEGylated biopharmaceuticals have caused PEG accumulation and cellular vacuolization in various tissues, including the choroid plexus, in pre-clinical studies.
- There is concern that PEG accumulation and the formation of these vacuoles may lead to adverse outcomes for PEGylated biopharmaceuticals 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 and kidney

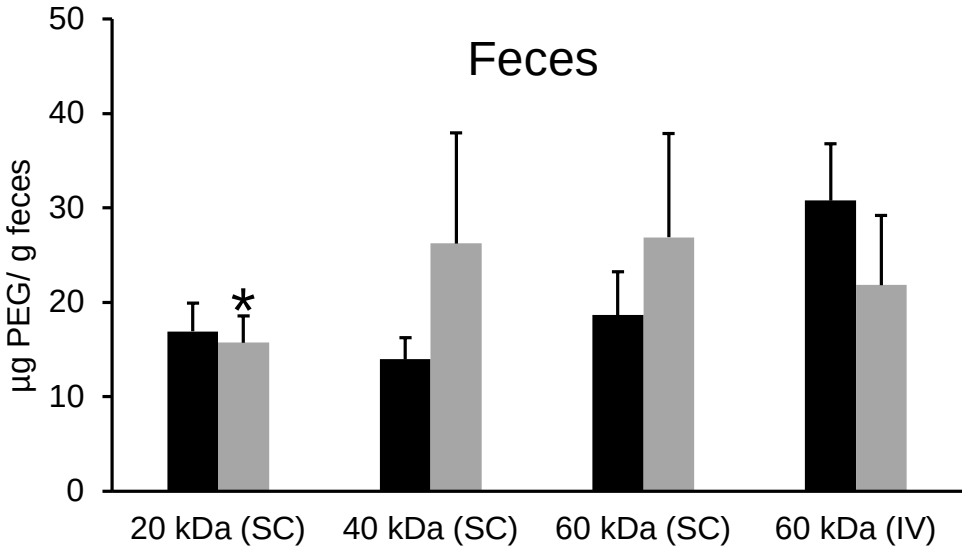
PEGYLATED BIOPHARMACEUTICALS EXPERIMENTAL DESIGN

- Assess the toxicities resulting from weekly repeated subcutaneous or intravenous injections of high-molecular-weight PEGs (20, 40, and 60 kDa) for 24 weeks to Sprague-Dawley rats.
- Evaluate the toxicokinetic profile of high-molecular-weight PEGs given as a single subcutaneous or intravenous dose to Sprague-Dawley rats.
- Evaluate the bioaccumulation of high-molecular-weight PEGs in organs/tissues of Sprague-Dawley rats upon repeat subcutaneous or intravenous injection of the test articles for 24 weeks.

PEGYLATED BIOPHARMACEUTICALS
LEVEL OF PEGs IN PLASMA, URINE, & FECES





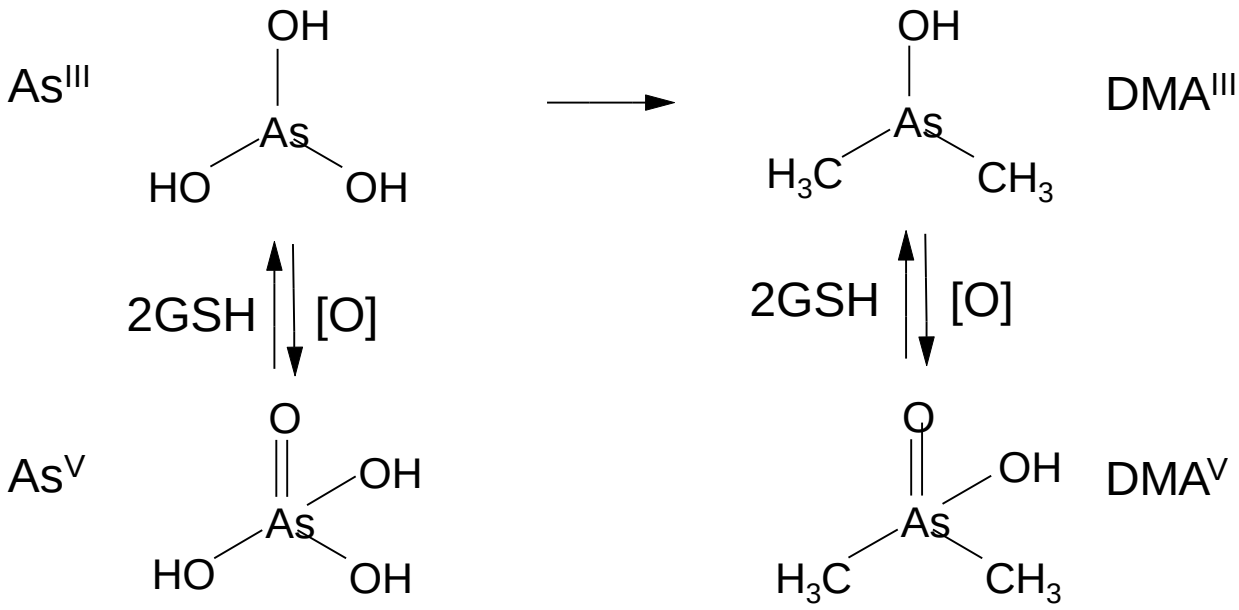


FUTURE PROJECT #1
ARSENIC BIOASSAY
(in collaboration with CFSAN)

- Average arsenic content in drinking water in the U.S. is 2 ppb, with some areas having concentrations >1000 ppb
- EPA maximum contaminant level and WHO guideline value for inorganic arsenic in drinking water is 10 ppb
- Estimated mean daily exposure to inorganic arsenic in U.S.
  - ✓ Adults, 0.08 - 0.20 µg/kg bw/day
  - ✓ Children, ages 1 - 6, 0.11 - 0.32 µg/kg bw/day
  - ✓ Children, < 1 year old, 0.24 - 1.19 µg/kg bw/day

ARSENIC: PHARMACOKINETICS SUMMARY

- iAs is readily absorbed from the GI tract (DMA^{V} also)
- Metabolism is dominated by DMA^{V}
- Non-linear kinetics at doses > 50 µg/kg bw
- DMA^{V} can be reduced to DMA^{III}, which can react with sulfhydryls in proteins
- Very poor lactational transfer of arsenic species



ARSENIC: OUTSTANDING ISSUES AND DATA GAPS

- Inorganic arsenic (iAs)
  - ✓ Unusual dose-response for lung tumors
  - ✓ Lack of tumors at additional sites (liver, adrenal cortex, ovary)
  - ✓ Poor lactational transfer

- Dimethylarsinic acid (DMA^{V})
  - ✓ No experiments conducted with perinatal exposure
  - ✓ Poor lactational transfer

ARSENIC: PROPOSED BIOASSAY AND MECHANISTIC STUDIES

- **"Whole life" exposure bioassay**
  - ✓ Dams and sires before and during breeding, and dams during pregnancy; drinking water
  - ✓ Pups - Postnatal days 1 - 21; gavage
  - ✓ Weaning - 2 years; drinking water
  - ✓ iAs and DMA^V

- **Mechanistic studies**
  - ✓ Internal dosimetry
  - ✓ Epigenetic alterations
  - ✓ Microbiome alterations
  - ✓ Hotspot cancer driver mutation analyses

FUTURE PROJECT #2
NON-ALCOHOLIC FATTY LIVER DISEASE

- **Background**
  - ✓ NAFLD is the most prevalent form of chronic liver disease in the United States
  - ✓ Pronounced sex differences in the susceptibility to NAFLD
  - ✓ Extensive individual variability in the susceptibility to NAFLD
  - ✓ Great difficulties with early diagnosis and disease staging
  - ✓ No currently FDA-approved therapies for NAFLD

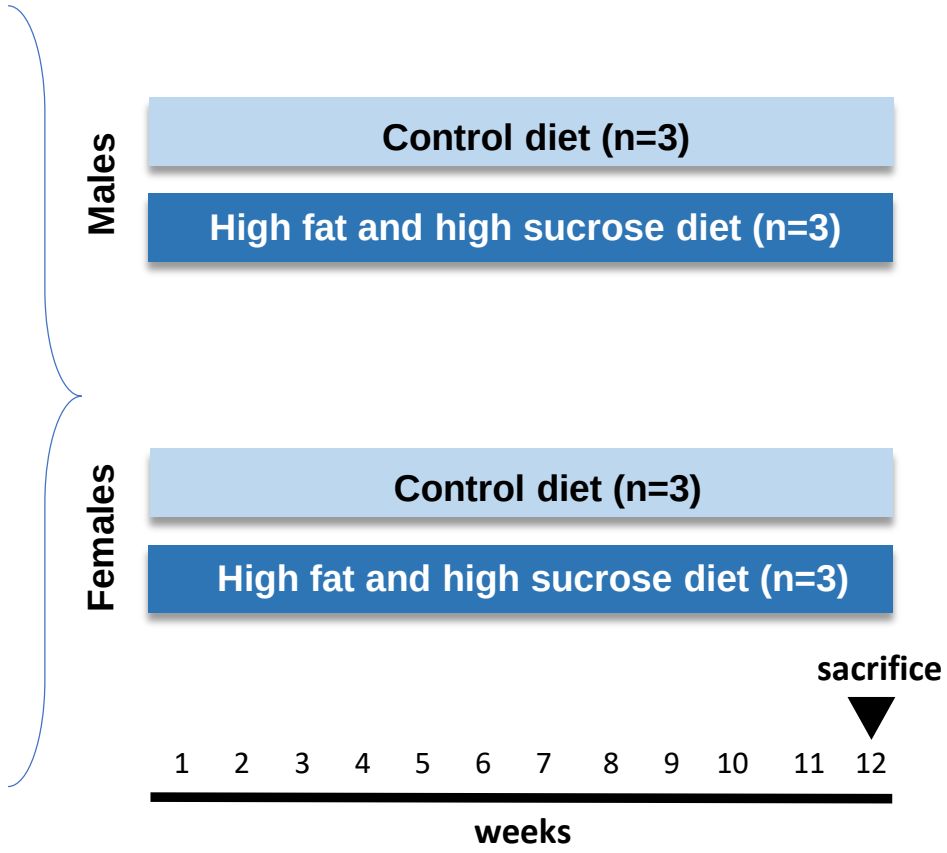
- **Objectives**
  - ✓ Determine genomic and genetic determinants of the different sex and individual susceptibility to NAFLD
  - ✓ Develop and evaluate novel biomarkers for NAFLD diagnosis and monitoring

E7635: GENETICS AND EPIGENETICS OF NAFLD SUSCEPTIBILITY USING COLLABORATIVE CROSS MICE

STUDY DESIGN

25 Collaborative Cross Mouse Strains

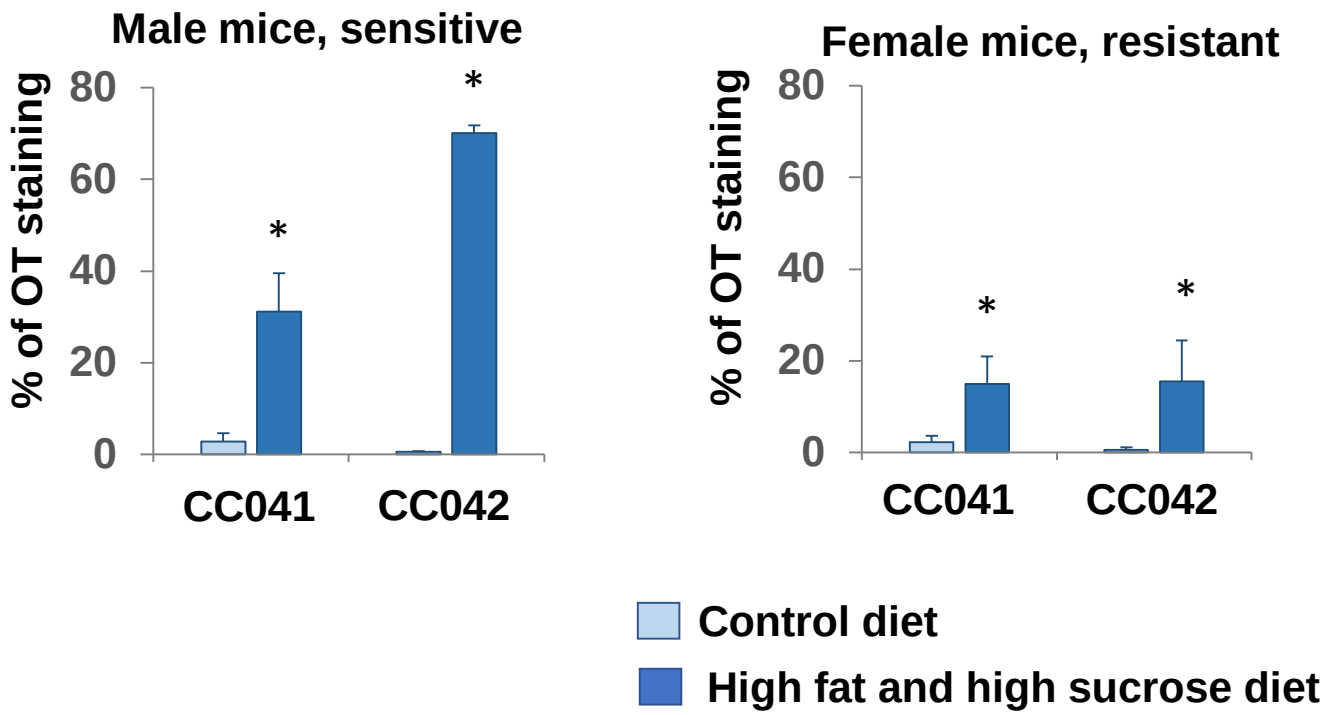
CC001/UncJ	CC026/GeniUncJ
CC002/UncJ	CC032/GeniUncJ
CC003/UncJ	CC037/TauUncJ
CC004/TauUncJ	CC040/TauUncJ
CC005/TauUncJ	CC041/GeniUncJ
CC006/TauUncJ	CC042/GeniUncJ
CC009/UncJ	CC043/GeniUncJ
CC010/GeniUncJ	CC051/TauUncJ
CC011/UncJ	CC060/UncJ
CC012/UncJ	CC061/GeniUncJ
CC013/GeniUncJ	CC068/TauUncJ
CC019/TauUncJ	CC080/TauUncJ
CC025/GeniUncJ	



E7635: GENETICS AND EPIGENETICS OF NAFLD SUSCEPTIBILITY USING COLLABORATIVE CROSS MICE

RESULTS

Extent of liver steatosis



GENOMIC AND GENETIC DETERMINANTS OF THE SUSCEPTIBILITY TO NAFLD

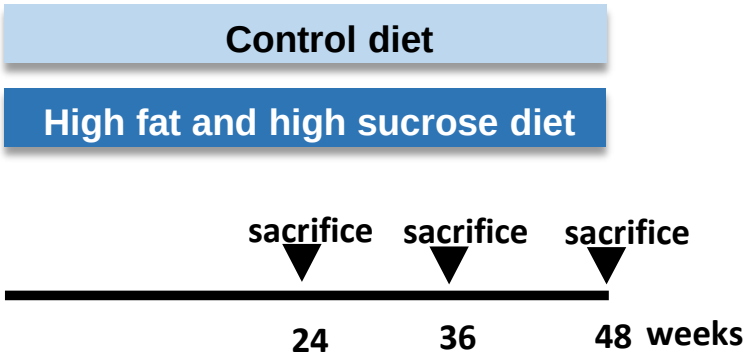
Investigate determinants of the sex-specific susceptibility to NAFLD

Males, sensitive

CC041	CC042

Females, resistant

CC041	CC042

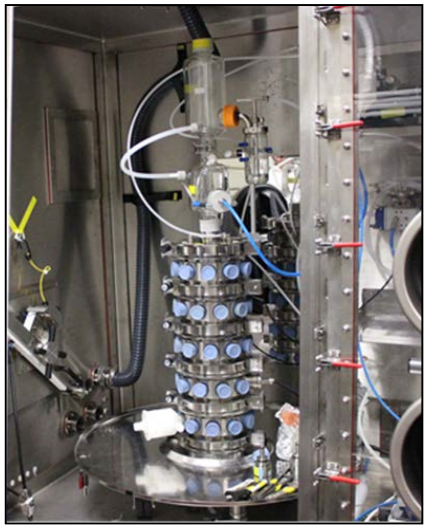


ANALYSES

- Histopathology
- Clinical biochemistry
- Transcriptome
- Epigenome
- Metabolome
- Microbiome

FUTURE PROJECT #3
CTP/NCTR INHALATION TOXICOLOGY CORE FACILITY

Rat restraint tube



Inhalation tower

Inhalation exposure lab

CURRENT AND FUTURE STUDIES IN INHALATION TOXICOLOGY CORE

- **Completed or ongoing**
  - ✓ NNK single-dose pharmacokinetic study
  - ✓ NNK 14-day nose-only inhalation toxicity study
  - ✓ NNK 90-day nose-only inhalation toxicity study
  - ✓ Nicotine single-dose pharmacokinetic study

DIVISION OF BIOCHEMICAL TOXICOLOGY CHALLENGES

- Postdoctoral fellows and visiting scientists
- Decreased funding from the National Toxicology Program
- Integration of the inhalation core into the division

QUESTIONS, COMMENTS, AND/OR SUGGESTIONS