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Studies to Identify Environmental Cardiotoxins and Susceptibilities to Cardiotoxicity

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NTP Board of Scientific Counselors
December 9 -10, 2009

Rank	Cause of Death	No. of deaths	% of all deaths
1.	Heart Diseases	652,091	26.6
2.	Cancer	559,312	22.8
3.	Cerebrovascular diseases	143,579	5.9
4.	Chronic lower respiratory diseases	130,933	5.3
5.	Accidents (Unintentional injuries)	117,809	4.8
6.	Diabetes mellitus	75,119	3.1
7.	Influenza and pneumonia	63,001	2.6
8.	Alzheimer disease	71,599	2.9
9.	Nephritis	43,901	1.8

Source: US Mortality Public Use Data Tape 2002, National Center for Health Statistics, Centers for Disease Control and Prevention, 2009.

Goals

- **Identify host susceptibility genes for cardiac disease**
- **Key components of the project**
  - Genetic diversity in multiple mouse strains
  - Prototype cardiotoxins
  - Quantitative biomarkers of cardiac toxicity
  - Cardiac phenotypes in control mice
- **Collaborations between NIEHS/NTP research groups**

genetic component + toxic component = disease

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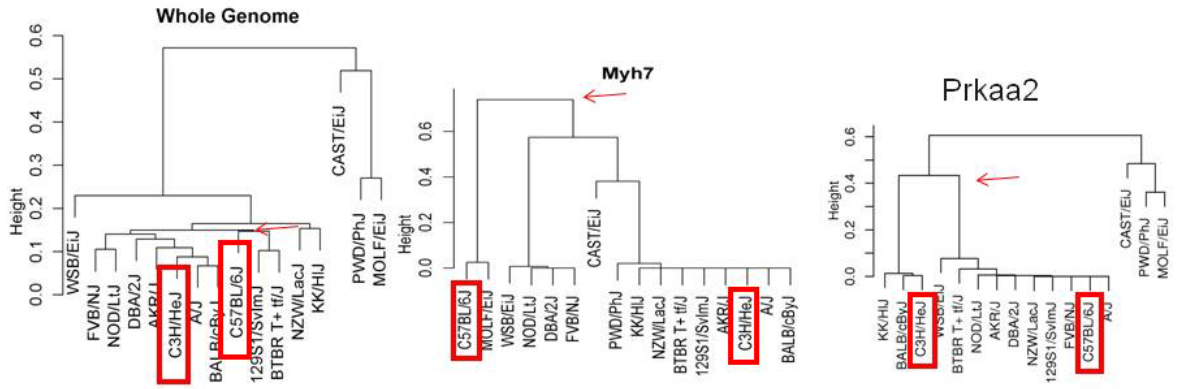
Genetic Diversity



http://mouse.perlegen.com/mouse/browser.html

Prototype Cardiotoxin 1: Bis(2-chloroethoxy)methane (CEM)

Starting material for synthesis of polysulfide elastomers
found in water ways and industrial wastes

CEM causes cardiotoxicity in rats and mice within a few days of dosing

- Histopathology
- EM
- Heart gene transcript patterns
- Serum biomarkers
- ECG

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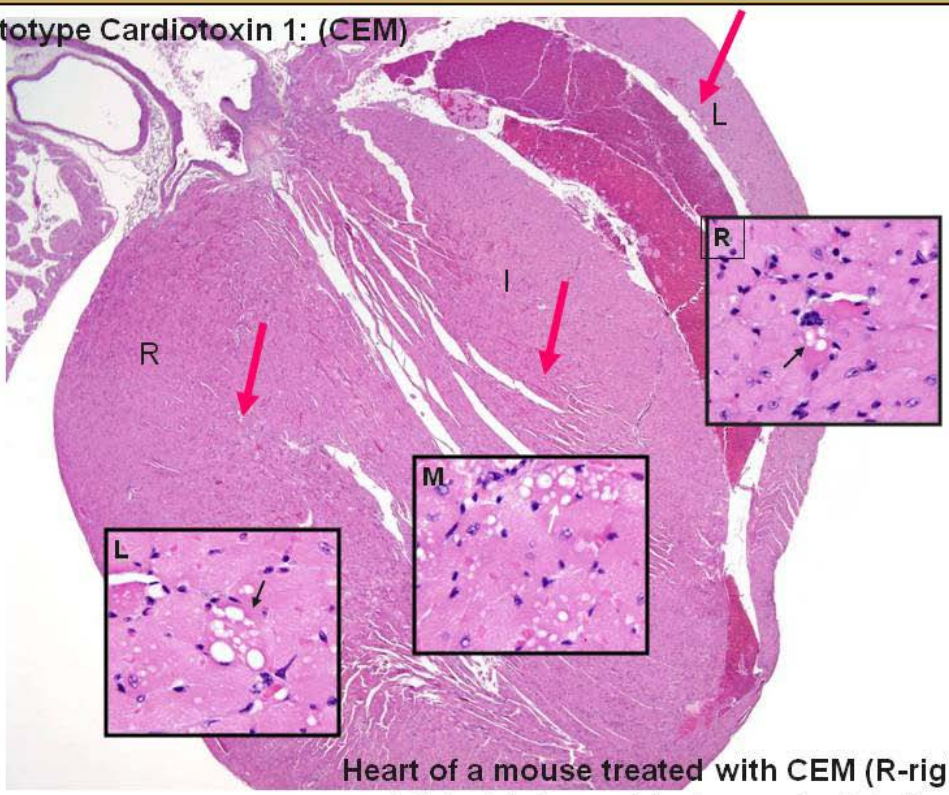
Prototype Cardiotoxin 1: (CEM)



Heart of a mouse treated with CEM (R-right ventricle; I-interventricular septa; L-left ventricle)

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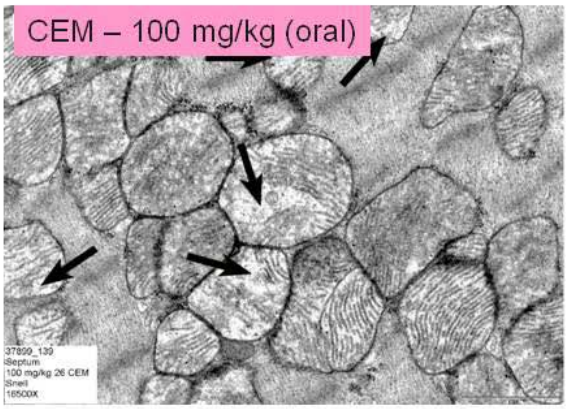
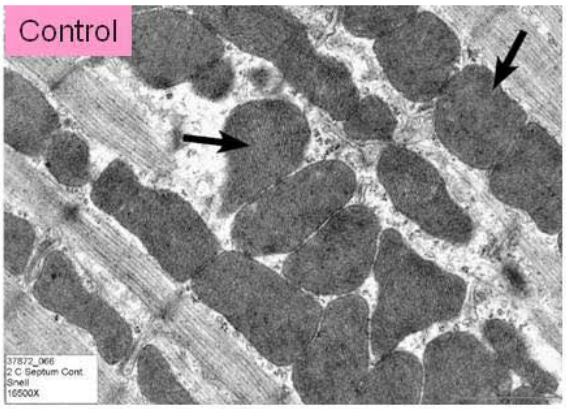
Electron Microscopy Evaluation



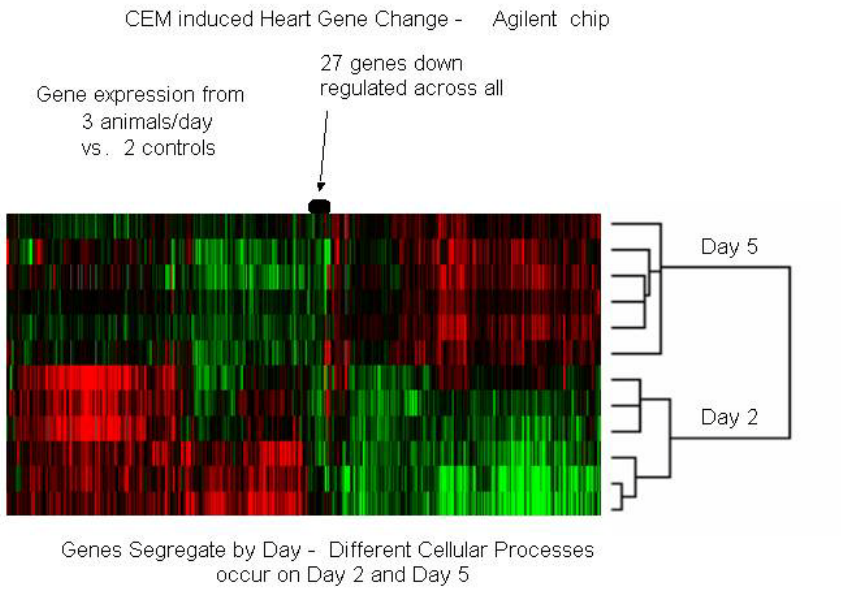


Damaged mitochondria with broken cristae

Prototype Cardiotoxin 1: (CEM)

Genomic Response of the Heart following CEM Treatment

CEM-induced Gene Transcript Patterns in the Heart



Prototype Cardiotoxin 1: (CEM)

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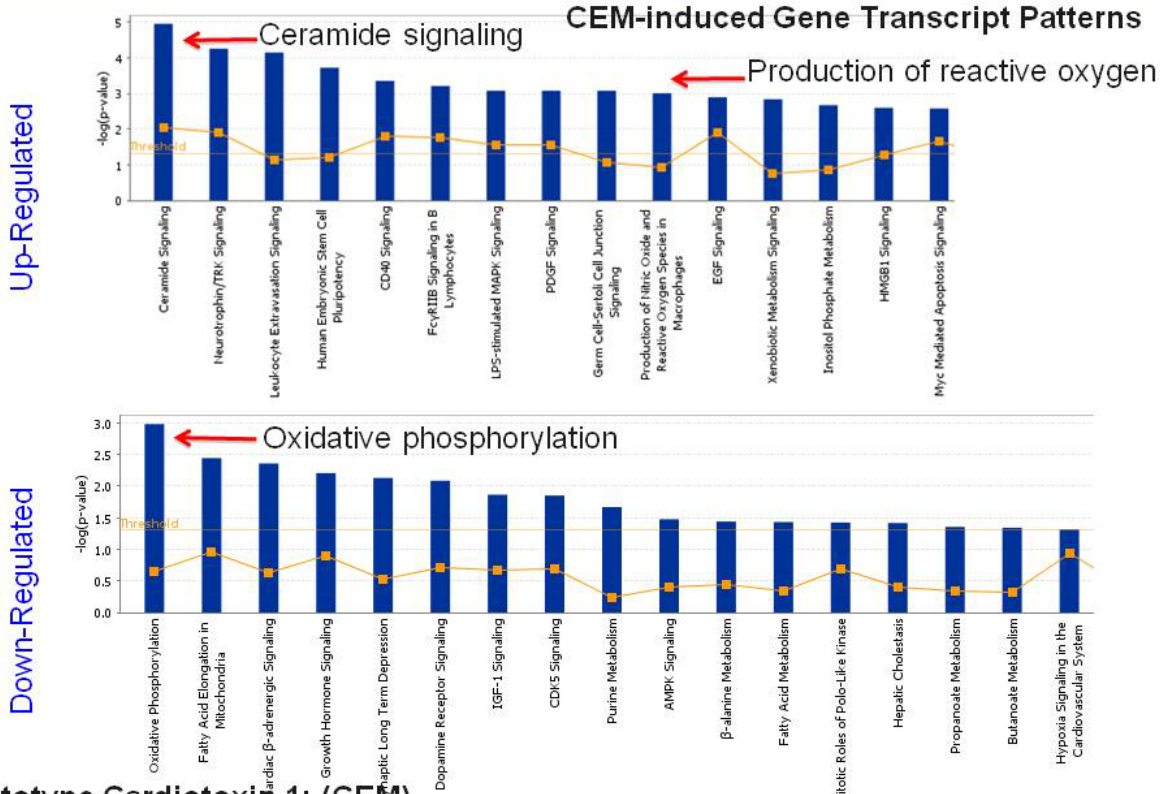
CEM-induced Gene Transcript Patterns



Prototype Cardiotoxin 1: (CEM)

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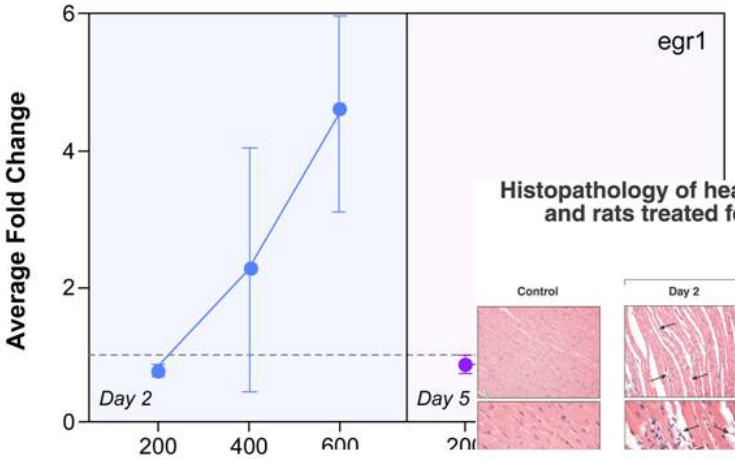
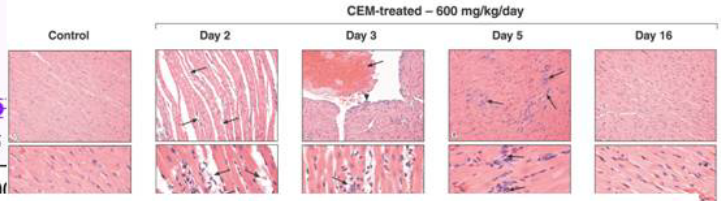
Prototype Cardiotoxin 1: CEM
Early growth response factor (egr1)



Histopathology of heart on days 2, 3, 5, and 16. F344 controls and rats treated for up to 16 days with 600 mg/kg/day.



Egr-1, a master switch coordinating upregulation of divergent gene families underlying ischemic stress

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Mechanism of CEM Heart Damage

$Cl\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2\text{-}Cl$
Bis(2-chloroethoxy)methane → $HOOC\text{-}CH_2\text{-}S\text{-}CH_2\text{-}COOH$
Thiodiglycolic Acid

Mitochondria Damage due to:

- **Interference with fatty acid metabolism**
  - sequestering carnitine and inhibiting utilization and transport of long chain fatty acids
- **Decrease in dehydrogenase levels and ATP synthase levels**

↓

Decreased Energy Supply

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Quantitative Biomarkers of Cardiotoxicity

TOXICOLOGICAL SCIENCES **100(1)**, 29–35 (2007)
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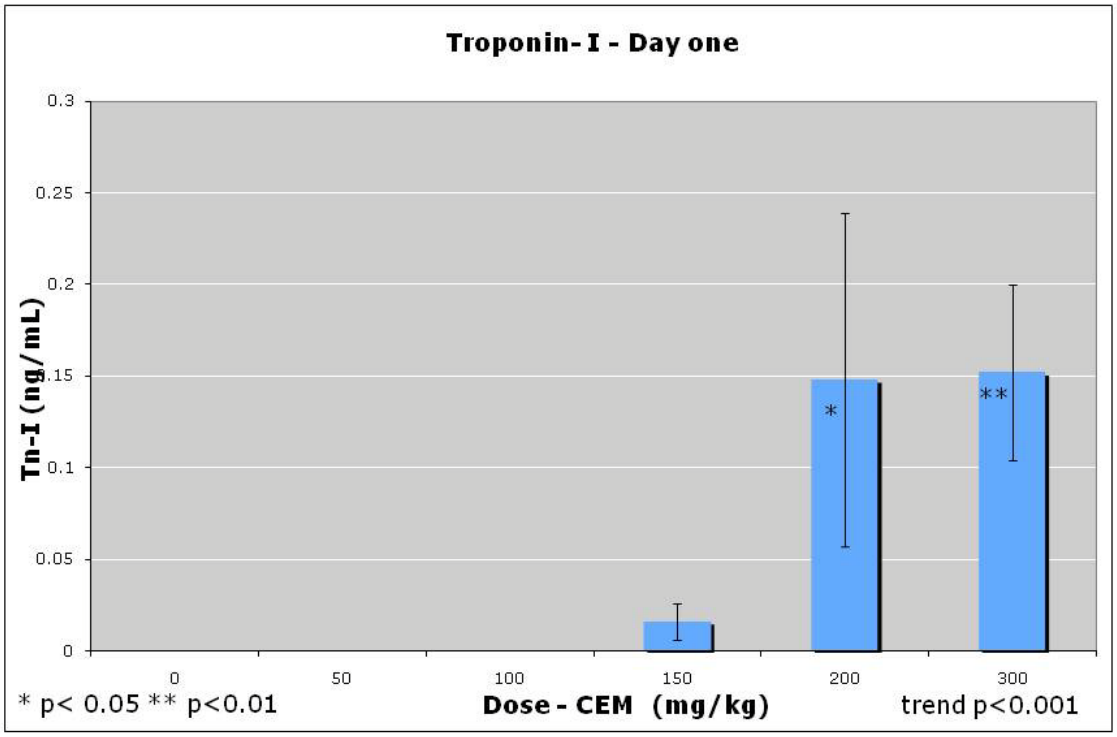
FORUM

Inclusion of Biomarkers for Detecting Perturbations in the Heart and Lung and Lipid/Carbohydrate Metabolism in National Toxicology Program Studies

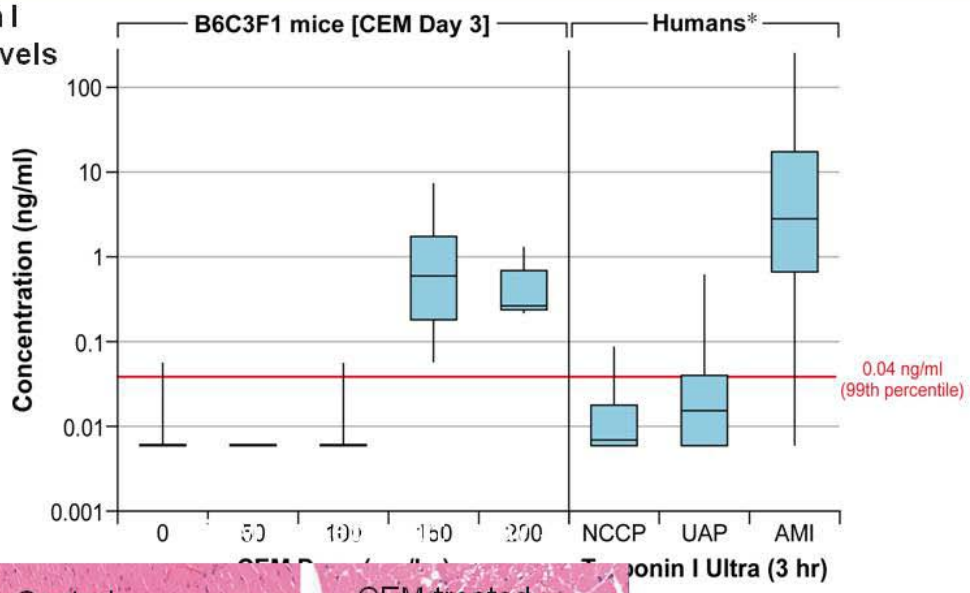
Quantitative Serum Biomarker of Cardiotoxins

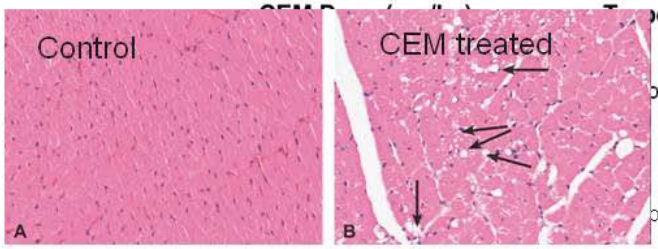
- TnI levels after chemical exposure in B6C3F1 mice
  - One or three day exposures
    - Samples taken four hours after chemical exposure
  - Comparison to TnI levels in human disease

Quantitative Serum Biomarker of Cardiotoxins



Troponin I Serum levels





of acute myocardial Infarction.

on

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Prototype Cardiotoxin 2: Ephedrine/Caffeine

Oral Ephedrine Exposure → Rapid absorption so that toxic levels are obtained within one hour of dosing → a/b adrenergic agonist - release of catecholamines

↓

Vasoconstriction (V)

↓

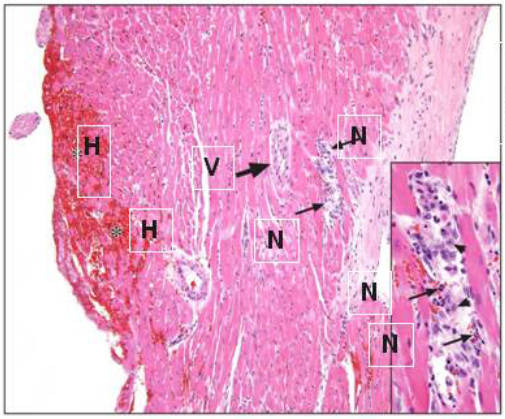
Increase HR and QT_c interval

↓

Myocardial ischemia

↓

Necrosis and Apoptosis (N) → Hemorrhage (H) → Sudden Death



Caffeine
Release of Intracellular
Ca^{++} Stores

Resolution by
Inflammation
Fibrosis

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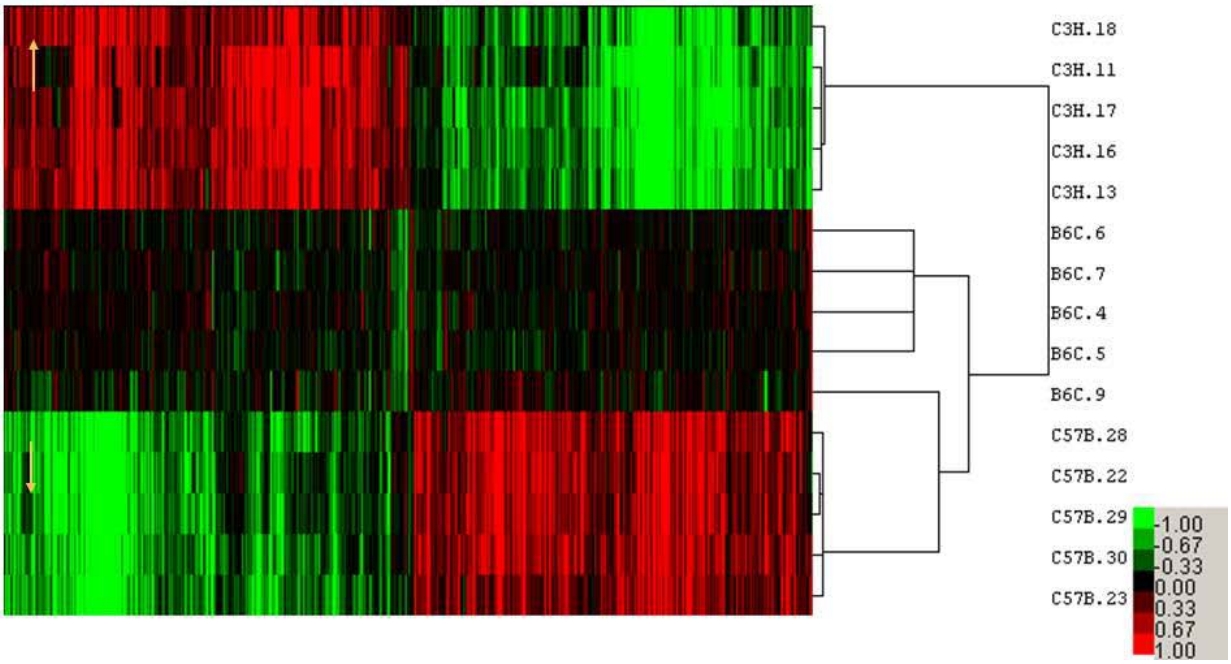
Characterizing Cardiac Gene Transcript Pattern in Control (C3H/HeJ, B6C3F1/J, C57BL/6J) Mice



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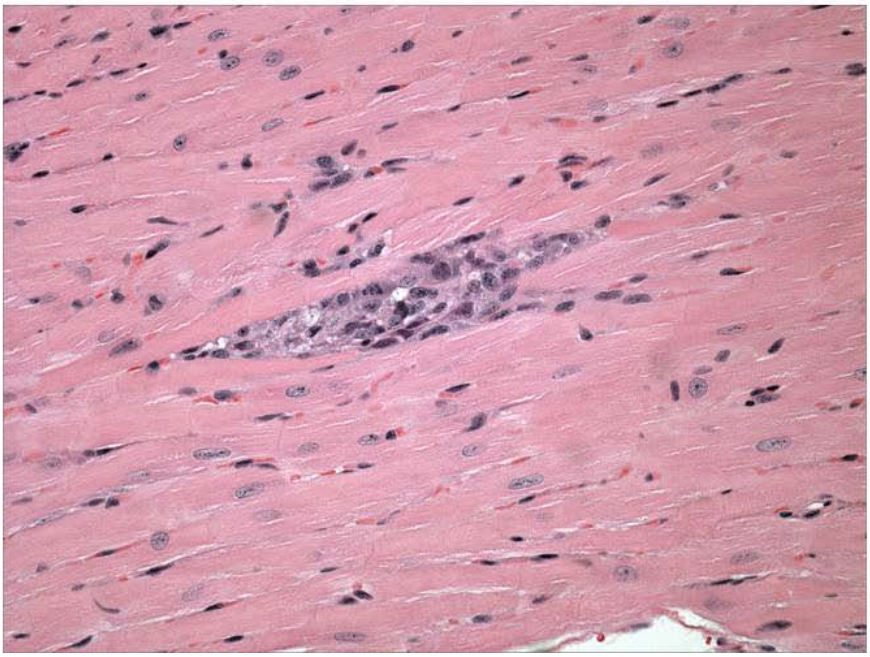
Histopathology of Heart in Control Mice (C3H/HeJ) showing Cardiac Phenotype

Focus of disrupted myocardial fibers in ventricular wall with infiltration by mononuclear cells, primarily histiocytes (x40)



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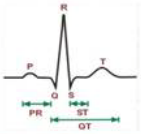
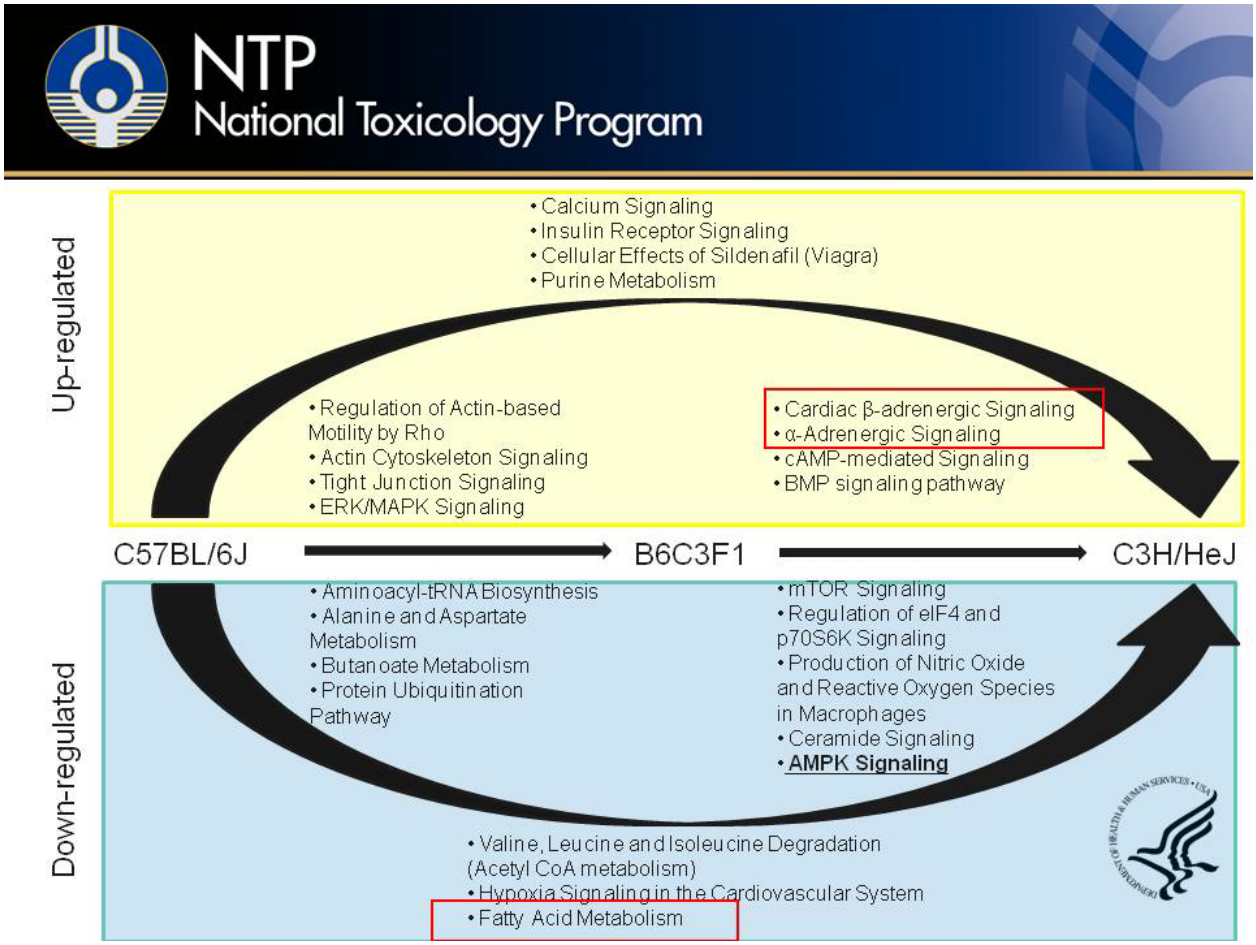
Background Cardiac Phenotypes in Control Mice

Electrocardiogram Analysis □□

	C3H/HeJ	B6C3F1/J	C57BL/6J
HR (bpm)	↑651□6	597±9	597±6
RR interval (msec)	↓94.2□2	100±3.3	101.8±1.9
QT_c Bazett's	↑187.1□1.2	175.4±1.1	183.2±2.1
R_{amp} mV	0.46□0.01	0.40□0.02	0.57□0.02
Body Temp °C	36.3□0.1	36.9□0.1	36.7□0.1



QT interval - time it takes the heart's electrical system to recharge after each beat (repolarization) before generating the next signal to activate ventricles;
Bazett's forumula: QT_c = QT/sqrt RR (R-R interval in seconds)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Up-regulated
• Calcium Signaling
• Insulin Receptor Signaling
• Cellular Effects of Sildenafil (Viagra)
• Purine Metabolism
• Regulation of Actin-based Motility by Rho
• Actin Cytoskeleton Signaling
• Tight Junction Signaling
• ERK/MAPK Signaling
• Cardiac β-adrenergic Signaling
• α-Adrenergic Signaling
• cAMP-mediated Signaling
• BMP signaling pathway
C57BL/6J
B6C3F1
C3H/HeJ
Down-regulated
• Aminoacyl-tRNA Biosynthesis
• Alanine and Aspartate Metabolism
• Butanoate Metabolism
• Protein Ubiquitination Pathway
• mTOR Signaling
• Regulation of eIF4 and p70S6K Signaling
• Production of Nitric Oxide and Reactive Oxygen Species in Macrophages
• Ceramide Signaling
• AMPK Signaling
• Valine, Leucine and Isoleucine Degradation (Acetyl CoA metabolism)
• Hypoxia Signaling in the Cardiovascular System
• Fatty Acid Metabolism

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In silico Approach - Identifying candidate host susceptibility genes using heart gene transcript patterns from control C3H/HeJ, B6C3F1/J, C57BL/6J mice

45101 probe sets ~ 14000 genes

↓ ANOVA, C3H/HeJ vs. C57BL/6J vs. B6C3F1J

3074 probe sets ~ 2377 genes

↓ Expression QTL from C3H/HeJ x C57BL/6J cross (GeneNetwork)

380 probe sets = 311 genes

↓ Association with cardiac function / disease (Database mining)
- Databases: Copub, GeneCards, GAD, HUGE Navigator

50 probe sets = 40 genes

↓ Found in cardiac necrosis QTL (Ivandic et al., 1996)

9 probe sets = 7 genes

↓ Expressed in cardiac tissue (GeneAtlas)

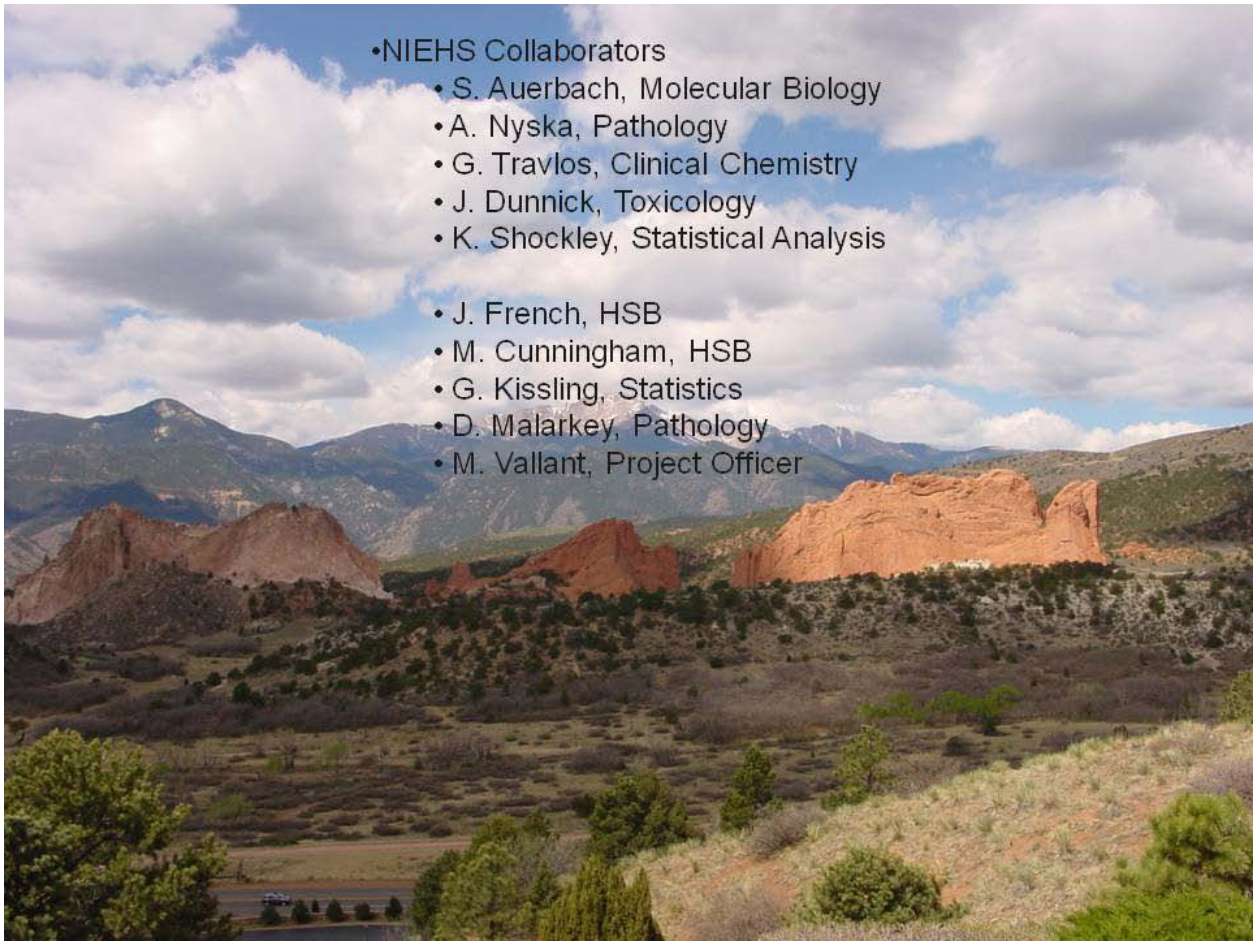
Prkaa2, Calr3, Il15

Future Directions

- Susceptibility to chemical-induced cardiotoxicity in multiple mouse strains
  - Three mouse strains exposed to prototype cardiotoxins - CEM and ephedrine/caffeine
  - Multiple mouse strain response to cardiotoxins
- Study of a broader range of response to cardiotoxins
  - AIDS drugs
- Effect of age & sex on cardiotoxicity
- Identify cardiac disease susceptibility gene candidates

Summary

- **Genetic diversity**
  - C3H/HeJ, B6C3F1/J and C57Bl/6J mice
- **Prototype cardiotoxins**
  - CEM and ephedrine/caffeine
- **Quantitative biomarkers of cardiac disease**
  - Serum biomarkers - TnI and MyL3
- **Candidate host susceptibility genes**
  - Prkaa2, Calr3, Il15
- **Multiple mouse strain studies**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