Presentation 10 – Mariana Morris

Gulf War-related Research at Wright State University

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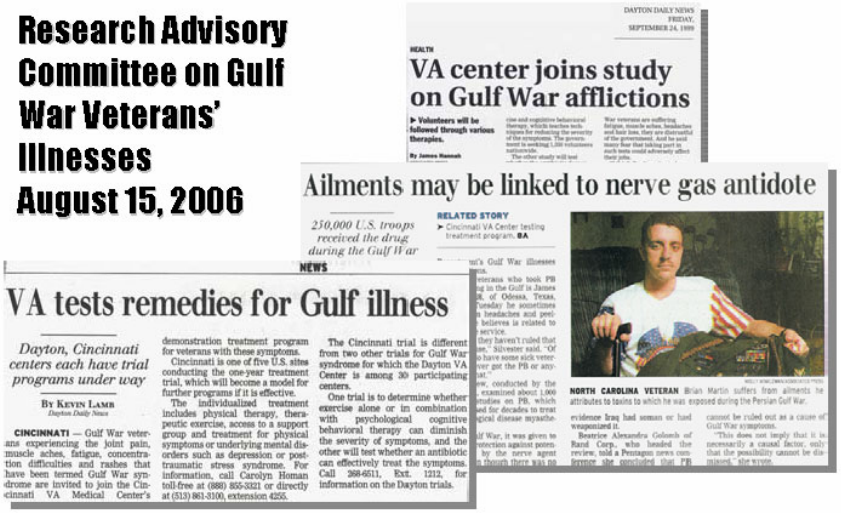
Wright State University

The Mission:

To understand the basis of toxicity from environmental, chemical and life-style interactions and to use this to study and develop countermeasures

Research Advisory Committee on Gulf War Veterans' Illnesses
August 15, 2006



DAYTON DAILY NEWS
FRIDAY,
SEPTEMBER 24, 1999

HEALTH

VA center joins study on Gulf War afflictions

▶ Volunteers will be followed through various therapies.

By James Hannah

Ailments may be linked to nerve gas antidote

250,000 U.S. troops received the drug during the Gulf War

RELATED STORY
➤ Cincinnati VA Center testing treatment program, B4

NEWS

VA tests remedies for Gulf illness

Dayton, Cincinnati centers each have trial programs under way

By Kevin Lamb
Dayton Daily News

CINCINNATI — Gulf War veterans experiencing the joint pain, muscle aches, fatigue, concentration difficulties and rashes that have been termed Gulf War syndrome are invited to join the Cincinnati VA Medical Center's demonstration treatment program for veterans with these symptoms.

Cincinnati is one of five U.S. sites conducting the one-year treatment trial, which will become a model for further programs if it is effective.

The individualized treatment includes physical therapy, therapeutic exercise, access to a support group and treatment for physical symptoms or underlying mental disorders such as depression or post-traumatic stress syndrome. For information, call Carolyn Homan toll-free at (888) 855-3321 or directly at (513) 861-3100, extension 4255.

The Cincinnati trial is different from two other trials for Gulf War syndrome for which the Dayton VA Center is among 30 participating centers.

One trial is to determine whether exercise alone or in combination with psychological cognitive behavioral therapy can diminish the severity of symptoms, and the other will test whether an antibiotic can effectively treat the symptoms. Call 368-6511, Ext. 1212, for information on the Dayton trials.

NORTH CAROLINA VETERAN Brian Martin suffers from ailments he attributes to toxins to which he was exposed during the Persian Gulf War.

evidence Iraq had sarin or had weaponized it.

Beatrice Alexandra Golomb of Rand Corp., who headed the review, told a Pentagon news conference she concluded that PB

cannot be ruled out as a cause of Gulf War symptoms.

"This does not imply that it is necessarily a causal factor, only that the possibility cannot be dismissed," she wrote.

COUNTERTOX
Research Partners

- Boonshoft School of Medicine
  - Pharmacology and Toxicology
  - Biochemistry and Molecular Biology
- Wright-Patterson AFB, Air Force Research Laboratory
- Dayton VA Medical Center
- Battelle Biomedical Research Center
- Rea Clinic
- Cenomed Inc

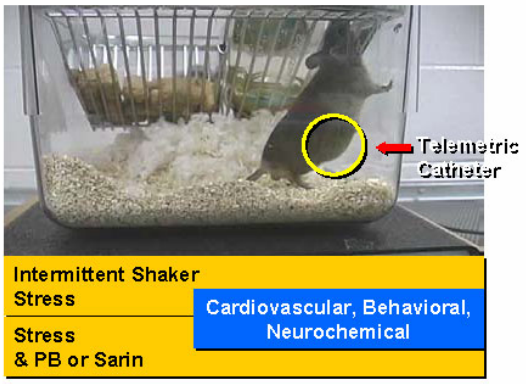
Core Research Facilities

- Center for Genomics
- Proteomics Analysis Laboratory
- Integrative Cardiovascular Laboratory (ICL)
- Chemical Warfare Agent Research Facility (CWF)
- Biosafety Laboratories
- Animal Resources Laboratory
- Magnetic Resonance Laboratory

Major Research Topics: Sarin Exposure

- Behavior
- Autonomic neural function
- Genomic biomarkers
- Chemical sensitivity in humans and sarin metabolism
- Proteomic biomarkers

Experimental Stress Model

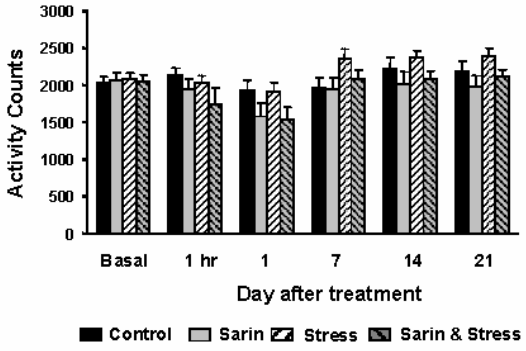


METHODS
Stress & Sarin Exposure

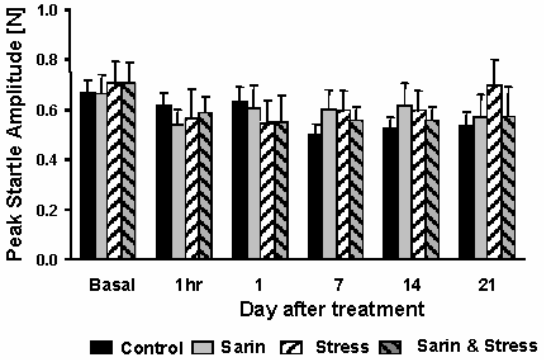
- Mice exposed to intermittent shaker stress for 7 days
- Sarin was injected sc for 3 days (64 µg/kg, 0.4xLD_{50})
- Groups: Control, Sarin, Sarin & Stress, Stress
- Evidence for adrenal dysfunction, altered fear potentiation and self-multilation

Lucot et. al

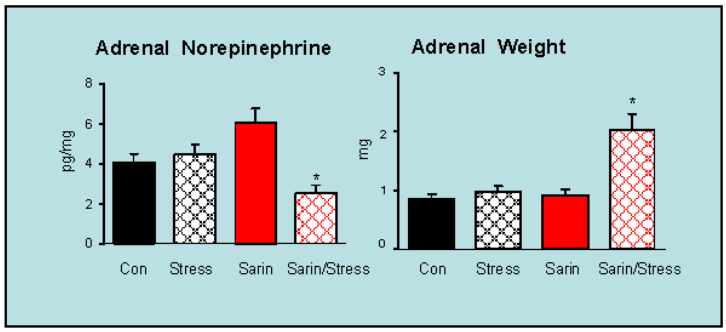
Sarin & Stress on Activity



Sarin & Stress on Acoustic Startle Response



Sarin and Stress
Adrenal Function



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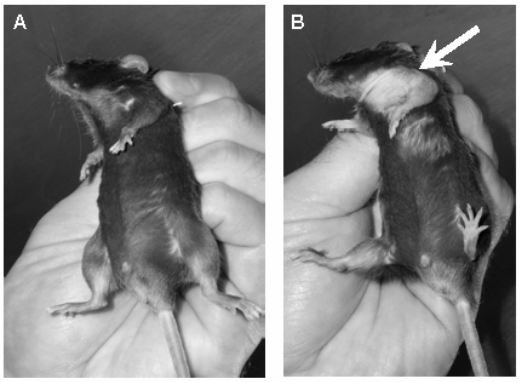
Sarin & Stress
Evidence for Self-Mutilation



Sarin & stress produced self mutilation in a subset of mice (~ 30%). This was not observed in the other groups.

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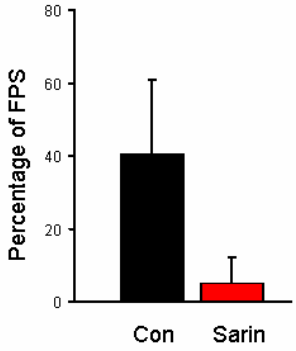
Sarin and Fear Potentiation of Startle Response



Behavioral response prevented in sarin treated mice

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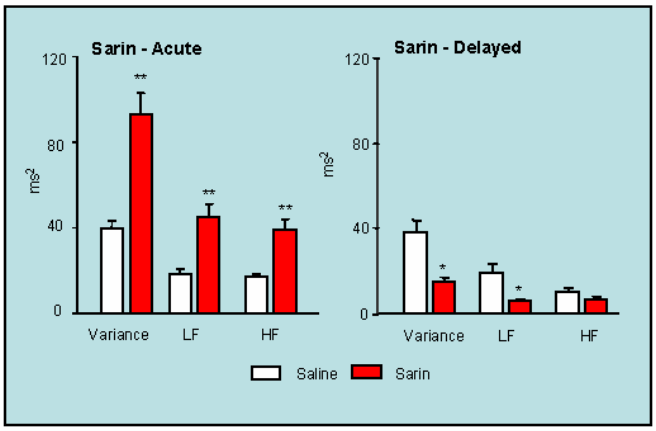
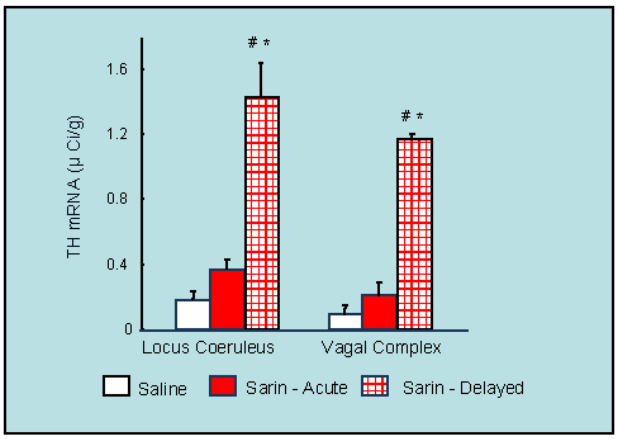
METHODS
Sarin & Autonomic Function

- Mice with telemetric carotid catheters
- Sarin was administered sc for 2 days (8 µg/kg, 0.05LD_{50})
- Cardiovascular parameters measured from 1 to 10 weeks after sarin
- Evidence for delayed autonomic dysfunction, similar to that seen with heart failure

Delayed Effect of Sarin on Heart Rate Variance and Frequency Domains



Low Dose Sarin Produces Delayed Changes in Brainstem Amine Function

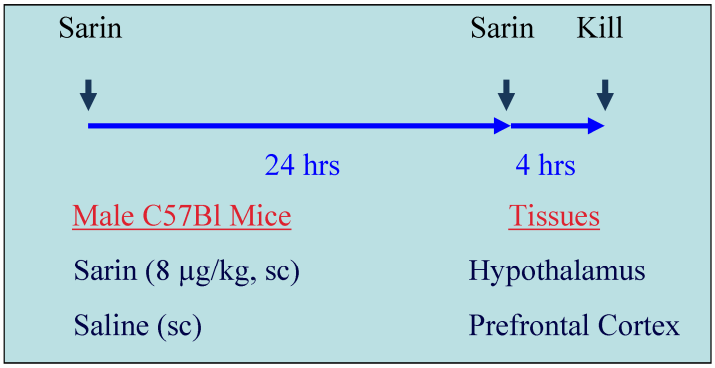


Gene Array Analysis

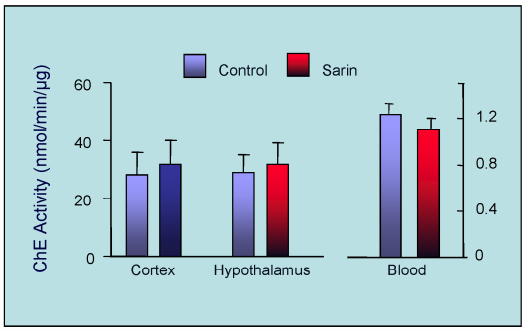
Gene Array Method

- Affymetrix GeneChip® (oligonucleotide based array)
- Mouse genome U74A (v2) chip
- Microarray Suite (v 5.0)
- Affymetrix Data Mining Tool (v 2.0)
- GeneSpring (v 6.1)
- PathwayAssist (v 2.5)

Experimental Protocol
Low Dose Sarin on Genomic Expression



Low Dose Sarin
Brain and Blood Cholinesterase



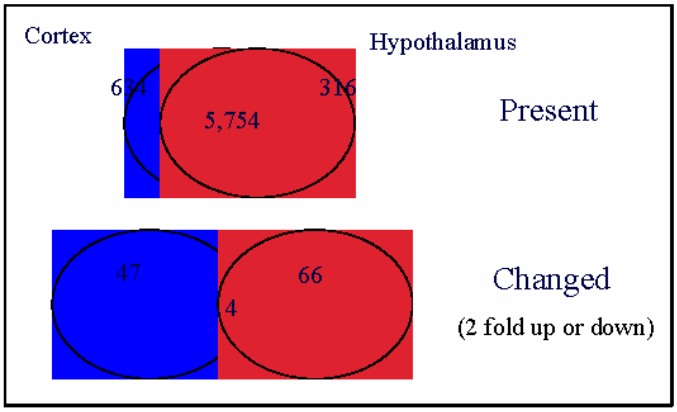
Data Mining (GeneSpring)

- Raw intensity and flags (P, M & A) from DMT
- Normalize all 14 chips to the median of controls
- Eliminate genes that were not present in at least 2 samples (6,388 in cortex & 6,070 in hypothalamus)
- Select genes increased or decreased (two-fold) Hcompared to control (70 in cortex & 51 in the hypothalamus)

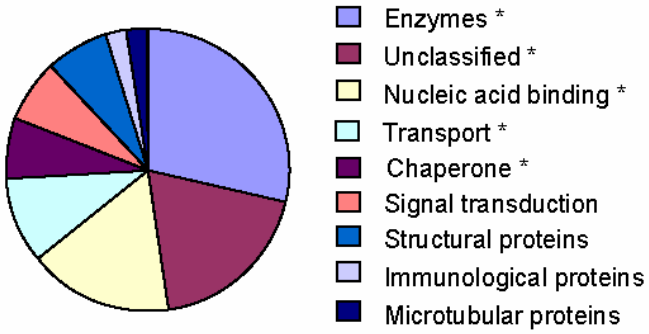
Low Dose Sarin
Effect on Cortex and Hypothalamus

Tissue	Increase	Decrease
Cortex	51	19
Hypothalamus	31	20

Genomics: Comparison of Hypothalamus to Cortex



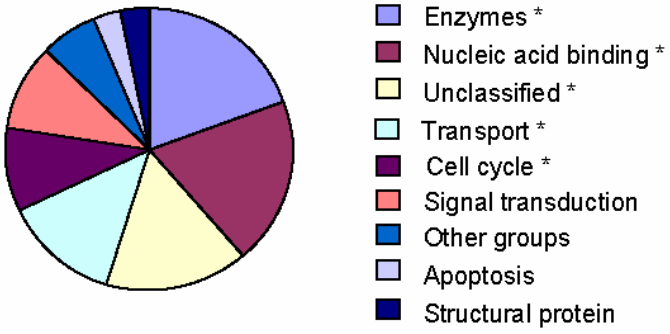
Classes of Genomic Changes Hypothalamic Increases



Sarin Increases Hypothalamic:

- Glial cell derived neurotrophic factor (GNDF)
- Serotonin transporter (SLC6A4)
- Tyrosine/Tryptophan activation protein (YWHAQ)
- PG F2 receptor negative regulator (PTGFRN)
- Doublecortin (DCX)

Classes of Genomic Changes Hypothalamic Decreases



Sarin Decreases Hypothalamic:

- PG D2 synthase (PTGDS)
- Voltage gated K+ channel (KCND2)
- Artemin of Glial cell line-Derived Neurotrophic Factor (GDNF) family (ARTN)
- Ankyrin repeat & SOCS box containing (ASB3)

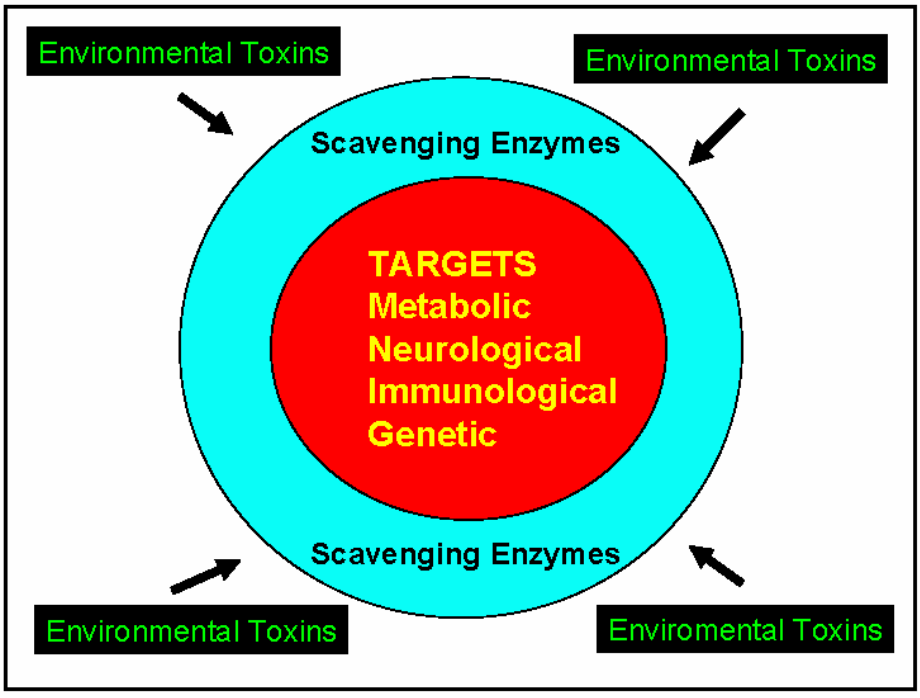
Low Dose Sarin & Genomics CONCLUSIONS

- There are brain changes in genetic expression at a sarin dose that does not change AChE activity
- Cortex and hypothalamus respond differently to acute sarin exposure.
- Detailed analysis (genomic and proteomic) will provide mechanistic insights into low dose sarin toxicity

Chemical Metabolism in Humans with Chemical Sensitivity

- Hypothesis: Chemical sensitivity is linked to deficiencies in scavenging enzymes.
- Corollary: Chemically sensitive individuals should have altered levels of scavenging enzymes

Alter et. al



Activities Measured

Formaldehyde ⟶ Formate

1) Aldehyde Dehydrogenase (ALD): NAD
2) chi Alcohol Dehydrogenase (xADH): NAD, GSH

Paraoxon ⟶ P - Nitrophenol + Diethyl Phosphate

Paraoxonase: Ca^{2+}

Phenyl Acetate ⟶ Phenol + Acetate

Arylesterase: Ca^{2+}

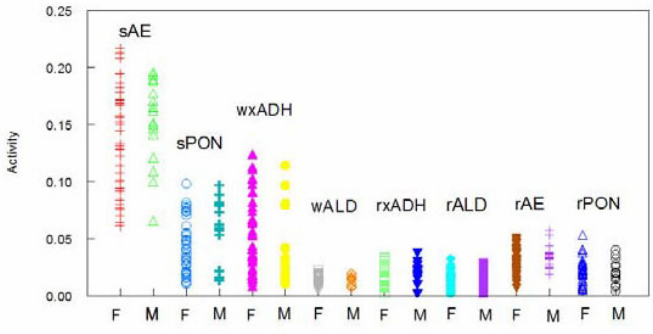
Sample Population

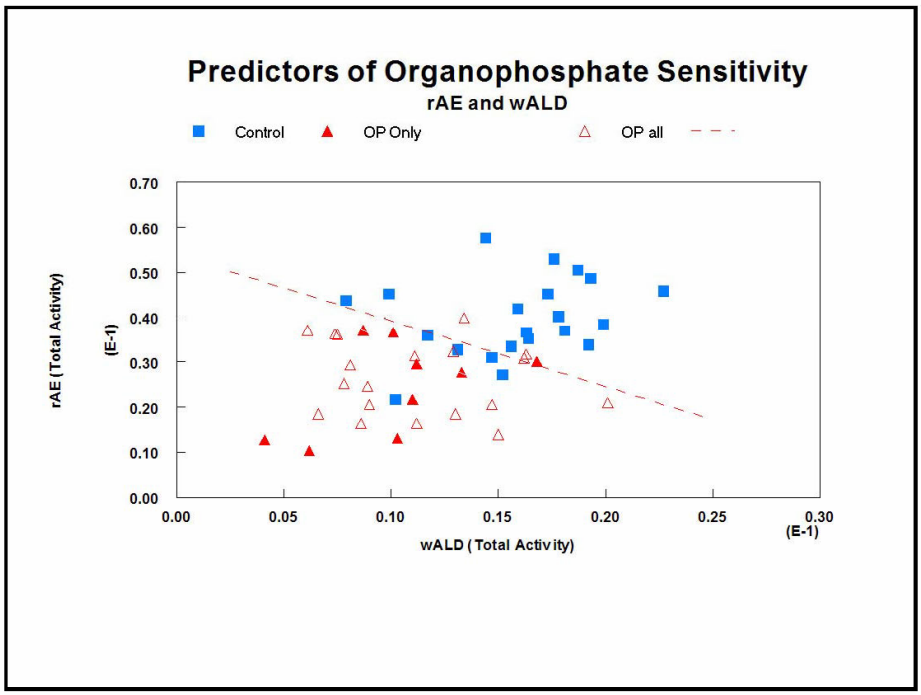
- Blood samples from chemically sensitive (formaldehyde or organophosphate) persons
- Rea Clinic, Dallas, TX
- Experiments were not performed directly with humans

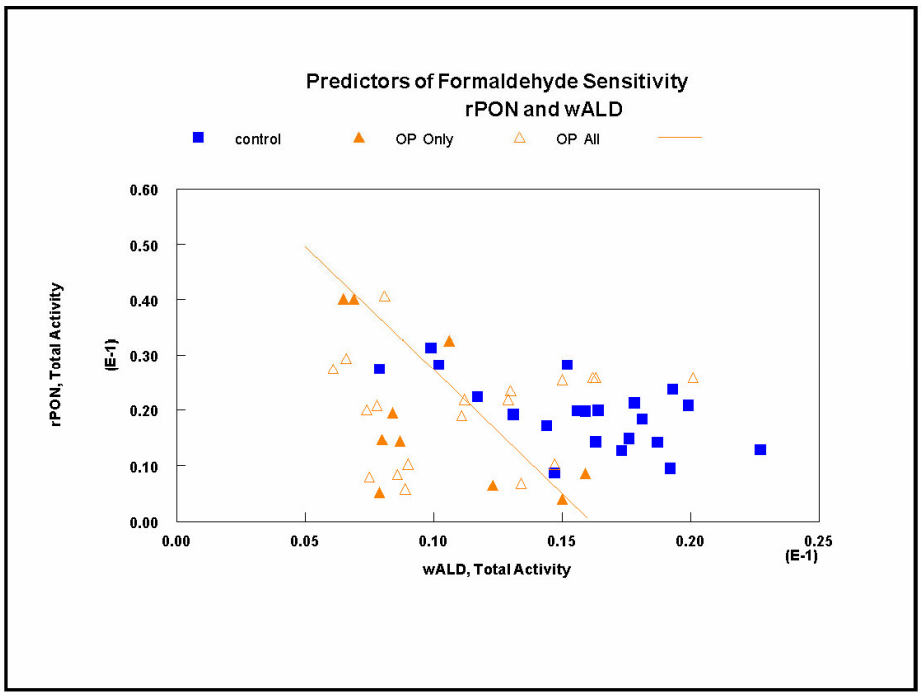
Abreviations

- Chi Alcohol Dehydrogenase: xADH
- Aldehyde Dehydrogenase: ALD
- Paraoxonase: PON
- Arylesterase: AE
- Red blood cell: r
- White blood cell: w
- Serum: s
- Organophosphate: OP

Gender Dependence of Activity




Predictors of Organophosphate Sensitivity
rAE and wALD
Control
OP Only
OP all
rAE (Total Activity)
(E-1)
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.00
0.05
0.10
0.15
0.20
0.25
0.30
(E-1)
wALD (Total Activity)


Predictors of Formaldehyde Sensitivity
rPON and wALD
control
OP Only
OP All
rPON, Total Activity
(E-1)
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.00
0.05
0.10
0.15
0.20
0.25
(E-1)
wALD, Total Activity

Biomarkers for Chemical Sensitivity

- NO age dependence
- NO gender dependence
- Ethnic dependence – little data
- BIOCHEMICAL MARKERS FOR ORGANOPHOSPHATE AND FORMALDEHYDE SENSITIVITY