Research Advisory Committee on Gulf War Veterans' Illnesses

June 16-17, 2003 Committee Meeting Minutes

U.S. Department of Veterans Affairs
810 Vermont Avenue, NW; Room 230
Washington, DC

DEPARTMENT of VETERANS AFFAIRS

Research Advisory Committee on Gulf War Veterans' Illnesses
VA Eastern Kansas Healthcare System (T-GW)
2200 S.W. Gage Blvd. Topeka, KS 66622

I hereby certify the following minutes as being an accurate record of what transpired at the June 16-17, 2003, meeting of the Research Advisory Committee on Gulf War Veterans' Illnesses.

_____

/signed/
James H. Binns,
Chairman
Research Advisory Committee on Gulf War Veterans' Illnesses

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Attendance Record

Members of the Committee
James H. Binns, Chairman
Nicola Cherry
Beatrice Golomb
Joel Graves
Robert W. Haley
Marguerite Knox
William J. Meggs
Pierre J. Pellier
Steve Robinson
Steve Smithson
Lea Steele

Consultant to the Committee
Jack Melling

Designated Federal Official
Laura O'Shea

Guest Speakers
Rogene Henderson
Michael Kilpatrick
Keith Rhodes
Antonio Sastre
Jennifer Vasterling
John Vogel
Roberta White

Abbreviations

AMS	Accelerator mass spectrometry
AChE	Acetylcholinesterase
BChE	Butyrylcholinesterase
CNS	Central nervous system
CO	Carbon monoxide
DoD	U.S. Department of Defense
DFP	Diisopropylfluorophosphate
GWI	Gulf War illnesses
IOM	Institute of Medicine
LLNL	Lawrence Livermore National Laboratories
MCS	Multiple chemical sensitivity
MRI	Midwest Research Institute
PON	Paraoxonase
PTN	Parathion
PER	Permethrin
PTSD	Post-traumatic stress disorder
PB	Pyridostigmine bromide
VA	U.S. Department of Veterans Affairs
VABHS	VA Boston Healthcare system

Meeting Agenda

Research Advisory Committee on Gulf War Veterans' Illnesses
Committee Meeting, June 16-17, 2003

June 16, 2003
Department of Veterans Affairs
810 Vermont Avenue, NW
Washington, DC
Room 230

8:30 a.m.	Welcome	Mr. James Binns
8:40 a.m.	Research Presentation	Antonio Sastre, Ph.D. Midwest Research Institute Kansas City, MO
10:10 a.m.	Discussion	
10:45 a.m.	Break	
11:00 a.m.	Research Presentation and Discussion	Roberta White, Ph.D., Boston Environmental Hazards Center, Boston, VA Healthcare System Medical Center, Boston, MA
11:30 a.m.	Research Presentation	John Vogel, Ph.D. Lawrence Livermore National Laboratories, Lawrence, CA
12.15 p.m.	Discussion	
12:45 p.m.	Lunch	
1:45 p.m.	Research Presentation	Rogene Henderson, Ph.D. Lovelace Respiratory Research Institute Albuquerque, NM
2:30 p.m.	Discussion	
3:00 p.m.	Summary Discussion	
3:30 p.m.	Break	
3:45 p.m.	VA Research Update	Nelda Wray, M.D. Chief R&D Officer, Department of Veterans Affairs
4:15 p.m.	Ongoing Research	Jennifer Vasterling, Ph.D. South Central MIRECC VAMC, New Orleans, LA
4:45 p.m.	Public Comments	
5:15 p.m.	Adjourn	

Tuesday, June 17, 2003
811 Vermont Ave.
Room 819

8:30 a.m.	Committee Staff Update	Dr. Beatrice Golomb; Dr. Lea Steele
8:45 a.m.	New Research Update	Dr. Beatrice Golomb
9:30 a.m.	GWVIS Update	Mr. Steven Robinson
9:45 a.m.	Public Affairs	Mr. Jeffrey Phillips, DASD
10:00 a.m.	Break	
10:15 a.m.	Presentations on Plume Modeling	Suchil Sharma, Ph.D., GAO Michael Kilpatrick, M.D., DOD
	Discussion	
11:30 a.m.	Lunch	
12:30 p.m.	Recommendations Discussion	
2:30 p.m.	Break	
2:45 p.m.	Work Plan	
3:15 p.m.	Public Comments	
3:45 p.m.	Adjourn	

Overview - June 16, 2003

Chairman James Binns opened the meeting with thanks to the presenters and committee members for the work that went into preparing the meeting. Mr. Binns introduced Dr. Antonio Sastre.

Physiological and Genetic Aspects of Autonomic Dysfunction in Gulf War Veterans.

Antonio Sastre, PhD
Chief Life Scientist, Midwest Research Institute (MRI), Kansas City, MO

Dr. Sastre presented for the first time results of his Department of Defense-sponsored study encompassing tests of autonomic nervous system function in ill veterans compared to well counterparts. Some scientists had previously suggested that Gulf War illnesses (GWI) may be neurological in nature, but studies suggesting neurological problem had often relied on symptoms reported by the veterans themselves. Because ill veterans tested as normal on routine neurological tests, some scientists have pointed to the absence of "objective evidence" of neurological dysfunction.

Dr. Sastre stated that his research is important because it provides objective indicators of neurological dysfunction in ill Gulf War veterans and potentially provides a physiological basis for many of the symptoms associated with GWI. He discussed in detail the importance of the study design and methods used. (See Appendix A – Presentation 1.)

Dr. Haley of the Committee pronounced it an excellent example of how to conduct research. He noted the importance of basing the research on a previous epidemiological study, the Kansas study, so that this expensive process did not have to be repeated, of choosing subjects with defined problems rather than testing random veterans who happened to come in for help, and of using a multidisciplinary team to pick the correct tests to perform. He indicated that PON-1 genetic variability may prove to be more important than BChE.

Dr. Pellier commented that the study was very important because it establishes at least one physiological basis for GWI.

Mr. Binns asked Dr. Sastre whether his findings suggest any promising treatments or future research topics. Dr. Sastre responded that discussion of treatments would be speculative now. Follow-on research direction was clear, however. It is necessary to assess where in the autonomic nervous system "loop" the problem is occurring. For example, take the case of the response in heart rate and blood pressure that is supposed to occur when a person stands up. Is the brain receiving the right sensory data? Is the information being conveyed to the brain correctly, but is the brain interpreting the information or processing it incorrectly? Is the brain processing correctly, but are the commands being sent to the heart the correct ones? Or are all the brain commands correct, but is the problem in the heart or in the arterioles? Understanding the specific nature of the ANS pathology requires studies that can "open the loop", as physiologists might say, to identify each ANS component individually. These studies can be performed using well-recognized FDA-approved procedures.

A member of the Committee asked if a veteran failed several tests of autonomic function, might it then be possible to conclude that the veteran was autonomically impaired? Which tests would be most instructive if you could just do a few tests on a large number of veterans?

Dr. Sastre said that if only one test could be given, it would be the upright tilt test. It produced the most information on differences between ill and healthy veterans. But it would be necessary to do other tests, too.

Mr. Binns introduced Dr. John Vogel.

Effects of Exposure to Multiple Chemicals at Low Dose *in vivo*: Allowing Physiology into Toxicology

John S. Vogel, PhD
Senior Research Scientist, Lawrence Livermore National Laboratory (LLNL),
University of California

Dr Vogel's presentation covered two main areas: 1) a study on low levels of pesticide exposure, and 2) technology that can be applied in studying metabolic underpinnings of diseases or syndromes. (See Appendix A – Presentation 2.) He emphasized that interactions of compounds at extremely small concentrations were physiological but were observable only in whole animals (as opposed to *in vitro* studies). He also suggested that the types of technology being utilized at Lawrence Livermore to detect these interactions may be useful in understanding Gulf War illnesses, and that metabolomic-type approaches could provide hypotheses relating to pathophysiolological processes underlying these conditions.

In response to questions by the Committee, Dr. Vogel elaborated that metabolomics could be used to distinguish compounds found in pre- and post-deployment blood samples. While it might be too late to identify evidence of the exposures themselves, one might be able to detect effects of previous exposures.

Mr. Robinson noted that samples of blood from nine hundred Marines were taken and stored before and after the Gulf War, and that many of these Marines would still be alive.

Dr. Sastre observed that metabolomics can be difficult to apply.

Mr. Binns asked how relevant the pesticide exposures used in the study was to real-world conditions. Dr. Vogel said that the level was exceedingly relevant, the equivalent of pesticide exposure that occurs when a human ingests a commercially grown apple.

Chairman Binns introduced Dr. Roberta White.

Effects of Pyridostigmine Bromide and PTSD on Neuropsychological Function in GW Veterans

Roberta White, PhD
Boston VA Healthcare System Medical Center – Environmental Hazards Center

Dr. White presented results of a study conducted at VA's Boson Environmental Hazards Research Center on the role of pyridostigmine bromide and PTSD on neuropsychological function in treatment-seeking Gulf War veterans. (See Appendix A – Presentation 3.) The results of the study indicated that Gulf War veterans performed worse than nondeployed veterans on measures assessing attention, motor and visuomotor skills, visual memory, and mood and motivation. The study also indicated that: 1) PTSD diagnosis was significantly associated with mood indices, but not significantly associated with cognitive functioning; 2) self-reported PB use was significantly associated with executive system functioning; and 3) there were no interactive effects of PB use and a diagnosis of PTSD in this group of veterans.

In response to Committee members' questions, Dr. White discussed follow-up studies of the Ft. Devens cohort, issues concerning treatment-seekers vs. non-treatment seekers, vulnerability/risk factors and future directions for research. She reported that when veterans in her study were followed up, they perceived their symptoms as worsening over time, and that they performed worse on neuropsychological tests.

Chairman Binns introduced Dr. Rogene Henderson.

Effects of Inhalation Exposure to Low Levels of Sarin in Fischer 344 Rats.

Rogene Henderson, PhD
Lovelace Respiratory Research Institute, Albuquerque, NM

Dr. Henderson presented results of three studies relating to neurological and immunological effects of subclinical exposures to inhaled sarin, conducted at Lovelace Respiratory Research Institute. (See Appendix A – Presentation 4.)

Following Dr. Henderson's presentation, she was asked what markers might be looked for in ill veterans. She said that sarin induces the expression of cytokines in the brain. She also noted that some compounds can be transferred from the olfactory bulb directly into the brain.

Mr. Binns asked what follow-on research Dr. Henderson foresaw doing in response to these findings. Dr. Henderson replied that there was a great deal they should be doing, but that there was presently no funding to follow up.

Mr. Robinson said that there was $50 million for research in the Assistant Secretary for Health Affairs budget at the Department of Defense that is not being spent.

Mr. Binns asked about possible treatment implications of this research. Dr. Henderson replied that it provided basic information that might, with additional research, ultimately lead to identification of treatments.

Mr. Binns opened the discussion to include all research studies presented that day.

Dr. Haley speculated as to whether Gulf War illness could simply be a permanent case of reduced muscarinic receptors.

Dr. Melling noted that Dr. Henderson's study together with Dr. Sastre's study provided very convincing evidence of the reality of Gulf War illness and the involvement of the autonomic nervous system.

Dr. Meggs noted that research presented at a Japanese meeting earlier in the year had shown long-standing cognitive deficits after organophosphate exposures, in situations where there was no stress present.

Dr. Haley noted that it was critical to do follow-up studies. It might just be necessary to stimulate reactivation of the muscarinic receptors. He indicated he had not seen a loss of brain cells in his research.

Dr. Meggs noted a disturbing progression of the illness. Research should look at the animals over time, and try to determine how to arrest the process.

Dr. Sastre stated that PTSD could affect the autonomic nervous system. He cautioned that a binding site is not necessarily coupled to a G-protein or that they might have been coupled prior to the insult.

Dr. White said that neuroimaging research has shown structural differences in subgroups of ill Gulf veterans. A study supported by the CDC showed a lower volume of white matter in veterans with a high level of complaints. Usually, one would expect to see lesions, but not lower volume. She also suggested there is a need to incorporate information on genetic risk factors. Gulf War illness is a very complex issue and she indicated an interest in doing additional studies on the Ft. Devens cohort.

Dr. Sastre observed that earlier studies of PB showed very low levels of side effects. It was also tested with heat. However, the test conditions were nothing that resembled battlefield conditions or where exposures like pesticides were present.

Mr. Graves said that he experienced no side effects from PB at the time he took the pills. Would it be possible to detect effects now?

Dr. Vogel said that the tools they have at LLNL could help with quantification of these studies. They have done AMS work with humans, and can also do studies on brain fluids.

Mr. Graves observed that the Committee is narrowing down what is happening in Gulf War illness.

Relationship between Illnesses in Gulf War Veterans and Acetylcholinesterase Levels

Dr. Nelda Wray, MD, MPH
Chief Research and Development Officer, Department of Veterans Affairs

Dr. Nelda Wray provided a research update on a collaborative study between VA and Dr. Hermona Soreq investigating acetylcholinesterase (AChE) levels in Gulf War veterans. (See Appendix A – Presentation 5.) The study had been undertaken and fast-tracked in response to a suggestion by the Committee following a presentation made by Dr. Soreq at the February, 2003, Committee meeting. Dr. Wray anticipated that preliminary results of the study would be available the coming summer.

In discussion following the report, a Committee member reminded Dr. Wray that the Committee's interest in Dr. Soreq's work was to determine whether an atypical variant form of AChE, AChE-R, was present in abnormally high levels in symptomatic Gulf War veterans, as opposed to AChE levels.

Prospective Assessment of Neurocognition in Future Gulf-Deployed and Gulf-Nondeployed Military Personnel

Jennifer Vasterling, PhD
South Central MIRECC VA Medical Center, New Orleans, LA

Dr. Vasterling provided information about a study recently initiated at the VA Medical Center in New Orleans that will provide assessment of neurocognitive function among military personnel before and after deployment to Iraq, and in a comparison group of nondeployed veterans. (See Appendix A - Presentation 6.) Discussion followed concerning the importance of prospective data collection.

Public Comments

Mr. Binns invited members of the public who had signed in to address the Committee.

Mr. Dan Fahey addressed the subject of depleted uranium (DU), a topic he has been following since 1993. He expressed concern that government representatives had lied about the occurrence of lymphoma identified in a Gulf veteran who had embedded depleted uranium fragments. He also voiced concern that government was only studying a small number of cases of DU-exposed veterans. He recommended a study of the 900 veterans identified by the Department of Defense as having been highly exposed to DU. He also said it is likely that some personnel in the current Iraq war are being exposed to DU but that the VA brochure does not mention DU or the availability of DU testing in its notification to veterans.

Ms. Denise Nichols made several recommendations to the Committee.

1. Keep up the website: schedules, Power Point presentations from meetings, etc.
2. VA clinicians need access to the website, to the Secretary's videotape.
3. Diagnostic data should be collected from VA: blood flow, heart rate. What are the rates of ICD-9 codes?
4. The Committee should consider the work of leading clinicians, not just researchers, including Dr. William Rae and Dr. David Berg.
5. Intravenous drips of glutathione has proved helpful, also CoQ10.
6. Look at magnesium levels, other data not considered by VA clinicians.

Mr. Albert Donnay noted that VA paid no attention to multiple chemical sensitivity (MCS), despite the condition being identified by VA as a major illness among Gulf War veterans in Dr. Kang's 1998 study. He indicated that the study had shown that 15% of ill veterans have MCS vs. 5% of controls. By contrast, VA did look for chronic fatigue syndrome and fibromyalgia in ill Gulf War veterans, although fewer veterans reported these conditions.

Mr. Donnay discussed carbon monoxide (CO) as a possible cause of Gulf War illness. He said that when he has checked the breath of ill veterans, all have had elevated levels of CO. He stated that oxygen therapy is an effective treatment for CO. He said that normabaric oxygen works fine, as documented in German research. He had been working with six civilian doctors using oxygen to treat MCS. Dr. Rae uses oxygen for 19 days, which is not long enough in these doctors' experience. Mr. Donnay noted that Dr. White has published on MCS in Gulf War veterans and CO poisoning in civilians. He submitted a written statement. (See Appendix B – Public Submission 1.)

Mr. Binns adjourned the first day of the meeting.

Overview - June 17, 2003

Committee Staff Update

Chairman Binns opened the meeting by giving the Committee a staffing update. The roles of Dr. Lea Steele and Dr. Beatrice Golomb were discussed. Dr. Golomb will now be concentrating on the science and will no longer be responsible for committee staff administration. Dr. Steele will be joining the staff as its scientific director, providing full time leadership for the committee staff beginning in September. The committee staff operation is moving from San Diego to Topeka. In comparison to the current operation, three times the level of scientific expertise will now be available to interface with the scientific community.

Presentation by Secretary of Veterans Affairs, Anthony J. Principi

Secretary of Veterans Affairs Anthony J. Principi addressed the Committee. He expressed his frustration with the current level of understanding of Gulf War illnesses, and indicated he is hopeful that the Committee will make important contributions in this area. He stated that he has asked the Institutes of Medicine to study further the long-term effects of low-level exposures to sarin nerve gas.

Presentation by Deputy Secretary for Public Affairs, Jeffrey Phillips

Deputy Secretary for Public Affairs, Jeffrey Phillips, played Secretary Principi's videotaped appeal to VA researchers to submit proposals for studies to study Gulf War illnesses. For FY2004, twice the level of VA funding would be available for this type of research, as compared to any previous year. Mr. Phillips said the tape would be widely publicized and disseminated within the VA community. All physicians and researchers would have the opportunity to view the tape using streaming video on the web, VA's satellite television system, or hard copies distributed to VA medical centers.

General Accounting Office (GAO) Report on Plume Modeling of Khamisiyah Exposures

Keith Rhodes
U.S. General Accounting Office

Mr. Rhodes presented a preliminary report on GAO's ongoing assessment of DoD plume modeling of the dispersion of chemical agents resulting from the U.S. demolition of Iraqi chemical munitions at Khamisiyah, Iraq, in March of 1991. The GAO investigation determined that several aspects of DOD's most recent modeling effort had neglected to consider important factors or had been based on unsupported assumptions and therefore could not be supported as being reliable or definitive. Mr. Rhodes indicated that in light of this information, it was not possible to know with any accuracy the total number or which individuals were or were not exposed to chemical agents as a result of the Khamisiyah demolitions. He also suggested that sufficient data are not available to produce an adequate model, and that epidemiologic studies that had relied on plume modeling efforts were not likely to have produced valid results.

Dr. Michael Kilpatrick, Deputy Director of the Deployment Health Support Directorate at the Department of Defense, responded that since the GAO findings were preliminary and DoD had not had an opportunity to review the final report, he could not comment on the GAO presentation. He described the modeling choices made by DoD analysts with respect to the Khamisiyah plume.

There was a discussion among Committee members about whether the Committee should compose a recommendation to the Secretary in regard to problems that may have resulted from VA researchers who had published studies, which relied on Khamisiyah modeling data that may have been inaccurate, and therefore led to erroneous conclusions. It was ultimately agreed to wait until the GAO report is available in final form before making any related recommendations.

Review of recent (and recently identified) Gulf War research

Dr. Golomb gave a presentation summarizing new Gulf War illness-related research published since the last meeting. (See Appendix A – Presentation 7.)

Birth Defects Research

Dr. Golomb presented a report on research on the prevalence of birth defects in children of Gulf War veterans. (See Appendix A - Presentation 8.)

Discussion of Recommendations for Committee Report

Mr. Binns stated that feedback from Committee members on the draft report indicated that it required further work. It was essential that the report provide a clear statement of the relevant science. This effort would require taking advantage of the new Committee staff resources, which would not be in place until September. Meanwhile, it was also desirable for the Committee quickly to get draft recommendations in front of VA decision-makers who will be considering FY2005 budgeting decisions and FY2004 research funding decisions. A draft executive summary including the recommendations should be completed by the staff and provided to the Secretary in the next six-to-eight weeks. The Committee will be asked to review and comment on the preparation of the draft executive summary. The full report is expected to be available early in 2004.

Mr. Binns said that recommendation topics to be further considered today include birth defects, vaccines, and research priorities. He presented the draft recommendations on birth defects from the current draft of the report, as follows:

1) Follow-up analysis and research should determine rates of birth defects in ill Gulf War veterans (not just all Gulf War veterans), and in subgroups of ill veterans

2) Continued surveillance for birth defects in children of Gulf War veterans should be conducted. Questions on children's health should be incorporated in surveys of Gulf War veterans.

3) Such research should look at the health of children of all ages, not just up to one year, including learning and behavioral disorders.

4) VA should closely follow and consult with the investigators of the ongoing British study of birth defects in Gulf War veterans, which appears to incorporate these recommendations.

Dr. Cherry commented that the risk of birth defects in the studies reviewed by Dr. Golomb was very low. Dr. Golomb said that there was a need, however, to look at older children for health problems including behavioral disorders, which has not been done.

Dr. Pellier said that there was generally less risk in having children earlier, before the chromosomal risk goes up. Dr. Haley also noted the risk was low. Dr. Pellier observed that there are toxicology studies for acetylcholinesterase-inhibiting drugs.

Dr. Golomb stated that there were many articles that suggested that these types of problems were associated with acetylcholinesterase inhibitors.

Mr. Robinson suggested that the Committee hear from Ms. Betty Mekdici, who maintains a birth defects registry.

Dr. Cherry suggested inviting the British researchers who are studying birth defects in Gulf War veterans to present their results before the Committee.

Dr. Haley noted that Dr. Kang's study is currently validating the existence of reported birth defects through a review of actual medical records. There may be opportunity to get additional analysis from this study population. Dr. Kang's data should be made available to other researchers. Results of the additional analyses from the Kang study should be made available to the public. Mr. Graves suggested that the birth defect data assembled by Dr. Kang should be analyzed by unit.

Dr. Melling cautioned that the language of the recommendation with respect to the risk for birth defects should not be unduly alarming, given the reported research results. Dr. Cherry agreed. There were no indications, based on existing studies, that there was a reason for veterans to not have children because of concerns related to an increased risk of birth defects.

Dr. Meggs said that future studies should look for increased levels of illnesses in veterans' offspring.

It was agreed to incorporate the consensus of the Committee's comments in the recommendations, for further review by the Committee.

Mr. Binns presented the draft recommendations pertaining to vaccines. Research should compare the health experiences of military units shipped the anthrax vaccine and units not shipped the anthrax vaccine on topics such as:

- Who is sick
- Cytokine profiles
- Any acute reaction at the time of vaccination
- Squalene antibodies, including studies of ill veterans versus well veterans

The recommendations were discussed and commented upon as follows:

- Similar follow-up studies should be conducted of veterans who received the anthrax vaccination at Dover Air Force Base, as well as at Tripler, and in Korea. Details of existing epidemiological studies related to use of the anthrax vaccine should be made public. There should be a randomized trial of the new anthrax vaccine and health questions pertinent to symptoms affecting Gulf War veterans should be added to vaccine studies currently being planned by federal agencies.

- Comprehensive records of all vaccinations administered by the military should be kept, and active surveillance should be conducted for both short-term and long-term effects, including outcome measures sensitive to cognitive changes.

- While research regarding new vaccines is important for the public good and to the health of future military personnel, it is less important to Gulf War illnesses research than, for example, studies into neurological mechanisms involved in Gulf War illnesses. Funding for studies of the effects of vaccines not used during the Gulf War should therefore not come from the "up to $20 million" available in FY2004. An appropriate source for funding in this area might more rightly come from unspent medical research funds believed to be available at DoD.

- The possibility of studying possible adverse effects of receiving multiple simultaneous vaccines was discussed. The Committee would look at results reported in the Australian study on this topic. Researchers could collect the blood of returning veterans from Iraq and study markers of immune status. Shot records are now computerized.

A revised draft of the recommendations related to vaccines, reflecting Committee members' above comments, would be prepared for review by the Committee.

Mr. Binns stated that the next topic to be discussed was research priorities and objectives.

There was strong agreement among Committee members that Gulf War illnesses research should be given priority among projects to be funded under the Secretary's special FY2004 deployment health research initiative. In comparison with any other recent deployment, there were more deployed to the Gulf War, more became ill as a result of deployment, and the need for additional research was great because the mechanisms and treatments for these illnesses are not understood. This research is also potentially of great relevance for future deployments. Moreover, as a result of recent research, promising opportunities for research breakthroughs have been identified, and scientists could take advantage of these opportunities. Therefore, it was reasonable that the lion's share of the $20 million to be allocated for Gulf War illnesses research. A similar or higher amount should be budgeted for FY2005. The Committee also encouraged other initiatives that would benefit ill Gulf War veterans.

Within Gulf War illnesses research, treatments research should be given the highest priority. Only VA has the patient population needed to study treatments. The Treatment Development Center(s) conceptualized by the Committee should be established and funded at $2.5 million in FY 2004 and $4.0 million in FY2005. Goals should be set to have eight treatments under data development by the end of FY2004 and two treatments in clinical trials. Small-scale trials should be used to avoid spending all funds on one trial.

The federal government, including VA, should prioritize the use of research resources for studies in areas that show the most promise, emphasizing research topics in which breakthroughs had been made and on scientists who had developed promising preliminary work. Examples of topics to pursue include markers, neurological mechanisms, effects of acetylcholinesterase inhibitors, and genetic linkages to Gulf War illnesses. Examples of laboratories to support include those at the U.S. Army Institute of Chemical Defense, Lovelace Respiratory Research Institute, and Lawrence Livermore National Laboratory.

An example of a topic not to pursue is stress. While physiological stress and psychological factors are important subjects for research as relate to deployment health in general, and some studies suggest that stress can increase the effects of toxic exposures, research proposals into the illnesses of Gulf War

veterans should not be funded if their primary focus is on psychological stress or other psychological causes of illness.

Research should pursue new technologies for identifying effects of low-level chemical exposures and detecting physiological abnormalities underlying Gulf War illnesses. Examples might include technologies such has those described by Dr. Vogel at Lawrence Livermore, Dr. Soreq in Jerusalem, and proteomics and/or genomics research.

Recent research emerging from the study of Gulf War illnesses also has important implications for chemical defense. There is currently a $1.6 billion annual investment at NIH in medical defenses against bioterrorism. There should be a comparable effort to develop safe and effective treatments against chemical agents.

Public Comments

Ms. Denise Nichols supported the idea that additional study should be done of birth defects. She also endorsed the proposed treatment research recommendations. She advocated posting the Committee's work plan on its website and otherwise using the website to make information about research and the activities of the Committee more open.

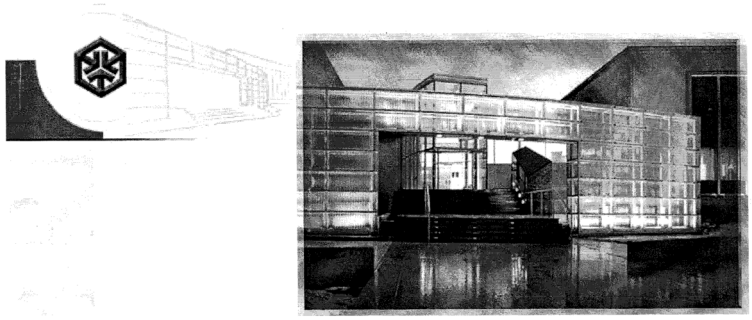
Dr. Rich Van Konynenburg noted that he had attended the last meeting of the Committee and had submitted a paper on the importance of glutathione. He thought this supplement had improved the health of chronic fatigue and fibromyalgia patients, although it was not a cure. It is possible to test for glutathione levels. Doctors using glutathione therapy include Paul Cheney, MD, Grace Ziem, MD, and Patricia Salvato, MD.

Mr. Scott Walker noted that he had attended the last meeting of the Committee and spoken about the increased absorption of vitamin and mineral supplements when taken in liquid form. He presented written results of a survey of ill veterans who used supplements provided by Mr. Walker. Of twenty-two summaries returned by veterans, twenty-one reported improvement. These results demonstrate that nutrition should be considered as a treatment for Gulf War illnesses.

Mr. Binns adjourned the meeting.

Appendix A

Presentation 1 – Antonio Sastre



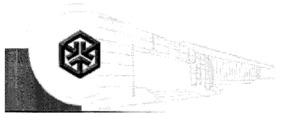
Physiological and Genetic Aspects of Autonomic Dysfunction in Gulf War Veterans

Antonio Sastre, Ph.D.
Senior Advisor, Life Sciences, Midwest Research Institute

Presentation to the U.S. Department of Veterans Affairs
Research Advisory Committee on Gulf War Veterans' Illnesses
June 16, 2003

Introduction and Terminology

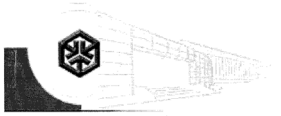
- **Physiological and Genetic Aspects of Autonomic Dysfunction in Gulf War Veterans**
  - Physiological: referring to the objective, quantitative measure of function of organ systems of the body
  - Genetic: dealing with inherited components of DNA which are eventually transcribed into proteins
  - Autonomic: the involuntary, autonomic nervous system (ANS), as distinct from the conscious or somatic (voluntary) nervous system
  - Dysfunction: meaning a failure to function within the range considered consistent with health



Overview of the Project

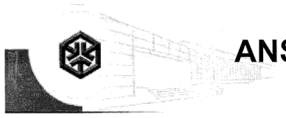
Two integrated studies (total of 387 veterans) provided evidence that:

- Veterans suffering from Gulf War Illness (GWI) differ from controls on a broad range of objective physiological measures of ANS function.
- In addition to case/control differences, ANS measures also differ significantly by BChE genotype.
- Genotype interacts with case status: ANS responses are different in GWI cases vs. controls depending on genotype.
- Genotype interacts with exposures: Veterans with variant BChE genotypes appear to be at substantially increased risk for GWI, but only if they experienced certain exposures.



General Introduction

- The ability of the body to respond to environmental and internal perturbations depends on non-conscious input and feedback from the autonomic nervous system (ANS).
- Perturbations include:
  - Gravity
  - Temperature
  - Exercise
  - Stressors (internal and external, including exposures)
- The ANS is composed of the sympathetic (mostly adrenergic) and parasympathetic (cholinergic) branches.
- These branches have generally opposing actions in target tissues, but often with an asymmetric distribution: the parasympathetic is much more selective.



ANS Feedback and Integration

- The ANS receives feedback information from multiple sensors - intra-arterial pressure sensors, pulmonary stretch receptors, skin thermal sensors, etc.
- Feedback information is sent via *afferent* nerves to pontine and medullary centers, where it is integrated and in some cases modulated by descending input.
- *Efferent* output then goes via cranial, vagus or sacral nerves, or through the intermediolateral columns of the spinal cord, the sympathetic ganglia, and the sympathetic nerves to the target organs.

Everyday Example of ANS Integration

- When rising from a supine (horizontal) or sitting position to standing, we take for granted that we are ***not*** going to faint.
- The following complex sequence of events occurs ***without any conscious awareness***:
  - Gravity immediately pulls blood down to our feet
  - Lowered blood pressure is sensed in aortic and carotid baroreceptors and info conveyed to pons and medulla
  - Increased sympathetic firing to resistance arterioles increases mean arterial pressure, and to lower leg veins to help increase venous return
  - Increased efferent sympathetic firing to SA node increases heart rate; increased firing to ventricles increases force of contraction; both combine to increase cardiac output

Why is ANS Function Important?

- Autonomic function, such as that described for standing up, is involved in multiple essential body processes.
- We take those body processes, and their autonomic regulation, for granted. Examples:
  - Pupillary adjustment to changes in light intensity
  - Processing and digestion of food
  - Sugar and fat metabolism
  - Thermoregulation
  - Blood pressure control
  - Aspects of reproductive function
- We only become aware of the importance of autonomic processes when there is some degree of autonomic dysfunction.

Complexity of What Can Go Wrong (cf. Kapoor, JAMA, 5/7/2003)

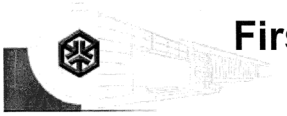
- Neurally-mediated syncope (fainting) induced by tilt-testing, can occur by at least 3 kinds of distinct mechanisms, which must be differentiated:
  - Neurally-mediated sudden hypotension, bradycardia, or both when the volunteer is kept upright;
  - Postural orthostatic tachycardia syndrome: tachycardia upon volunteer being brought upright that persists through procedure;
  - Dysautonomia: gradual decrease in blood pressure with little or no change in heart rate during the procedure.

Optimization of Tilt Testing Protocols

- There is no standardized approach for tilt-testing across medical centers, whether in time, tilt angle, or use of vasoactive agents.
- Highly-aggressive protocols have lower specificity and may be at increased risk for false-positives.
- Our approach, after an exhaustive examination of the clinical and experimental literature, was a protocol that maximized our ability to obtain useful data and preserve specificity while eliminating discomfort for the volunteers.

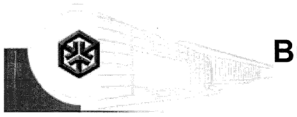
Syndromes and Illnesses

- A number of 'post-war syndromes' have been described.
- The unexplained symptoms and conditions reported by Gulf War Veterans (GWV) have been labeled 'Gulf War Syndrome' by media reports.
  - *Problem*: Review panels have concluded that there is no single, unique syndrome linked to Gulf War service.
  - *But*: Investigators using different study designs describe a fairly consistent set of symptom types and illness categories that occur at higher rates in GWV.
- Hence, we will refer to these symptom types and illness categories as Gulf War Illness (GWI).



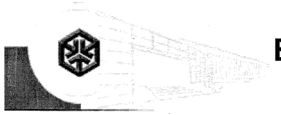
First Component of Our Hypotheses

- We hypothesized that sufferers of Gulf War Illness (GWI) i.e., cases, would differ in **ANS reactivity** from suitable controls.



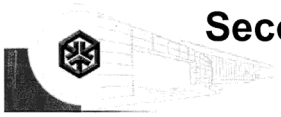
Butyrylcholinesterase (1)

- Butyrylcholinesterase (BChE) is a soluble, circulating and tissue enzyme that hydrolyzes acetylcholine (ACh) and several other drug classes; it is believed to serve as a 'scavenger' for those drug classes.
- BChE exhibits considerable genetic variation in humans.
- BChE's genetic variation is of importance in clinical medicine; carriers of some mutations will exhibit considerable morbidity upon exposure to paralytic drugs used during surgery.



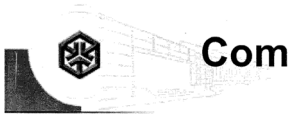
Butyrylcholinesterase (2)

- The long-held belief that BChE does not play a role in normal cholinergic physiology is under re-examination as a result of Lockridge's results with the AChE(-/-) double knock-out mouse.
- Soreq and others have conjectured non-enzymatic roles for BChE in mammals and other higher vertebrates.
- At the time we began our study there were preliminary data suggesting a link between carriers of low-velocity BChE genetic variants and GWI.



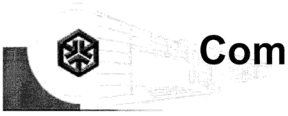
Second Component of Our Hypotheses

- We hypothesized that our data would support the conjectured link between **carriers of low-velocity BChE genetic variants** and **GWI**.
- We hypothesized that ANS reactivity would also differ depending on the **genotype of BChE.**
- We further hypothesized that **case status and BChE genotype would interact**, as revealed in response to ANS stressors.



Complementary Studies (1)

- To test the various aspects of our hypotheses, we designed two complementary, interdependent studies:
- *Study 1* was designed to
  - Obtain BChE genetic data, demographics, exposure and epidemiologic data on a substantial number of cases and controls
  - Test whether a link exists between carriers of low-velocity BChE genetic variants and GWI, using a rigorous case definition
  - Identify a number carriers of low-velocity BChE genetic variants who could be invited to participate in physiologic tests in *Study 2*

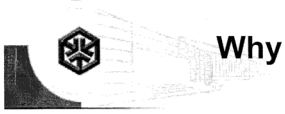


Complementary Studies (2)

- *Study 2* was designed to:
  - Examine GWV cases and controls, as well as non-deployed (PGW-era) veteran controls
    - who served with only one of two Army units
    - deployed 8/1990 – 7/1991
    - enlisted personnel only
  - Examine BChE variant carriers from *Study 1,* and determine genetics of new volunteers
  - Examine ANS normal function and reactivity through a multi-faceted *battery* of tests

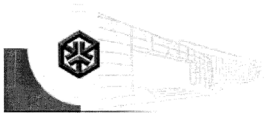
Why a ***Battery*** of ANS Tests (1) ?

- The ANS consists of numerous interacting feedback loops with distinct neuroanatomical pathways, integrative and regulatory centers, several neurotransmitters, and dozens of physical triggers and regulators.
- No one test, or small number of tests, can capture all the functions of the ANS.
- It is too easy to obtain false negative or misleading results in ANS studies through an unduly restrictive choice of tests.
- Most medical and research centers use a battery of tests customized for specific purposes.



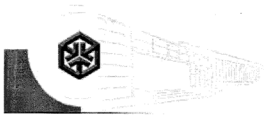
Why a ***Battery*** of ANS Tests (2) ?

- For example, based on Valsalva and temperature tests only, Sharief et al (*Neurology* ***59***:1518 [2002]) reported "…no objective abnormalities of autonomic nervous system…in symptomatic veterans." (p.1524)
- If we had only performed Valsalva maneuvers, our data would have shown no difference between case and control veterans.
- As will be seen below, a conclusion of no difference in autonomic function between cases and controls for our volunteers would have been ***erroneous***.



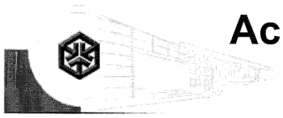
Is a Battery of Tests a 'Fishing Expedition'?

- Concerns can be legitimately be raised about multiple tests leading to spurious results.
- Due to the recognized complexity of the ANS, especially with respect to cardiovascular reflexes, ANS test batteries have been developed and guidelines issued in Consensus Statements by the American Autonomic Society and the American Academy of Neurology.
- When examining and analyzing the data, one looks for ***consistency*** and ***coherence*** of findings across tests, corrects statistically for multiple comparisons (when present), and maintains healthy skepticism.



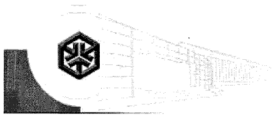
The Research Team

- All work was performed jointly with:
  - Drs. Mary R. Cook (**co-PI** at MRI; overall design and Physiology) and Mary M. Gerkovich (Statistics at MRI)
  - Dr. Lea Steele (Epidemiology; Kansas Commission on Veterans Affairs, Kansas Health Institute)
  - Dr. Oksana Lockridge (Mol. Bio. and Biochem; Eppley Institute, University of Nebraska, Omaha)
- And with the highly-skilled assistance of:
  - Dr. J. Hackman
  - Mrs. D. D. Dozier, Mr. S. Hoffman, Mrs. R. C. Peterson, Mrs. K. Whitson



Acknowledgements and Disclaimers

- All protocols were approved by the Midwest Research Institute's Institutional Review Board and by the Surgeon General of the Army's Human Subjects Research Review Board.
- This research was supported by the U.S. Army Medical Research and Materiel Command under contract USAMRAA DAMD17-00C-0018. Opinions, interpretations, conclusions and recommendations are those of the investigators and do not necessarily reflect an official position or opinion of the U.S. Army.



Study Design: ANS *Study 1*

- **Population-based**: *Random* sample of Gulf veterans living in Kansas City area (from Kansas Gulf veterans database, DOD-provided list of Missouri Gulf veterans)

  Case/Control Design: Cases met Kansas "Gulf War Illness" case definition; CDC definition also assessed

- **Final sample**: 304 Gulf War veterans: 144 cases, 160 controls

- Participants screened by telephone interview to determine study eligibility, filled out questionnaire, came to study site to provide blood sample

Study Design: ANS *Study 1*

Eligibility Criteria: both Cases and Controls:

- **Lived in Kansas City area**
- **Deployed to Persian Gulf** any time between August 1990 and July 1991
- **Never diagnosed** by physician for: diabetes, heart disease, stroke, lupus, multiple sclerosis, cancer, liver disease, conditions associated with psychosis
- **Not hospitalized** since the Gulf War for: alcohol or drug dependence, PTSD

Case Definition: Gulf War Illness (GWI)

Kansas GWI Definition

- Empirically derived from 1998 study of 2,030 Kansas Gulf War-era veterans
- Based on symptoms reported from 6 highly correlated domains, in pattern that differentiates Gulf War veterans from similar veterans who did not serve in the Gulf War
- Requires multiple or moderately severe symptoms in at least 3 of 6 domains:
  - » ***Neuro/cognitive/mood symptoms***
  - » ***Fatigue/sleep problems***
  - » ***Pain symptoms***
  - » ***Gastrointestinal symptoms***
  - » ***Respiratory symptoms***
  - » ***Skin problems***

Study 1 Methods - Biochemistry and Molecular Biology of BChE

- Phenotyping by velocity with benzoylcholine as substrate, followed by
- Inhibition profile with dibucaine, sodium fluoride, and RO 2-0683 (dimethylcarbamate of [2-hydroxy-5-phenylbenzyl]-trimethylammonium bromide)
- For ambiguous phenotypes, genotyping is performed:
  - Amplification of genomic DNA
  - Four primers for 2 PCR amplifications are created
  - A amplification creates a Mae III restriction site when the K-variant ACA codon (Thr 539) is present
  - B amplification creates a Bgl I restriction site when the GCA codon (Ala 539) is present

Study 1 Methods - Biochemistry and Molecular Biology of BChE (2)

- Not necessary to genotype samples that phenotype as heterozygous for the A variant (Asp 70->Gly) because dibucaine inhibition is extremely accurate.
- Samples that phenotype as heterozygous for the F variant were sequenced to determine which of the three reported mutations were responsible for F resistance.
- One sample was found whose inhibition values were a novel set; a single mutation was found in one allele. Codon 70 had C in place of G, changing Asp 70 (GAT) to His (CAT), nucleotide 208G->C. No other mutations were found in the coding region. The presence of the mutation was confirmed by repeating the PCR and sequencing in both directions.
- The k_{cat} value for benzoylcholine was determined by measuring Vmax and titrating the active sites with chlorpyrifos oxon.

ANS *Study 1* Results: Genotype

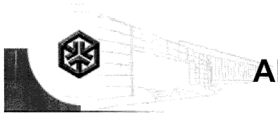
Association of GWI with BChE Genotype

BChE Genotype	% Cases (n=144)	% Controls (n=160)
U/U	62 %	62 %
U/K	28 %	29 %
K/K	5 %	4 %
U/AK	3 %	3 %
U/A	1 %	1 %
A/F	0 %	1 %
AK/F	1 %	0 %

ANS *Study 1* Results: Genotype

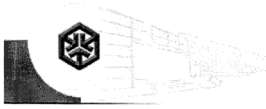
Association of GWI with Variant Status

BChE Genotype	% Cases (n=144)	% Controls (n=160)	OR (95% C.I.)
U/U and U/K	90%	91%	1.0
All other variants	10%	9%	1.12 (0.52 - 2.44)



ANS *Study 1* Results: Conclusions

1. **Overall, *no* independent association was found between BChE genotype and Gulf War illness**



ANS *Study 1* Results: Locations/Experiences in Theater

- "Exposure" information was obtained from questions asked about specific locations and experiences (as opposed to exposures) in theater
- For example, we asked: ***"Did you come into direct contact with destroyed enemy vehicles?"***
- We did **not** ask: ***"Were you exposed to depleted uranium?"***

ANS *Study 1* Results: Locations

Association of GWI with Locations in Theater

	% Cases (n=144)	% Controls (n=160)	OR (95% C.I.)
Kuwait	64 %	39 %	**2.80** (1.75 -- 4.49)
Eastern Saudi Arabia	89 %	76 %	**2.59** (1.35 – 4.96)
Iraq	46 %	32 %	**1.80** (1.12 – 2.89)
Bahrain	57 %	55 %	1.09 (0.69 – 1.73)
Northern Saudi Arabia	22 %	23 %	0.94 (0.54 – 1.64)
Western Saudi Arabia	10 %	12 %	0.81 (0.39 – 1.68)
At sea in the Persian Gulf	9 %	18%	**0.44** (0.21 – 0.90)

ANS *Study 1* Results: Exposures

Association of GWI with Experiences in Theater

	% Cases (n=144)	% Controls (n=160)	OR (95% C.I.)
Wore uniform treated with pesticides	27 %	9 %	**3.72** (1,91 – 7.21)
Took NAPP pills (Pyridostigmine Br; PB)	72 %	44 %	**3.21** (1.91 – 7.21)
Used pesticide cream/spray on skin	57 %	31 %	**2.89** (1.80 – 4.64)
Saw Iraqis/civilians badly wounded or killed	65 %	40 %	**2.71** (1.70 – 4.31)
Contact w/destroyed enemy vehicles	60 %	36 %	**2.63** (1.65 – 4.18)
Saw/contact with dead animals	54 %	34 %	**2.20** (1.38 – 3.51)
Received 1 or more shots in arm in theater	73 %	58 %	**2.00** (1.21 – 3.29)
Directly involved in ground combat	32 %	25 %	1.42 (0.86 – 2.36)
Saw living area sprayed/fogged with pesticides	22 %	17 %	1.33 (0.74 – 2.37)
Saw U.S. troops badly wounded or killed	39 %	33 %	1.31 (0.82 – 2.11)
Heard chemical alarms sound	59 %	53 %	1.31 (0.83 – 2.07)

ANS *Study 1* Results: Conclusions

1. Overall, *no* independent association of BChE was found between BChE genotype and Gulf War illness

2. **Some locations and exposures in theater *were* associated with increased GWI risk**

ANS *Study 1* Results: Interaction of Genotype with Exposures

- BChE genotype, alone, was not associated with an increased risk of GWI.
- But illness risk was higher for veterans who carried variant BChE genes who also reported several specific exposures in theater.
- For our analyses, non-variants were genotypes U/U and U/K; all others were considered "variants."

ANS *Study 1* Results: Interaction of Genotype with Exposures

Association of GWI with Exposures

	NonVariant Subjects Only OR (95% C.I.)
Directly involved in ground combat	1.18 (0.69 – 2.02)
Saw Iraqis/civilians badly wounded or killed	2.34 (1.44 – 3.80)
Contact with POWs	2.26 (1.39 - 3.67)
Frequently had < 4 hours sleep in 24 hours	1.89 (1.15 – 3.08)
Received 1 or more shots in arm in theater	1.71 (1.02 – 2.86)
Saw/contact with dead animals	1.75 (1.07 – 2.85)
Took NAPP pills (PB)	2.68 (1.62 – 4.44)

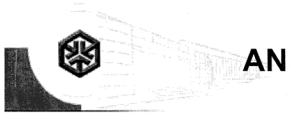
[NOTE: Associations in non-variant veterans are weaker than in the study population as a whole]

ANS *Study 1* Results

Interaction of Genotype with Exposures

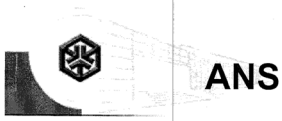
	Non-Variant Subjects Only (n= 276) OR (95% C.I.)	BChE Variant Subjects Only (n = 28) OR (95% C.I.)
Directly involved in ground combat	1.18 (0.69 – 2.02)	8.00 (1.28 – 50.04)
Saw Iraqis/civilians badly wounded or killed	2.34 (1.44 – 3.80)	15.00 (2.26 – 99.64)
Contact with POWs	2.26 (1.39 - 3.67)	15.00 (2.26 – 99.64)
Frequently had < 4 hours sleep in 24 hours	1.89 (1.15 – 3.08)	18.33 (2.52 -133.26)
Received 1 or more shots in arm in theater	1.71 (1.02 – 2.86)	23.40* (1.15 -475.56)
Saw/contact with dead animals	1.75 (1.07 – 2.85)	36.00 (4.33 -299.02)
Took NAPP pills (PB)	2.68 (1.62 – 4.44)	40.00 (3.58 -447.04)

*No unexposed cases: OR estimate added .5 to 0 cell



ANS *Study 1* Results: Conclusions

1. Overall, *no* independent association of BChE genotype with Gulf War illness
2. Some locations and exposures in theater *were* associated with increased GWI risk
3. **For those with BChE variant genotypes, some exposures appear to be linked to a large increase in risk of illness.**



ANS *Study 1*: Remaining Analyses

1. Identify exposure "clusters," e.g. multiple exposures occurring in the same veterans, and possible links with locations in theater.
2. Assess confounding due to co-occurrence of multiple exposures with one another, to determine independent associations of exposures with illness and with BChE genotype.

Study Design: ANS *Study 2*

- **Case/Control Design**: Cases met Kansas "Gulf War Illness" case definition; controls did not
- **Study 2 case/control sample**:
  - » 49 Gulf War veteran cases
  - » 19 Gulf War veteran controls
  - » 23 non-deployed (PGW-era) veteran controls
- **Variant Sample**:
  - » 23 BChE variant subjects from Study 1
- Participants screened by telephone interview to determine study eligibility, filled out questionnaire, came to study site for 3.5-hour battery of physiological testing, and provided blood samples

Study Design: ANS *Study 2*

Study 2 veterans much more homogenous re: locations and experiences in theater than Study 1 veterans

Eligibility Criteria: Study 2 Case/Control Sample

- Deployed some time between August 1990-July 1991 (PGW subjects)
- Served with one of two units (PGW subjects)
- Enlisted only
- Army only
- 10% female
- Never diagnosed by physician for: diabetes, heart disease, stroke, lupus, multiple sclerosis, cancer, liver disease, conditions associated with psychosis
- Not hospitalized since the Gulf War for: alcohol or drug dependence, PTSD

Biochemistry and Molecular Biology Methods

- Same as Study 1

Study Design - *Study 2* ANS Battery - Direct Measures

- Endpoints that were measured and digitized continuously at 256 Hz:
  - ECG (Lead II)
  - Non-invasive radial arterial tonometry (Colin)
  - Respiration (strain gauge)
- Endpoints measured at the end of the test session digitized at 1,024 Hz:
  - Quantitative electromyography from the *orbicularis oculi* for amplitude of the startle reflex and its inhibition by a pre-pulse (PPI)

Study Design - *Study 2* ANS Battery - Derived Measures (1)

- From Lead II ECG:
  - Mean Heart Rate (MHR)
  - Fourier Spectral Heart Rate Variability (S-HRV)
    - Total Power
    - Absolute and Percent Low Frequency Power
    - Absolute and Percent High Frequency Power
    - Low to High Frequency Power Ratio
  - Time-Domain Heart Rate Variability (TD-HRV)
    - SDNN
    - rMSSD
    - SDSD
    - %NN

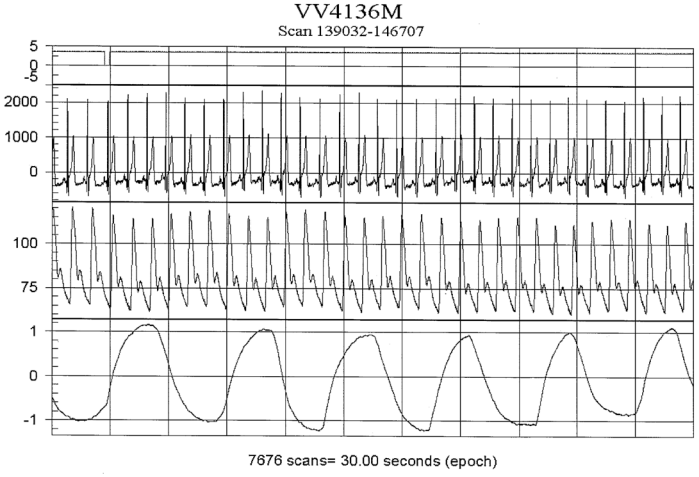
Study Design - *Study 2* ANS Battery - Derived Measures (2)

- From Non-invasive radial arterial tonometry:
  - Mean Blood Pressure (MBP)
  - Mean Systolic Pressure (SP)
  - Mean Diastolic Pressure (DP)
  - Peak Systolic Pressure for a given challenge
  - Trough Diastolic Pressure for a given challenge
- From ECG and tonometry combined:
  - Estimate of pulse transit time
  - Changes in pulse transit time with challenges

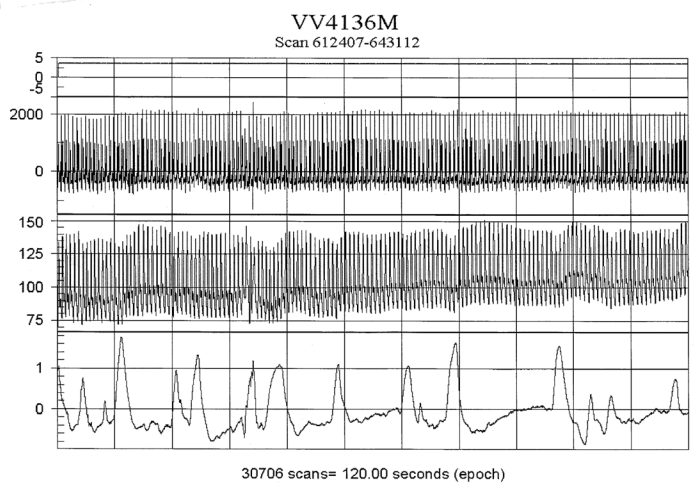
ANS Stressors

- Stressors (separated by appropriate baselines and recovery periods):
  - Deep breathing
  - Paced breathing
  - Hand grip
  - Mental Arithmetic
  - Valsalva maneuver (3x)
  - Stressful event: recall and tell
  - Tilt-up (80°, 20 min)
  - Tilt-down

Example of Real-Time Beat-to-Beat Data: Baseline



Example of Real-Time Beat-to-Beat Data: Mental Arithmetic



It is Important to Demonstrate Robust Responses from Baseline

- Elements of test batteries need to exhibit robust responses to the stressor from the baselines.
- The more robust the response, the more likely that a difference between cases and controls, *if there is one*, can be detected reliably.
- We observed robust and significant responses from baseline to seven of our eight stressors.
- Examples:

Physiological Effects (Mean, SD) of the Autonomic Reactivity Battery (ARB)

Task	Variable	p <	Baseline	Trial 1	Trial 2
Hand Grip	Mean HR	.0001	68.6(9.7)	70.3(9.8)	75.2(10.7)
	SD HR	.0004	2.6(1.6)	2.9(1.2)	2.8(1.4)
	Mean BP	.0001	91.5(10.9)	98.2(10.2)	107.7(14.7)
	SBP	.0001	127.2(13.5)	134(13.5)	143.5(18.2)
	DBP	.0001	72.8(10.1)	78.9(9.4)	86.7(13.2)

Physiological Effects of the ARB (2)

Task	Variable	p <	Baseline	Trial 1	Trial 2
Arithmetic	Mean HR	.0001	67.3(9.8)	73.2(10.2)	
	SD HR	.0001	2.9(1.4)	3.6(1.4)	
	Mean BP	.0001	91.5(9.6)	98.5(11.2)	
	SBP	.0001	125.7(12.8)	134.2(14.8)	
	DBP	.0001	73.3(9.1)	79.4(10.1)	

Physiological Effects of the ARB (3)

Task	Variable	p <	Baseline	Trial 1	Trial 2
Valsalva	Mean HR	.0001	68.7(9.7)	72.2(9.8)	70.9(9.6)
	SD HR	.0001	2.5(1.4)	9.0(4.4)	9.0(4.5)
	Mean BP	.0001	91.7(11.1)	100.2(9.3)	98.7(8.9)
	SBP	.0001	127.5(13.6)	136.7(13.8)	134.4(12.5
	DBP	.0001	72.9(10.2)	83(8.3)	81.5(8.3)

Physiological Effects of the ARB (4)

Task	Variable	p <	Baseline	Trial 1	Trial 2
Recall of stress	Mean HR	.0001	67.3(9.9)	73.2(10.5)	
	Mean BP	.0001	94.6(9.6)	98.1(10.3)	
	SBP	.0001	129.9(13.1)	134.3(13.5)	
	DBP	.0004	76(8.8)	78.6(8.9)	
	Power	.0001	28.7(12.4)	34.9(13.4)	
	ABS LF	.0001	11.2(5.2)	13.1(5.4)	

Physiological Effects of the ARB (5)

Task	Variable	p <	Baseline	Trial 1	Trial 2
Initial Up-Tilt	Mean HR	.0001	67.2(10.1)	81.7(10.9)	
	DBP	.0001	78.2(9.2)	83.9(9.2)	
	Power	.0001	35.7(14.6)	44.7(15.9)	
	ABS LF	.0001	14.1(7)	20.9(9.4)	
	ABS HF	.0006	13.3(7.4)	10.7(5.9)	
	L/H ratio	.0001	1.22(.5)	2.15(.8)	

Physiological Effects of the ARB (6)

Task	Variable	p <	Baseline	Trial 1	Trial 2
Initial Up-Tilt	%LF	.0001	39.2(7.7)	45.7(8.8)	
	%HF	.0001	36(9.7)	23.4(7.3)	
	SDNN	.0009	50.4(23.3)	57.1(23.7)	
	RMSSD	.0001	39.7(29.6)	23.5(16)	
	SDSD	.0001	39.8(29.6)	23.5(16.0)	
	%NN	.0001	15.2(18.1)	5.3(9.4)	

Selected ANS Results

- Some of the most striking results on case-control differences in ANS reactivity were observed in responses to four of the stressors:
  - Stressful event recall and telling
  - Orthostatic up-tilt (80°, initial and 20 min)
  - Orthostatic down-tilt (initial and 10 min)
  - Prepulse Inhibition and Startle reflex
- Examples:

Case/Control Differences in Physiological Response to the ARB (Example 1)

Task	Variable	p<	Cases	Deployed Controls	Nondeployed Controls
Initial Up-Tilt	SDNN	.02	49(25.1)	56.8(19.7)	61.4(21.7)*
	RMSSD	.008	27.5(22.7)	28.7(15.9)	43.1(32.4)*
	Abs LF	.007	15.8(8.9)	20.7(9.6)	18.4(7.7)
	%NN	.007	8.1(14.0)	8.8(10.9)	16.2(19.1)*

*Pairwise comparison showed that the marked Group differed from the Case Group at $p < .05$

Case/Control Differences in Physiological Response to the ARB (Example 2)

Task	Variable	p<	Cases	Deployed Controls	Nondeployed Controls
Prepulse Inhibition	Startle	.003	330(370)	611(464)*	639(552)*
	Prepulse	.002	12.1(31.3)	22.7(40.3)	65.8(104.6)*
	PPI score	.012	6.7(24.2)	3.4(6.3)	15.4(30.3)*

*Pairwise comparison showed that the marked Group differed from the Case Group at $p < .05$

Effects of BChE Genetic Status

- We examined the role of BChE variants in the responses to the ANS battery independent of case-control status.
- We also examined any possible interaction between case-control status and BChE variant status.
- Examples:

Variant/Nonvariant Differences in Physiological Responses to the ARB

Task	Variable	p <	Variant	Nonvariant
Baseline	Mean HR	.02	64.2(7.6)	69.1(9.6)
	ABS LF	.006	13.2(4.9)	10.4(5.0)
	L/H ratio	.04	1.4(.7)	1.1(.4)
	%LF	.004	42.3(8.2)	38(6.2)
	SDNN	.008	53.6(29.4)	39.9(25.5)
Breathing	Mean HR	.02	63.5(7.1)	68.4(9.1)

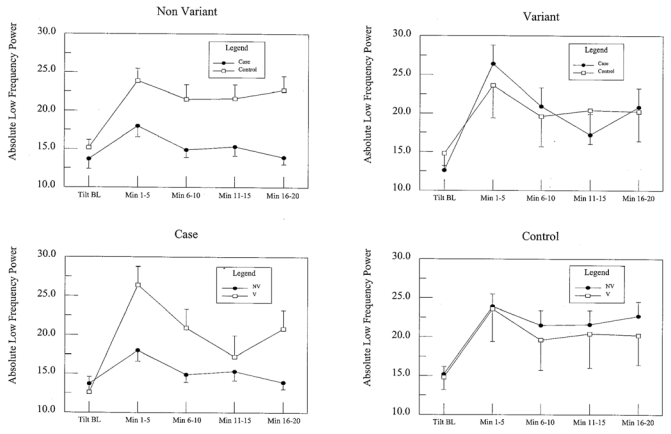
Variant/Nonvariant Differences in Physiological Responses to the ARB (2)

Task	Variable	p <	Variant	Nonvariant
Hand Grip	Mean HR	.02	66.4(8.7)	71.6(10.4)
Arithmetic	Mean HR	.04	66.4(10.1)	70.6(10.3)
Valsalva	Mean HR	.008	65.7(7.3)	71(9.7)
Stressful event recall	Mean HR	.02	65.3(8.9)	70.6(10.4)
	Power	.04	36.7(14.7)	31.5(13.2)
	ABS LF	.05	13.9(5.4)	12(5.3)
	SDNN	.0006	73.7(35)	54.2(28)
	RMSSD	.02	56(49.7)	38(34.8)
	SDSD	.02	56.1(49.8)	38.1(34.9)

Variant/Nonvariant Differences in Physiological Responses to the ARB (3)

Task	Variable	p <	Variant	Nonvariant
Initial Up-Tilt	Mean HR	.02	69.8(12.7)	74.8(12.7)
Initial Down-Tilt	Mean HR	.03	64.4(8.7)	68.6(9.8)
Recovery	Mean HR	.02	61.6(9.1)	66.2(9.9)
	DBP	.05	70.6(10.2)	74.2(9.9)
Prepulse Inhibition	Startle	.007	738(599)	475(469)
	PPI Score	.04	11.8(19.9)	5.9(20.4)

ANS Response and Variant Status Interaction



Summary of ANS *Study 2* Results

Association of GWI with ANS Physiologic Measures

Test	Response Measures that Differed between Cases and Controls
Baseline	Mean BP, L/H ratio, %HF, rMSSD, SDSD
Deep Breathing	Mean BP, DBP
Mental Arithmetic	DBP
Recall of stressful event	Mean HR, Mean BP,DBP,%HF, rMSSD,SDSD,%NN
Initial up-tilt	Mean BP, DBP, Power, ABS LF, ABS HF, L/H ratio, %HF, SDNN, rMSSD, SDSD, %NN
Up-Tilt @ 20 min	Mean BP, SBP, DBP, Power, ABS LF, %LF, SDNN, %NN
Initial down-tilt	Mean HR, ABS LF, SDNN, rMSSD, SDSD, %NN
10 min. down	Mean HR, DBP, Abs LF, SDNN, rMSSD, SDSD, %NN
Pre-pulse inhibition	Initial startle, auditory pulse, PPI score

ANS *Study 2* Results

Association of GWI with ANS Physiologic Measures

- ***No*** significant associations between case status and:
  - –Hand grip
  - –Valsalva maneuver

ANS *Study 2* Results: Conclusions (1)

1. Compared to controls, veterans with GWI show significant autonomic dysfunction, reflected in a broad range of objective measures.
2. GWI-associated physiological differences are found in both baseline (resting state) measures and in response to autonomic challenge.
3. Veterans with variant BChE genotypes differ autonomically from those with non-variant BChE genotypes, without regard to their GWI case status.

ANS *Study 2* Results: Conclusions (2)

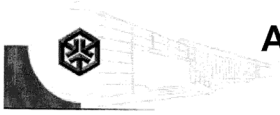
4. There are significant interactions between genotype and case status. That is, ANS responses in GWI cases vs. controls also depend on BChE genotype.
5. In general terms, autonomic reactivity appears to be blunted in GWI cases compared to healthy veterans, but there are significant exceptions to this generalization.
6. Additional analyses will combine deployed and non-deployed control groups (when appropriate) to further evaluate associations between GWI and ANS dysfunction.

ANS *Study 2:* Important Research Implication

- The results from Study 2 will allow us to design targeted follow-up studies capable of identifying specific components of the ANS that may be involved in the dysfunctions we have identified.
- Specifically, it will be possible to use ***ethical*** designs, with ***FDA-approved procedures*** and ***pharmaceuticals*** to examine:
  - Sympathetic efferents
  - Parasympathetic efferents
  - Ganglionic efferents
  - ANS Sensory **a**fferents

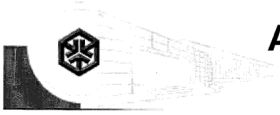
ANS Study Implications: Take-Home Points (1)

1. **Objective physiological tests indicate that Gulf War Illness is associated with autonomic dysfunction**.
   - Similar differences in heart rate variability measures have been found, in other studies, linked to eventual higher cardiac and overall mortality rates.
2. **Autonomic dysfunction is also associated with BChE genotype, regardless of case/control status.**
3. **Case/Control Status and BChE variant status interact.**



ANS Study Implications: Take-Home Points (2)

4. **Veterans with variant BChE genotypes appear to have a substantially increased risk of GWI in association with certain exposures.**
   - In the absence of these exposures, carriers of BChE variants were not at increased risk for GWI
   - Further study of genetic variants of other key enzymes, such as Serum Paraoxonase 1, is needed to help clarify the complex interactions between environmental exposures, physiologic ranges and genetic polymorphisms.



ANS Study Implications: Take-Home Points (3)

5. **Methodology is extremely important**. The clear results from our study depended on:
   a. **Interdisciplinary expertise.** Study designed and executed by a team that included experts in autonomic physiology, psychophysiology, molecular biology, and epidemiology.
   b. **Test battery.** Comprehensive and well-executed battery of physiological tests. One ANS measure (e.g. Valsalva test in prior study) doesn't tell the whole ANS story.
   c. **Case definition**. Use of a pre-determined and functional GWI case definition (e.g. CDC case definition gave results similar in direction, but with weaker associations)
   d. **Well-defined sample, veteran participation**. Sufficient numbers of veterans proactively contacted from defined **population-based** samples.

Presentation 2 - John Vogel

Effects of Exposure to Multiple Chemicals at Low Dose *in vivo*:
Allowing Physiology into Toxicology

- John S. Vogel, PhD, Senior Research Scientist
  - Center for Accelerator Mass Spectrometry
  - Lawrence Livermore National Laboratory
  - University of California
  - Livermore, CA

Toxic effects of multiple pesticides are not known for low doses.

- "Insufficient data exist to determine if effects of multiple OP pesticides can be extrapolated through dose responses that :
  - ... are additive.
  - ... sum toxicologicly equivalent doses.
  - ... are synergistic through activation or detoxicfication.
  - ... are antagonistic." *
  - ... are none of the above?

- Goal: design an assumption-free test of *in vivo* interactions of co-administered compounds at low doses.

* Common Mechanism of Toxicity: A Case Study of OP Pesticides. B.E. Mileson, et al. Tox. Sci. 41:8-20 (1998)

Extrapolated high dose OP data predict no significant low dose effects.

Constraining assumptions!

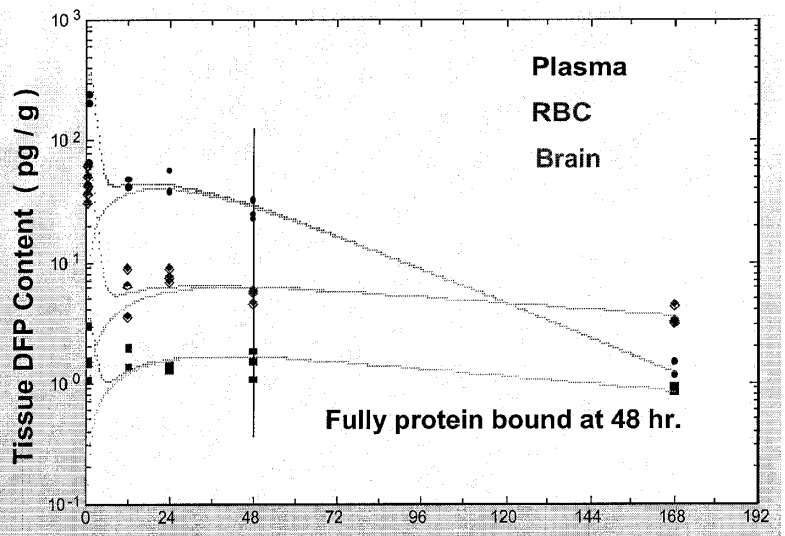
- **Dose Effects**
  - Measured index is linear with dose (e.g. AChE activity).
  - Intermediate metabolism and distribution are linear.
- **Mixture Effects**
  - Compounds act on same target molecule.
  - Compounds use the same molecular mechanism.
  - Compounds affect the same measured index.
  - Interaction is not the result of intermediate induction.

A "reporter" assay integrates physiologic and biochemical interactions.

- **Choose a quantifiable "toxic" end point.**
  - [^{14}C]-DFP binds firmly to specific enzymes.
  - Use a very sub-toxic dose.
- **Expose animals to realistic doses of compounds.**
  - Pesticides: parathion and permethrin.
  - Therapeutic: pyridostigmine bromide.
- **Control for confounding physiologic effects.**
  - Avoid stresses of handling or metabolism cages.
- **Quantify reporter in target tissues.**
  - Normalize response to reporter in plasma.

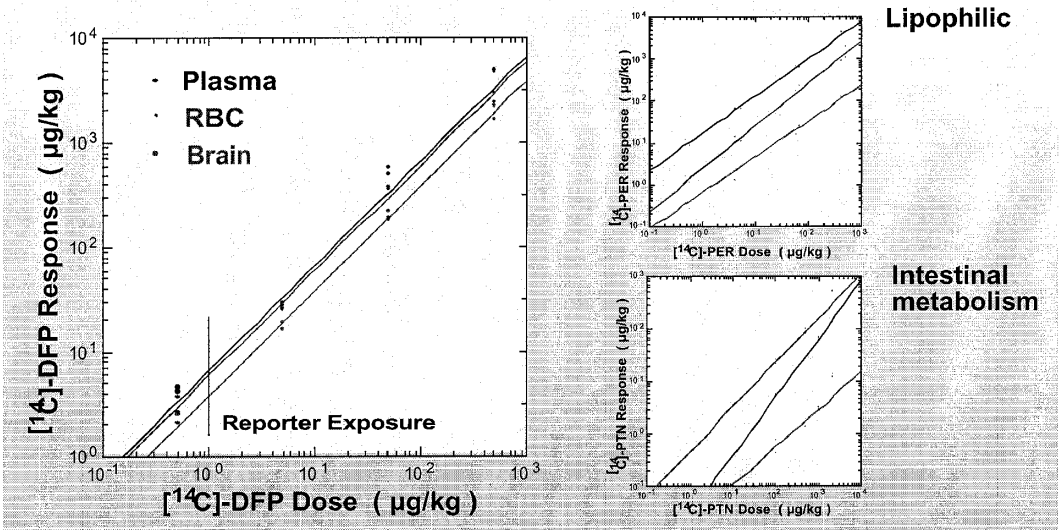
DFP - di isopropyl fluorophosphate, isofluorophate, ...
PTN - parathion
PER - permethrin
PYB - pyridostigmine bromide

DFP is quickly eliminated, but binds to exposed target proteins.



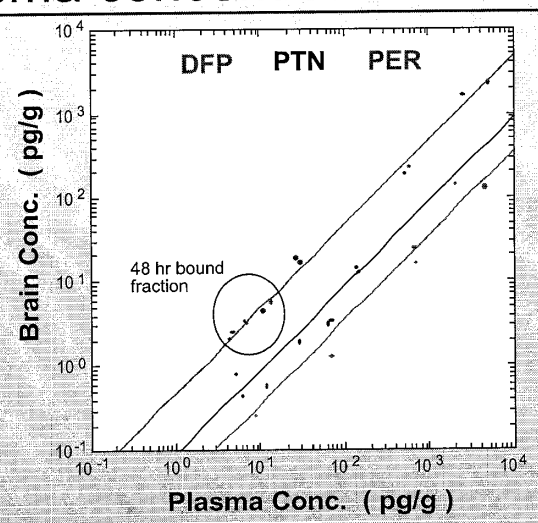
- Unbound DFP is metabolized.
- Bound DFP ^{14}C remains with the binding protein.

DFP was distributed linearly with dose by 1 hr after exposure through ingestion.



- Distribution rapidly equilibrated for DFP reporter.
- PER and PTN are not so well behaved.

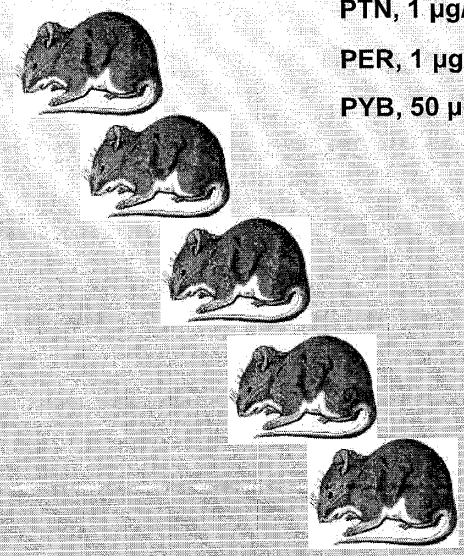
DFP brain concentrations were linear with plasma concentrations.



- Both DFP and PER are lipophilic.
- PER transfer to the brain was 10% that of DFP.
- Brain follows plasma for DFP.

Kinetics and dose response data helped design the mixture study.

CD2/F1 mice



Mixtures:

PTN, 1 µg/kg

PER, 1 µg/kg

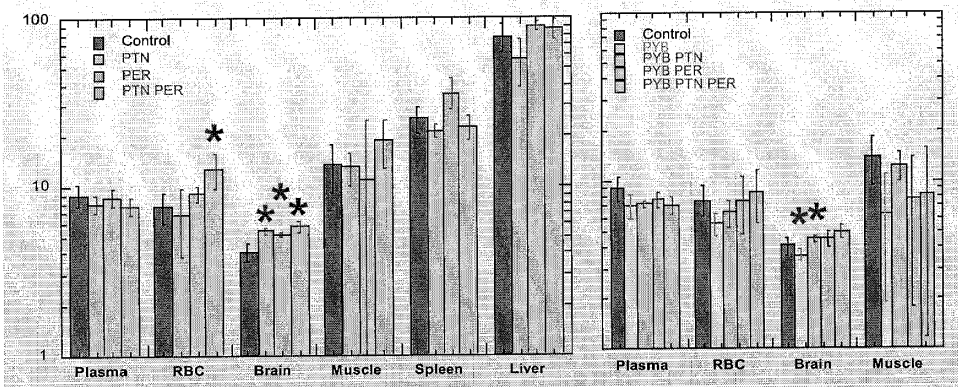
PYB, 50 µg/kg

PYB PTN PER
PYB PER
PYB PTN
PTN PER
PYB
PER
PTN

- 5 day exposure to mixture.
- in moist food, avoids dose loss
- no handling, reduces stress
- [^{14}C]-DFP reporter (5 nCi)

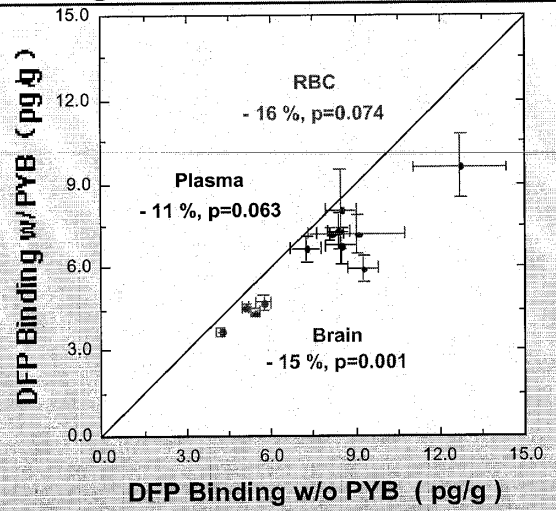
- 48 hr post DFP
  - brain
  - blood (RBC)
  - liver
  - spleen
  - muscle

Compounds increased brain DFP levels without affecting blood levels.



- Low companion doses produce no change in plasma and most tissue.
- Brain contains significantly more reporter compound.
- PYB significantly reduces reporter in brain.
- Other compounds increase reporter in brain even with PYB.

PYB provided a 15% system-wide protection against bound DFP.



- Plasma esterases are too numerous to succumb to competitive antagonism.
- Equivalence of competition across BBB is unlikely.

Mechanisms of interactions are not related to protein binding at low doses.

1. **Brain - plasma relation is linear for DFP in controls.**
   Brain increase not related to plasma concentration.
2. **Pesticides cross BBB less readily than DFP from plasma.**
   Pesticides did not induce synergistic protein binding.
3. **Pesticides increase brain DFP.**
   More plasma delivered to brain .

Hypothesis:

- PER and PTN induce nitric oxide synthase (hence, NO) through cholinergic receptors.
- NO increases brain blood flow at normal human pesticide exposures.

Mechanisms of interactions are not related to protein binding at low doses.

1. **PYB has low bioavailability and even lower brain access.**
   Plasma concentrations are low.
2. **Competitive binding in plasma would increase brain DFP.**
   Copious plasma esterases not overloaded.
3. **DFP binding decrease is systemwide at ≈ 15%.**
   Less DFP enters the system.

Hypothesis:

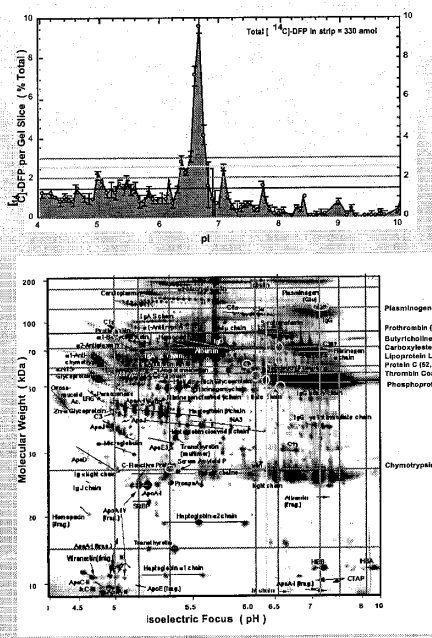
- PYB decreases intestinal peristalsis.
- Absorption of DFP also lowered by intestinal changes.

Conclusions from initial study.

- Interaction among compounds at low dose are physiological.
- Synergism /antagonism are only visible in whole animal.
- Reporter compound quantifies the effect of multiple exposures.
- Response involves a non-linear extrapolaion from high dose.

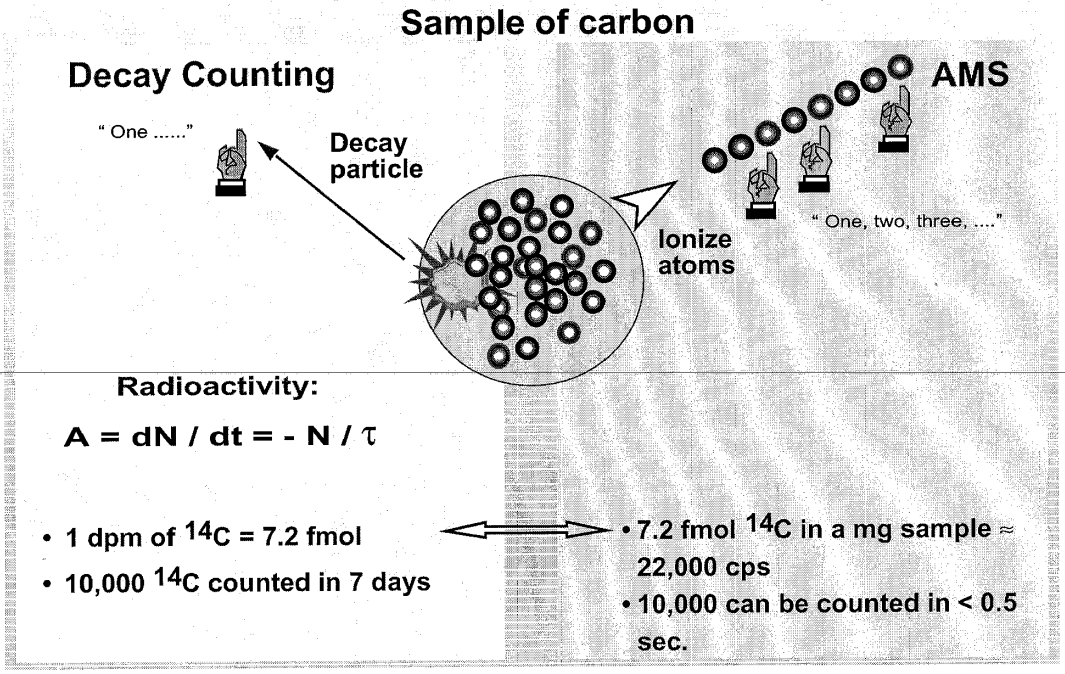
- Next Steps
  - Identify *in vivo* target proteins
  - Probe NOS levels with citruline formation from [^{14}C]-arginine

Virtual 2D gel AMS finds DFP protein targets at 1 µg/kg dose.

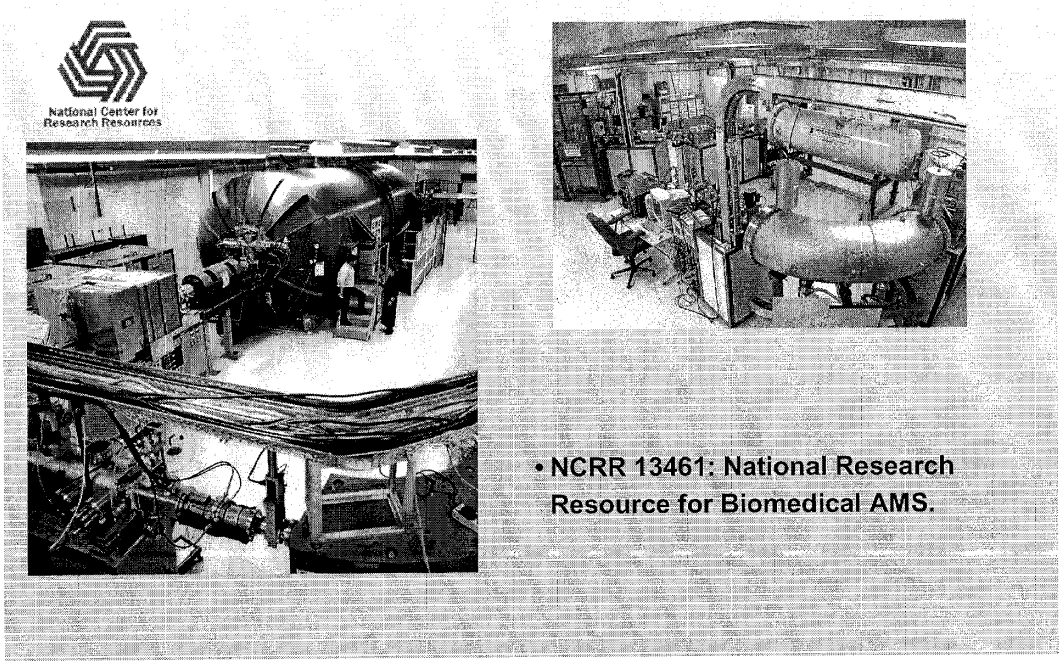


- Plasma proteins separated by molecular weight and isoelectric point.
- BChE is dominant target in plasma.

Low dose assays are made possible by high sensitivity of AMS for ^{14}C.



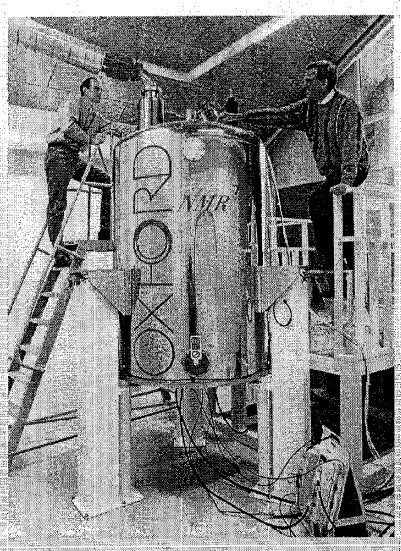
AMS is accessible through an NIH Research Resource at LLNL.



None of our animals became "radioactive waste".

- **§ 20.2005 Disposal of specific wastes.**
- **(a) A licensee may dispose of the following licensed material as if it were not radioactive:**
- **(1) 0.05 microcurie (1.85 kBq), or less, of hydrogen-3 or carbon-14 per gram of medium used for liquid scintillation counting; and**
- **(2) 0.05 microcurie (1.85 kBq), or less, of hydrogen-3 or carbon-14 per gram of animal tissue, averaged over the weight of the entire animal.**

Another large technology is being used for "hypothesis generation".

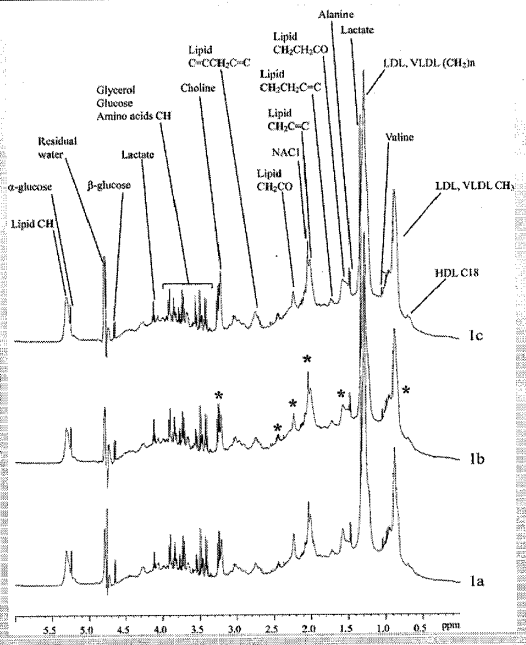


- **Nuclear Magnetic Resonance (NMR) is the basis for MRI.**
- **NMR provides exquisite resolution of chemical structures.**
- **100 µl of serum or urine can be scanned for all constituents.**

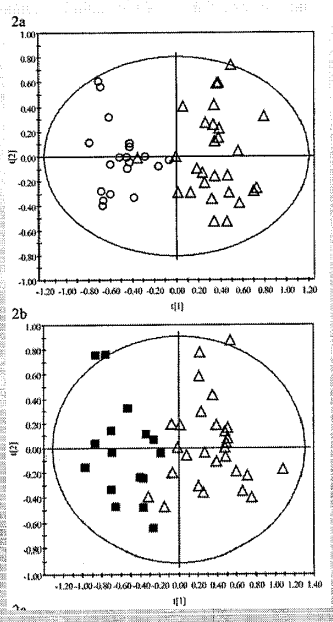
Plasma can be scanned for evidence of metabolic imbalances.

- **NMR spectra quantitate thousands of compounds.**
- **Only one scan (seconds) needed for each sample.**
- **Changes can be correlated to diagnoses.**

Metabolomics



Computers seek significant differences among defined classes of people.



- **Computers "train" on one set of diagnosed people.**
- **Find the "principal components" that distinguish the diagnosis.**
- **Principal components are the "most distinguishing" features designating membership in a class.**
- **Data are from a study of hypertensives (Imperial College London, Jeremy Nicholson)**

Acknowledgements

- LLNL
  - Bruce Buchholz
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  - Guomin Shan
- NIH
  - ES 09690 Chem. Mixtures
  - RR 13461 AMS Resource

Presentation 3 – Roberta White

Effects of Pyridostigmine Bromide and PTSD on Neuropsychological Function in GW Veterans

June 16, 2003; VACO; Washington, DC

Boston Environmental Hazards Center
RF White, PhD; K Sullivan, PhD; M Krengel, PhD; S Proctor, DSc; S Devine, PhD; T Heeren, PhD; J Vasterling, PhD

Introduction - 1

Gulf War illness symptoms generally include memory and concentration difficulties, fatigue, headache and joint pains.

Introduction - 2

Suggested causes of Gulf War illness include exposure to environmental toxicants (diesel fuels, pesticides, pyridostigmine bromide use, biological or chemical warfare agents) and acute stress reactions.

Introduction - 3

Due to limited record keeping by the military and GW veterans' lack of awareness concerning potential environmental contaminants, it has been virtually impossible to definitely assess exposures in the veteran group.

Introduction - 4

However, it seems likely that GW veterans would recall with some accuracy whether they used the anti-nerve gas agent pyridostigmine bromide (PB) due to the fact that the pills were self-administered.

Pyridostigmine Bromide -1

PB is an acetylcholine (ACh) inhibitor used in the GW to protect US veterans against chemical weapon attacks.

PB - 2

PB reversibly binds to ACh receptors in the peripheral nervous system, providing protection from chemical weapon exposures (soman, sarin) that irreversibly bind to ACh receptors.

PB - 3

PB generally does not cross the blood brain barrier or have centrally acting effects. However, evidence from animal models suggests that PB taken during periods of stress may affect the central nervous system (CNS). In humans, this might result in confusion, fatigue and cognitive difficulties.

Post-Traumatic Stress Disorder (PTSD)

Stress has been suspected as a cause of GW illness.

PTSD - 2

Severe stress reactions result in the diagnosis of PTSD. A chronic state of arousal may occur when individuals with PTSD are exposed to triggers similar to the original traumatic event.

PTSD - 3

Exposure to traumatic or stressful events has been associated with cognitive impairments such as diminished attention on tracking tests.

PTSD - 4

PTSD has been associated with memory and attention deficits in US Gulf War veterans.

Study Aims

- Assess the impact of PB use and PTSD diagnosis on cognitive functioning in GW veterans.
- Assess the separate impact of each exposure on cognition and then the combined effect of PTSD and PB use.

Participants - 1

Treatment seeking veterans from the VA Boston Healthcare System who were in the military during the time of the Gulf War (1990-1991) were eligible for study participation.

Participants - 2
GW-deployed veterans

1. Randomly selected GW veterans seeking treatment or diagnostic evaluation for any health complaint.
2. GW veterans clinically referred for a neuropsychological evaluation due to increased cognitive or health symptom complaints.

Participants - 3

A control group of VABHS treatment seeking GW-era veterans who were not deployed to the Gulf were randomly recruited and evaluated.

Participants - 4

A total of 207 GW-deployed participants were recruited for the study.

53 GW-era veterans participated.

Participants - 5
GW-deployed group

92 GW-deployed veterans reported using PB during the GW.

28 GW-deployed veterans met criteria for PTSD based on CAPS-DX criteria.

Participants - 6

Mean age and sex were different among GW-deployed and non-GW-deployed participants.

Mean age and sex were not significantly different between participants with and without PB use or those with and without PTSD diagnoses.

Methods - 1

All study participants underwent a semi-structured clinical interview, the Clinician Administered PTSD scale (CAPS-DX), and a neuropsychological test battery.

Methods - 2

PTSD diagnosis was made by the CAPS-DX, an instrument specifically designed for PTSD diagnosis.

Methods - 3

PB exposure was determined by a self-report questionnaire inquiring about environmental exposures while in the Gulf War.

Neuropsychological Test Battery - 1: General Intellectual Function

Wechsler Adult Intelligence Scale-Revised (WAIS-R), Information subtest

Neuropsychological Test Battery - 2: Attention and Executive Function

- Continuous Performance Test (CPT)—computerized
- WAIS-R Digit Spans
- Wechsler Memory Scale- Revised (WMS-R), Digit Spans
- Trail-making Test
- Stroop Test
- Paced Auditory Serial Arithmetic Test
- Wisconsin Card Sorting Test

Neuropsychological Test Battery - 3: Motor Function

- Finger Tapping Test
- Purdue Pegboard Test

Neuropsychological Test Battery - 4: Visuospatial Abilities

- WAIS-R Block Designs
- Hooper Visual Organization Test

Neuropsychological Test Battery - 5: Memory

- WMS-R Verbal Paired Associate Learning
- California Verbal Learning Test
- WMS Visual Reproductions, immediate and delayed recall
- Rey-Osterreith Complex Figure, immediate and delayed recall

Neuropsychological Test Battery - 6: Motivation

- Test of Memory Malingering
- Internal consistency measures

Neuropsychological Test Battery -7: Mood

- Profile of Mood States

Statistical Analyses -1

The significance of the findings were evaluated in a two-step process:

- Multivariate analyses of covariance were performed for each neuropsychological domain.
- Univariate analyses were performed for each specific test.

Statistical Analyses - 2

- Analyses for GW-deployed veterans (N=207) and non-deployed veterans (N=53) controlled for age and sex
- Effects of self-reported PB exposure (N=92) and PTSD diagnosis (N=28) were analyzed within the group of 207 GW-deployed veterans

Results: Deployed vs. non-deployed veterans

- Deployed veterans performed worse on measures assessing
  - Attention
  - Motor and visuomotor skills
  - Visual memory
  - Mood and motivation

Results - Domain Specific Analyses GW-deployed group

	PB P value	PTSD P value	PB x PTSD P value
Attention	.14	.53	.52
Executive Functions	.01*	.92	.91
Motor / Visuomotor	.89	.72	.82
Verbal Memory	.12	.19	.29
Visual Memory	.38	.53	.53
Mood and Memory	.09	.05*	.17

Results - Mood Scales GW-deployed group

MOOD	PB PTSD	Exposure No PTSD	No PB PTSD	Exposure No PTSD	PB P value	PTSD P value	PB x PTSD P value
POMS Tension (t)	51.8	42.5	58.3	43.3	.10	.006	.44
POMS Depression (t)	49.2	42.6	55.3	44.3	.38	.04	.61
POMS Anger (t)	53.5	47.7	57.8	49.3	.65	.15	.72
POMS Vigor (t)	50.1	52.3	47.3	54.8	.91	.37	.95
POMS Fatigue (t)	57.7	51.9	60.5	55.3	.03	.30	.61
POMS Confusion (t)	54.9	44.2	53.0	48.3	.20	.75	.20

Results - Executive Tasks GW-deployed group

	PB PTSD	Exposure No PTSD	No PB PTSD	Exposure No PTSD	PB P value	PTSD P value	PB x PTSD P value
WCST # Sorts	3.5	3.5	4.8	3.9	.01	.49	.40
Stroop Ğ Words	84.7	92.0	76.0	90.5	.68	.84	.75
Stroop Ğ Colors	65.3	65.9	64.5	71.7	.19	.88	.98
Stroop Ğ Word/Color	41.0	39.2	37.5	39.1	.43	.84	.73
PASAT-trials 1-4	124.1	121.2	107.0	123.3	.32	.76	.60
Trail Making B (time)	77.6	73.9	72.8	67.2	.34	.50	.55

Conclusions -1

PTSD diagnosis was significantly associated with the mood indices of POMS test (tension and depression). However, it was not significantly associated with cognitive functioning in this cohort.

Conclusions - 2

Self-reported PB use was significantly associated with executive system functioning in this cohort of GW veterans.

Conclusions - 3

There were no interaction effects of PB use and a diagnosis of self-reported PTSD in the group of veterans.

Follow-up study: Preliminary results (6/12/03)--1

- Paired t-tests show no changes in performance on most neuropsychological tests among GW-deployed veterans
- Exceptions (worse performance):
  - Digit Spans backward
  - Wisconsin Card Sorting Test
  - Purdue Pegboard

Follow-up study: Preliminary results (6/12/03) - 2

GW veterans report more symptoms than they did 3 years previously:

Symptom	Time 1	Time 2
Headaches	62%	80%
Fatigue	59%	88%
Forgetfulness	63%	81%
Joint pain	46%	88%
Skin rash	32%	61%

Follow-up study: Preliminary results (6/12/03) - 3

- Diagnosis of PTSD is associated with increased mood complaints but not diminished cognitive function
- Self-reported PB exposure is associated with lower scores on tests assessing visuomotor function (Trails B, Finger Tapping non-dominant hand, Block Designs) and possibly memory (CVLT-short delay)

History of Work

- Ft. Devens survey—1991 (N = 3000)
- Clinical study (VA)—1993-1994
- BEHC studies (VA)—1994-2000
- DoD study 1—1996-1999
- CDC studies—1997-2001
- DoD study 2—2000-2003

Ft. Devens results - 1 (June 3, 1994 - 2000)

- Self-reported PB exposure was *not* predictive of these classes of symptoms:
  - Gastrointestinal
  - Musculoskeletal
  - Neurological
  - Neuropsychological
  - Psychological

Ft. Devens result - 2

Self-reported PB exposure was *not* related to neuropsychological test performance

DISCUSSION

- Treatment-seekers vs. non-treatment seekers
- Vulnerability/risk factors
- Future directions

Presentation 4 – Rogene Henderson

EFFECTS OF INHALATION EXPOSURE TO LOW LEVELS OF SARIN IN FISCHER 344 RATS

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Contract No. DAMD17-97-C-7054

THE QUESTION

What are the potential health effects from single or repeated exposure to subclinical (acutely asymptomatic) levels of sarin? Does heat stress potentiate those health effects?

PRELIMINARY STUDY

- Purpose:
  - To determine if the exposure levels chosen on the basis of literature reports were sub-clinical.
- Approach:
  - Exposed four rats/group to 0, 0.2, 0.4 or 0.8 mg/m^3 sarin for 1 hr.
- Results:
  - No observed abnormal behaviors (salivation, lacrimation, red eye discharge, sneezing, excess face rubbing, excess urination, excess defecation, diarrhea, muscle tremors, muscle weakness, paralysis)
- Conclusion:
  - Exposure levels chosen are sub-clinical

EXPERIMENTAL DESIGN

- Animals:
  - Male, F344 rats, 10–11 weeks old
- Exposures:
  - Nose-only to 0, 0.2 or 0.4 mg/m^3 of sarin for 1 hour/day, for 1, 5 or 10 days
- Heat stress:
  - Exposures were done at either normal (25°C) temperature or under heat stress (32°C)
- Sacrifice times:
  - 1 day after exposure to observe acute effects
  - 30 days after exposure to determine if any effects are persistent
- Observations:
  - Body weights, activity patterns, body temperature, histopathology, apoptotic cells in the brain, brain cytokine levels (IL-1β, TNFα, IL-6), densities of brain muscarinic receptor sites
  - Limited observations on effects on the immune system

METHODS

- Exposures:
  - Nose-only, inside a glove box. Monitored by minicam (GC/FPD). Exhaust scrubbed by sodium hypochlorite bubbler.
- Pulmonary function during exposure:
  - Plethysmography exposure tubes that hold a Fleisch pneumotach connected to a validyne differential pressure transducer (DP45). The signal from the transducer indicates the volume displacement caused by breathing.
- Body temperature during exposure:
  - Rectal probes.
- Body temperature and activity after exposure:
  - Surgically implanted biotelemetry devices transmitted information to activity boards under the rat cages.

SARIN EXPOSURE LEVELS (mg/m^3, $\bar{X}$ ± SD)[a]

	Normal Temperature		High Temperature	
Days of	Low Level	High Level	Low Level	High Level
1	0.22 ± 0.04	0.38 ± 0.06	0.21 ± 0.03	0.42 ± 0.06
5	0.19 ± 0.02	0.37 ± 0.02	0.18 ± 0.03	0.37 ± 0.02
10	0.20 ± 0.01	0.42 ± 0.03	0.21 ± 0.01	0.42 ± 0.02

[a]The $\bar{X}$ ± SD values for the 1 day exposure were based on 15 measurements taken every 4 min during the 1 hr exposures. The values for repeated exposures are the mean and SD of the daily exposure values.

BODY TEMPERATURE DURING EXPOSURE

($\bar{X}$ ± SD, n = 4 animals)

	Control	0.2 mg/m^3	0.4 mg/m^3
Normal Temperature	36.5 ± 0.8	36.5 ± 0.8	36.8 ± 0.9
Heat Stress	37.7 ± 0.5	38.0 ± 0.7	38.0 ± 0.7

BODY WEIGHTS

(g, $\bar{X}$ ± SE)

	Before Exposure	End of Exposure	1 Month After Exposure
Sarin Exposure Group	n = 24	n = 12	n = 12
1–Day Exposure (NT)			
Control	216 ± 1.6	227 ± 2.9	272 ± 3.6
0.2 mg/m^3	213 ± 1.8	225 ± 1.7	270 ± 4.4
0.4 mg/m^3	213 ± 1.8	223 ± 1.8	267 ± 4.4
1–Day Exposure (HT)			
Control	222 ± 1.0	218 ± 1.9[a]	239 ± 4.9[a]
0.2 mg/m^3	220 ± 1.3	222 ± 2.1	247 ± 2.4[a]
0.4 mg/m^3	221 ± 1.2	225 ± 1.9	247 ± 2.3[a]

NT = normal temperature (25°C); HT = high temperature (32°C)
[a]Weight gain differs from NT, $P \leq 0.05$.

BODY WEIGHTS

(g, $\bar{X}$ ± SE)

Sarin Exposure Group	Before Exposure	End of Exposure	1 Month After Exposure
	n = 24	n = 12	n = 12
5–Day Exposure (NT)			
Control	213 ± 1.2	231 ± 1.3	281 ± 3.0
0.2 mg/m³	215 ± 1.6	231 ± 0.6	282 ± 5.4
0.4 mg/m³	214 ± 1.5	224 ± 3.0	279 ± 4.4
5–Day Exposure (HT)			
Control	214 ± 1.6	218 ± 1.4*	259 ± 4.2*
0.2 mg/m³	214 ± 1.8	220 ± 2.2*	260 ± 3.2*
0.4 mg/m³	214 ± 1.4	220 ± 0.6	253 ± 2.1*

NT = normal temperature (25°C); HT = high temperature (32°C)
*Weight gain differs from NT, P ≤ 0.05.

BODY WEIGHTS

(g, $\bar{X}$ ± SE)

Sarin Exposure Group	Before Exposure	End of Exposure	1 Month After Exposure
	n = 24	n = 12	n = 12
10–Day Exposure (NT)			
Control	222 ± 3.2	231 ± 2.7	295 ± 3.6
0.2 mg/m³	227 ± 1.9	229 ± 2.9	290 ± 3.2
0.4 mg/m³	224 ± 1.7	231 ± 3.5	296 ± 3.4
10–Day Exposure (HT)			
Control	210 ± 1.7	214 ± 2.5	242 ± 3.6*
0.2 mg/m³	210 ± 1.4	218 ± 2.4	248 ± 2.6*
0.4 mg/m³	211 ± 1.0	215 ± 1.6	241 ± 2.9*

NT = normal temperature (25°C); HT = high temperature (32°C)
*Weight gain differs from NT, P ≤ 0.05.

PULMONARY FUNCTION

(during exposure; $\bar{X}$ ± SE, n = 4)

Sarin Exposure Group	Frequency (breaths/min)	Tidal Volume ml	Minute Volume ml/min
1–Day Exposure (NT)			
Control	159 ± 16	1.5 ± 0.08	236 ± 23
0.2 mg/m³	169 ± 1	1.6 ± 0.12	267 ± 19
0.4 mg/m³	149 ± 1	1.6 ± 0.07	231 ± 11
1–Day Exposure (HT)			
Control	155 ± 7	1.5 ± 0.02	238 ± 13
0.2 mg/m³	149 ± 5	1.5 ± 0.03	223 ± 10
0.4 mg/m³	156 ± 5	1.6 ± 0.06	242 ± 13

NT = normal temperature (25°C); HT = high temperature (32°C)

PULMONARY FUNCTION

(during exposure; $\bar{X}$ ± SE, n = 4)

Sarin Exposure Group	Frequency (breaths/min)	Tidal Volume ml	Minute Volume ml/min
5–Day Exposure (NT)			
Control	160 ± 6	1.5 ± 0.07	236 ± 20
0.2 mg/m³	170 ± 3	1.5 ± 0.07	267 ± 19
0.4 mg/m³	165 ± 3	1.5 ± 0.08	252 ± 9
5–Day Exposure (HT)			
Control	166 ± 7	1.6 ± 0.13	258 ± 15
0.2 mg/m³	174 ± 9	1.5 ± 0.07	264 ± 23
0.4 mg/m³	172 ± 6	1.6 ± 0.06	269 ± 10

NT = normal temperature (25°C); HT = high temperature (32°C)

PULMONARY FUNCTION

(during exposure; $\bar{X} \pm SE$, n = 4)

Sarin Exposure Group	Frequency (breaths/min)	Tidal Volume ml	Minute Volume ml/min
10–Day Exposure (NT)			
Control	160 ± 2	1.5 ± 0.02	238 ± 5
0.2 mg/m³	154 ± 2	1.6 ± 0.02	247 ± 6
0.4 mg/m³	164 ± 2	1.5 ± 0.02	253 ± 6
10–Day Exposure (HT)			
Control	142 ± 2	1.6 ± 0.02	222 ± 4*
0.2 mg/m³	149 ± 2	1.6 ± 0.02	232 ± 4*
0.4 mg/m³	144 ± 2	1.6 ± 0.02	230 ± 4*

NT = normal temperature (25°C); HT = high temperature (32°C)

BLOOD CHOLINESTERASE IN RATS

Sarin Exposure (mg/m³)	1 Day of Exposure (n = 12)	
	RBC ChE ($\bar{X} \pm SE$)	Plasma ChE ($\bar{X} \pm SE$)
0	1.553 ± 0.040	0.236 ± 0.008
0.2	1.437 ± 0.040*	0.202 ± 0.006
0.4	1.387 ± 0.034*	0.189 ± 0.008

*Differs from control, $p \leq 0.05$

BLOOD CHOLINESTERASE IN RATS

Sarin Exposure (mg/m³)	5 Days of Exposure (n = 10-11)	
	RBC ChE ($\bar{X} \pm SE$)	Plasma ChE ($\bar{X} \pm SE$)
0	1.946 ± 0.064	0.162 ± 0.006
0.2		
0.4	0.804 ± 0.020*	0.140 ± 0.007†

*Differs from control, $p \leq 0.05$
†Differs from control, p = 0.055

BLOOD CHOLINESTERASE IN RATS

Sarin Exposure (mg/m³)	10 Days of Exposure (n = 10-11)	
	RBC ChE ($\bar{X} \pm SE$)	Plasma ChE ($\bar{X} \pm SE$)
0	1.681 ± 0.042	0.231 ± 0.011
0.2	1.125 ± 0.040*	0.227 ± 0.004
0.4	0.618 ± 0.037*	0.220 ± 0.012

*Differs from control, $p \leq 0.05$

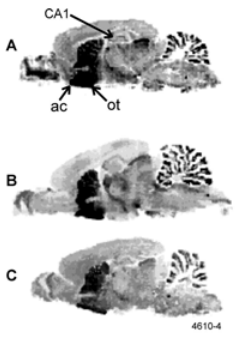
BRAIN CHOLINESTERASE ACTIVITY IN 5-DAY EXPOSED RATS, 1 DAY AFTER EXPOSURES

(U ChE activity/µg protein; $\overline{X} \pm$ SE, n = 3)

Sarin Exposure (mg/m^2)	NT (25°C)	HT (32°C)
0	5.41 ± 0.16	5.26 ± 0.06
0.2	5.15 ± 0.10	5.11 ± 0.15
0.4	5.66 ± 0.31	5.15 ± 0.20

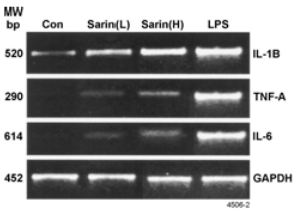
BRAIN AChE BY HISTOCHEMICAL STAINING

- AChE reduced in cerebral cortex, striatum and olfactory bulb.
- Loss of laminar pattern of AChE in hippocampus over CA1 and CA3 sectors.
- Diminished forebrain, but not brain stem AChE.



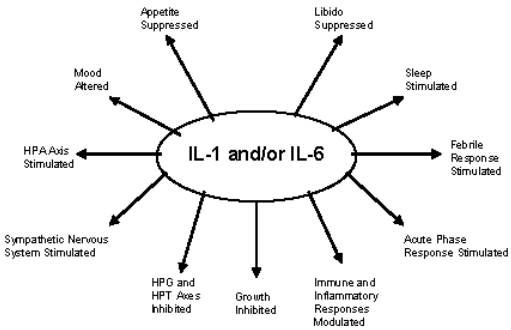
CYTOKINES IN BRAIN

IL-1β, IL-6 and TNF-α were all induced in a dose-dependent manner in the brains of rats exposed to sarin for 5 days, but not in rats exposed for 1 day.



Sarin induces the expression of IL-1β, IL-6, and TNF-α in the brain

CYTOKINES AND THE NEUROIMMUNE RESPONSE

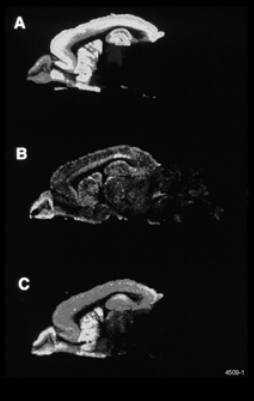


MUSCARINIC ACETYLCHOLINE RECEPTOR SITES (Coupled to G Proteins)

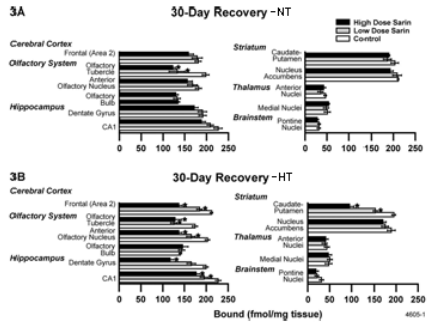
M1 – Cerebral cortex, forebrain, telecephalic structures, paralimbic cortical areas

M2 – All sensory and motor areas, cerebral cortex

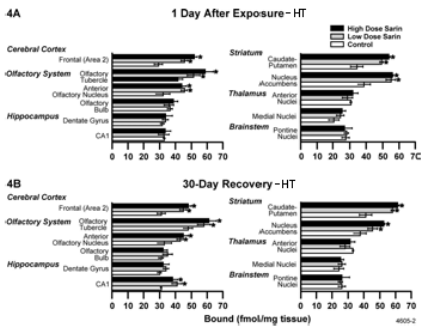
M3 – Forebrain, telecephalic structures, auditory areas of temporal lobe, hypothalamus



M1 RECEPTOR SITES



M3 RECEPTOR SITES



END OF EXPOSURE

	M1	M2	M3
Normal Temperature	no change	no change	no change
High Temperature	no change	no change	↑

frontal lobe
olfactory
tubercle
striatum

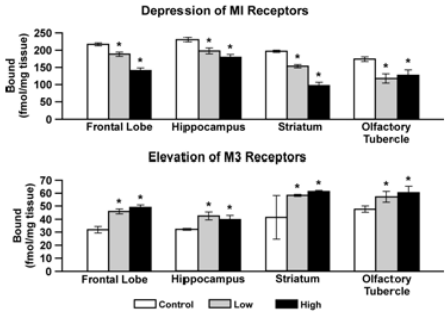
RAT BRAIN

Areas of increased M3 receptor sites.

THIRTY DAYS AFTER FIVE REPEATED 1 HR/DAY EXPOSURES

	M1	M2	M3
Normal Temperature	↓ olfactory tubercle	no change	no change
High Temperature	↓ Frontal Area 2 Olfactory tubercle Olfactory nucleus Hippocampus Striatum	no change	↑ Frontal Area 2 Olfactory tubercle Olfactory nucleus Hippocampus Striatum

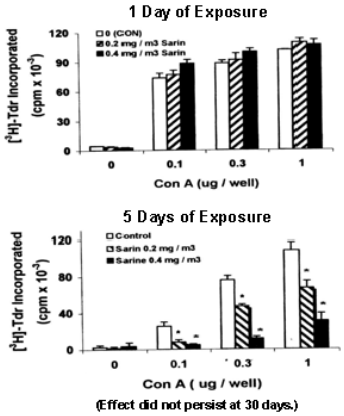
THIRTY DAYS AFTER FIVE REPEATED 1 HR/DAY EXPOSURES
(Heat Stress Conditions)



DOES SARIN AFFECT THE IMMUNE SYSTEM?

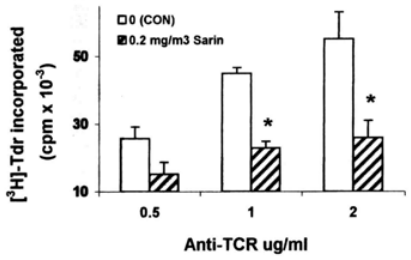
- Bidirectional communication between the brain and the immune system.
- Lymphoid tissues are innervated by both sympathetic and parasympathetic nerves. The function of these innervations is not clear.
- Cholinergic agents, including organophosphates, affect some immune parameters.

SARIN EXPOSURE INHIBITED T-CELL RESPONSE TO MITOGEN



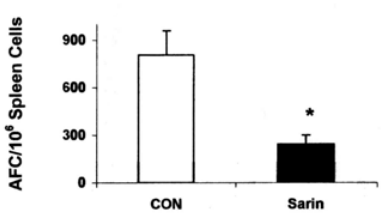
(Effect did not persist at 30 days.)

SARIN SUPPRESSES PROLIFERATIVE RESPONSE OF T-CELLS TO ANTIGEN

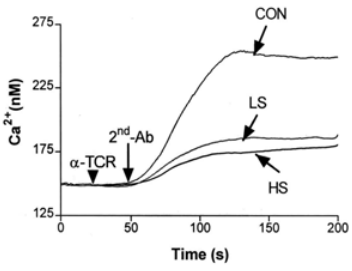


SARIN SUPPRESSES ANTIBODY RESPONSE TO SHEEP RED BLOOD CELLS

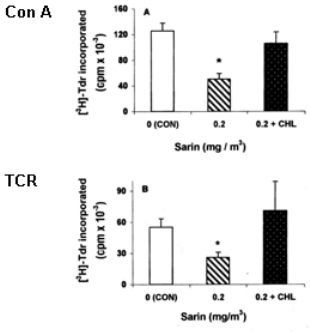
(0.4 mg/m³ sarin, 1 h/day, 5 days)

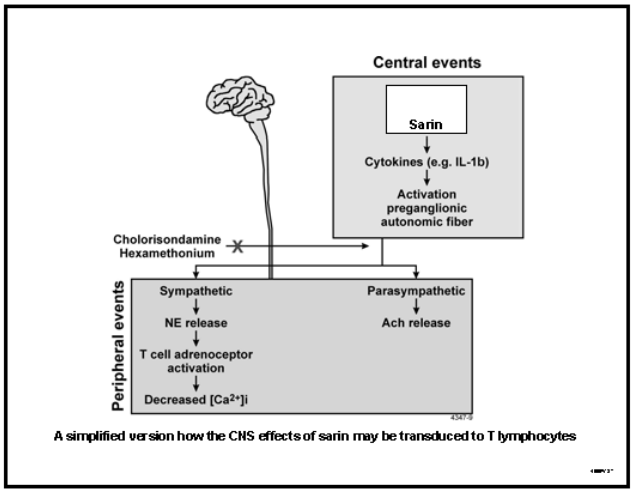


SARIN SUPPRESSES THE CALCIUM INCREASE THAT ACCOMPANIES Ag-MEDIATED T-CELL ACTIVATION

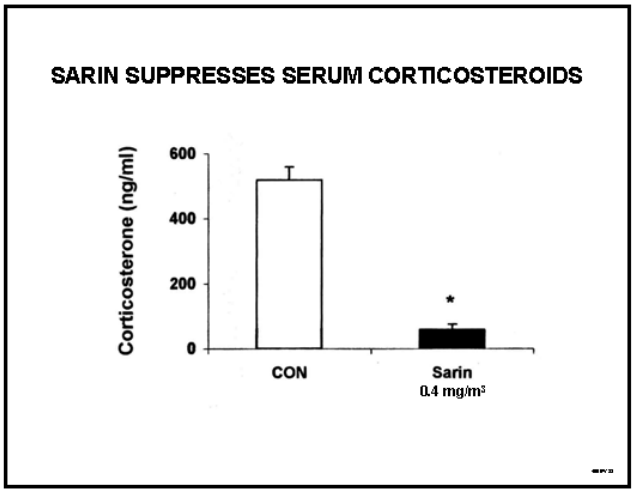


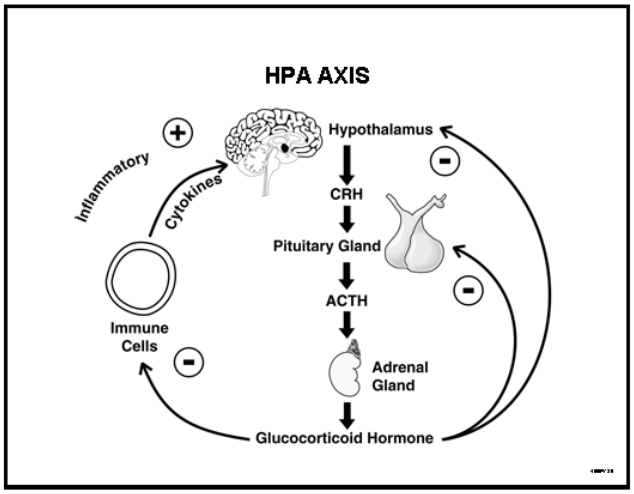
A GANGLIONIC BLOCKER ATTENUATES SARIN SUPPRESSION OF T-CELL PROLIFERATION

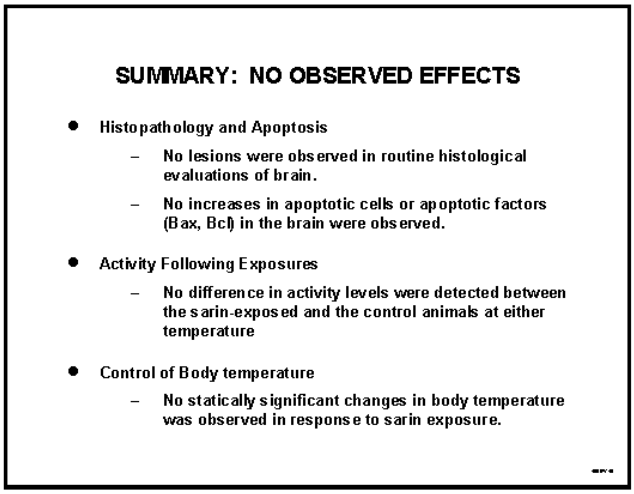




A simplified version how the CNS effects of sarin may be transduced to T lymphocytes







OBSERVED EFFECTS

- Subclinical exposures to sarin induced the expression of brain cytokines involved in neuroimmune responses.
- Combined exposure to sarin and heat stress caused a statistically significant increase in M3 receptor sites in the olfactory and adjacent areas of the brain that persisted for 30 days. There was a delayed (30 day) increase in M3 receptor sites in the hippocampus, a region of the brain important for memory and cognitive function.
- Sarin alone caused a decrease in M1 receptor sites in the olfactory tubercle. Sarin plus heat stress caused a delayed decrease in M1 receptor sites in the cerebral cortex, the olfactory region, the striatum and the hippocampus.

OBSERVED EFFECTS

- Repeated subclinical exposures to sarin inhibited antibody formation and antigen-mediated T cell proliferation.
- Changes in the T cell function may reflect impaired antigen-receptor-mediated signaling in T cells.
- Immunosuppression is not the result of increased CORT production; however, sarin may affect the immune system via the autonomic nervous system.
- Sarin and other cholinergic agents may decrease CORT levels, and serum CORT level may serve as biomarker for cholinergic exposure.

CONCLUSIONS

- Repeated subclinical exposure to sarin results in suppression of immune responses.
- Repeated subclinical exposure to sarin, especially under heat stress, results in persistent, as well as delayed, alterations in the density of acetyl choline receptor sites in areas of the brain responsible for memory and cognitive function.

REFERENCES

1. Henderson, R. F., E. B. Barr, W. B. Blackwell, C. R. Clark, C. A. Conn, R. Kalra, T. H. March, M. L. Sopori, Y. Tesfaigzi, M. G. Ménache and D. C. Mash: Response of Rats to Low Levels of Sarin. *Toxicol. Appl. Pharmacol. 184(2)*: 67-76, 2002.
2. Kalra, R., S. P. Singh, S. Razani-Boroujerdi, R. J. Langley, W. B. Blackwell, R. F. Henderson and M. L. Sopori: Subclinical Doses of the Nerve Gas Sarin Impair T Cell Responses Through the Autonomic Nervous System. *Toxicol. Appl. Pharmacol. 184(2)*: 82-87, 2002.
3. Conn, C. A., K. Dokladny, M. G. Ménache, E. B. Barr, W. Kozak, A. Kozak, M. Wachulec, K. Rudolph, M. J. Kluger and R. F. Henderson: Effects of Sarin on Temperature and Activity of Rats as a Model for Gulf War Syndrome Neuroregulatory Functions. *Toxicol. Appl. Pharmacol. 184(2)*: 77-81, 2002.

Presentation 5 – Nelda Wray

Relationship between
Illnesses in Gulf War Veterans and
Acetylcholinesterase Levels

Nelda P. Wray, M.D., M.P.H.
Chief Research and Development Officer
June 16, 2003

Principal Investigators

- Bradley Doebbeling, M.D., Iowa City VA Medical Center and University of Iowa
- Hermona Soreq, Ph.D., Hebrew University, Jerusalem

2

VA Collaboration with Dr. Soreq

- In Feb. 2003, Dr. Wray attended meeting of Research Advisory Committee on Gulf War Veterans' Illnesses.
- Very exciting presentation by Dr. Soreq, a leading researcher in field of neurotransmitters (chemicals that transmit signals in nervous system).
- Dr. Wray decided that VA should collaborate with Dr. Soreq to study illnesses in Gulf War veterans.
- This study was placed on fast track for planning and funding.

3

Background on Acetylcholinesterase and Illness

- Acetylcholinesterase (AChE) is an essential enzyme involved in transmission of signals between nerve cells.
- Abnormalities of AChE metabolism occur in neurological diseases:
  - Alzheimer's disease
  - Myasthenia gravis
- Some medications cause changes in AChE levels:
  - Some anti-depressants inhibit AChE activity (lower levels).
  - Some anti-psychotics induce release of AChE (higher levels).

4

Recent Study of Human AChE Levels by Dr. Soreq

- 470 members of American population.
- Strong correlation between levels of illness and serum AChE levels.
- As the reported levels of illness increased, the AChE levels decreased.
- No correlation between illness levels and levels of two other enzymes related to acetylcholine metabolism:
  - BuChE (Butyrylcholinesterase)
  - PON (Paraoxonase)

5

Exposure to AChE Inhibitors During the Gulf War

- **During Gulf War, troops were potentially exposed to a number of chemicals that inhibit AChE:**
  - Pyridostigmine bromide (PB)
  - Organophosphate pesticides (e.g., chlorpyrifos)
  - Low levels of nerve agents (e.g., sarin)
- **Dr. Soreq's hypothesis:**
  - Exposure to these chemicals could lead to long-term alteration of AChE function, which could lead to illnesses in some Gulf War veterans.

6

Objective of Collaborative VA-Israeli Study

- **To determine if serum levels of AChE are related to illnesses in Gulf War veterans and non-deployed veterans.**

7

Collaborative Roles of Research Team

- Dr. Doebbeling and Iowa scientists:
  - **Provide frozen serum samples**
  - **Provide data on illnesses in Gulf War and non-deployed veterans**
- Dr. Soreq and Israeli scientists:
  - **Analyze serum samples for levels of AChE**
- Statistical analysis by Cooperative Studies Program in West Haven, CT

8

Participants in Iowa Study

- Phase I:
  - In 1995-96, 3,695 veterans took part in telephone interviews.
  - 50% Gulf War veterans; 50% non-deployed veterans
- Phase II:
  - In 1999-2002, a subset of 580 veterans took part in detailed medical exams, including serum samples.
  - 50% Gulf War (GW) veterans; 50% non-deployed (ND) veterans
  - Four groups were evaluated (3 ill groups and one control group):
    - Ill: Cognitive dysfunction, Depression, or Chronic Widespread Pain (both GW and ND veterans)
    - Healthy controls: both GW and ND veterans
  - Frozen sera are available for these 580 veterans.

9

Planned Statistical Analysis

- Compare levels of AChE among Gulf War veterans vs. non-deployed veterans.
- Compare levels of AChE among ill veterans vs. healthy veterans.

10

Timeline of Study Development and Implementation

- **Feb. 2003: Dr. Wray meets Dr. Soreq and decides that VA should perform collaborative research with her.**
- **April 2003: Cooperative Studies Program planning meeting held with Dr. Soreq and Dr. Doebbeling.**
- **May 2003: Proposal written and IRB approval.**
- **June 2003: Shipment of serum samples to Israel.**
- **Late summer 2003: Preliminary results expected.**

11

Significance of Collaborative Research

- In 1999, RAND Corporation report on potential health effects of pyridostigmine bromide in Gulf War veterans.
  - Recommended that a study similar to this VA study with Dr. Soreq would be one of the definitive studies of illnesses in Gulf War veterans.
- This study could lead to better understanding of the underlying biochemical mechanism of illnesses in some Gulf War veterans.
- This collaborative research is responsive to recommendations of *Interim Report* of Research Advisory Committee on Gulf War Veterans' Illnesses.

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Presentation 6 – Jennifer Vasterling

Prospective Assessment of Neurocognition in Future Gulf-Deployed and Gulf-Nondeployed Military Personnel: A Pilot Study

Co-PIs: Jennifer J. Vasterling, Ph.D.
Susan P. Proctor, D.Sc.
Co-I: Robert Kane, Ph.D.
Consultants: Roberta F. White, Ph.D
Tim Heeren, Ph.D.
MAJ James Ness, Ph.D.

Funding: DoD/VA (USAMRMC-RADIII; VA Medical Research Service)

Scientific Advisory Board

COL Gary Gackstetter, DVM, MPH, Ph.D.

Matthew J. Friedman, MD, Ph.D.

COL Paul Amoroso, MD, MPH

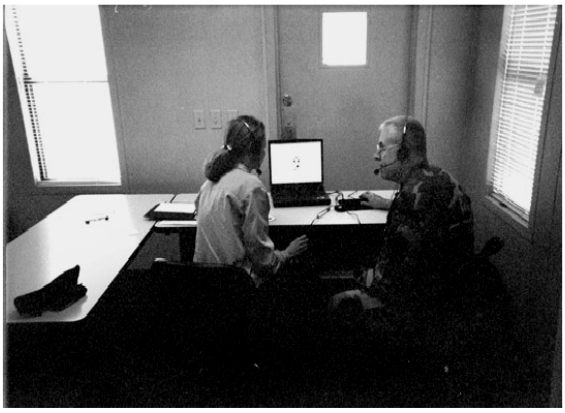
CDR Margaret A.K. Ryan, MD, MPH

Primary Objective

To examine prospectively neurocognitive outcomes related to Iraq deployment in deployed and comparable nondeployed Army troops.

Why Neurocognitive Outcome?

- Concentration and memory problems commonly reported among 1991 Gulf War veterans
- Adverse impact on daily life
- Some research suggesting CNS dysfunction in 1991 Gulf War veterans.
- Neuropsychological performance as an objective, "portable" screen of CNS integrity.



Why Prospective Assessment?

- Baseline and post-deployment assessments allow documentation of change over time.
- Addresses questions relative to pre-existing conditions.
- Assessment of change helps address interpretation of "subtle" deficits. (That is, what is minor to one person may represent a significant change to another.)

Design

- Prospective, longitudinal
  - Time 1: Baseline
  - Time 2: Post-deployment
- 3 primary samples of Army personnel:
  - Iraq- deployed (n = 600)
  - Non-deployed (n = 450)
  - (?) Sinai-deployed (n = 150)

Sample

- Iraq Deployed Sample (n = 600)
  - 4 units:
    - 2 combat/combat support
    - 2 service support
  - At least 1 unit Guard or Reserve
- Non-deployed Sample (n = 450)
  - 3 units:
    - 1 Active Duty combat/combat support
    - 1 Active Duty service support
    - 1 Guard or Reserve

Variable Domains

- Predeployment Variables

  Demographics, baseline cognition/mental status, prior trauma exposure, brain & nervous disease/risk factors, perception of unit cohesion, preparedness, and physical health, military variables

- Deployment Variables

  Deployment status, MOS, unit type, geographic location, objective environmental exposures, combat and stress exposure, self-reported environmental exposures

Neurocognitive Variables

- Functions robust to most acquired brain insults

  (e.g., vocabulary)

- Functions sensitive to potential deployment-related exposures

  (e.g., attention, working memory, learning, memory, motor, processing speed)

Data Sources

- Military Health and Personnel Records
- Military environmental exposure and geographic location data
- Self-report (deployment experiences, risk factors, health perception, mood & emotional symptoms)
- Objective neuropsychological performances

Data Analytic Approaches

- Repeated measures multivariate analysis of (co)variance to examine neurocognition over time by deployment group
- Multivariate stepwise regression to identify the unique contributions of independent variables to postdeployment cognitive performance (over deployment)

Units Assessed

- Combat Arms/Combat Support (Active Duty)
  n = 150
  98.7% (of 152)
- Combat Service Support (Active Duty)
  n = 151
  95.6% (of 158)
- Combat Support (National Guard)
  n = 53
  79.1% (of 67)
- Combat Service Support (Active Duty)
  n = 105
  71.4% (of 147)

Participant Characteristics

Age (yrs)	27.3 (7.2)	18-56
Education (yrs)	12.9 (1.4)	11 - 18
% women	16.6%	
Rank		
% E4 or below	59.0%	
% E5 – E9	35.1%	
% Officers	5.9%	
Race/Ethnicity		
% Caucasian	51.1%	
% African American	24.0%	
% Hispanic	15.0%	
% Other	9.9%	

Summary

- Prospective, longitudinal data collection, including baseline and post-deployment assessments
- Comparison of Iraq-deploying troops to nondeploying troops (and possibly Sinai-deploying troops)
- Primary outcome measure is neurocognitive performance

Presentation 7 – Beatrice Golomb

New Research Update 6-03

Beatrice Golomb, MD, PhD

Topics

- AChEi
- DU
- Vaccines
- Characterizing Illness
- Birth Defects (separately)

AChEi, Chemicals

Sarin delayed effects

- Adult male rats treated x3wks w/ either or both
- **Sarin (S) sc**: 62.5µg/kg, 0.5xLD(50) 3x/wk
- **PB po**: 80 mg/L in drinking water
- **Measure:** Passive avoidance, open field activity, acoustic startle, nociceptive threshold
- 2 wk: Sarin -> musc downreg in caudate/putamen & mesencephalon. Incr startle; Decr OFA
- 4 wk: no effect
- 16 wk: S incr, PB+S decr habituation in OFA. PB+S incr pain threshold. No change ChAT, AChE
- No effects of PB alone on *these* outcomes
- **Sxremin, O.U.**, *et al.*, *Delayed neurologic and behavioral effects of subtoxic doses of cholinesterase inhibitors.* J Pharmacol Exp Ther, 2003. **304**(3): p. 1111-9.

PB suppressed IL-8 cytokine release

- In vitro: Porcine skin flap model
- In vitro: Human epidermal keratinocytes
- Permethrin, DEET, both: + PB or DFP in medium (50 & 30 ng/ml)
- IL-8, TFalpha, PGE2 at1,2,4,8,12,24h
- IL-8 suppressed by PB at many times
- Effect on TNFalpha depends on vehicle

- **Monteiro-Riviere, N.A.**, et al, Pyridostigmine bromide modulates topical irritant-induced cytokine release from human epidermal keratinocytes and isolated perfused porcine skin. Toxicology, 2003. 183(1-3): p. 15-28.

DEET absorption enhanced by chems

- In vitro: Porcine skin flap & silastic diffusion
- (DEET flux sim to human skin, 2µg/cm2/h)
- PB or DFP or sulfur mustard or occlusion increase flux, to max of 5x

Tough to compare dose to that of PGWV

- * **Riviere, J.E.**, *et al.*, *Percutaneous absorption of topical N,N-diethyl-m-toluamide (DEET): effects of exposure variables and coadministered toxicants.* J Toxicol Environ Health A, 2003. **66**(2): p. 133-51.

GWV: PB assoc with cognitive dysfcn

- SS: 207 GW deployed & 53 era Veterans. (120 GWV referred for neuropsych evals; rest & era were treatment seeking veterans at Boston.)
- Exposures: PB: 44% GWV. PTSD: 13.5% overall.
- Tests: multiple neuropsych tests, dif domains
- Results: GWV worse on attention, motor, visuomotor, visual memory, mood, motivation (not exec fcn)
- PB exposed: worse on overall exec fcn, and card sort
- PTSD exposed: worse on depression, tension, POMS
- No change if exclude those with poor motivation score

- * **Sullivan K** et al. Cognitive Functioning in Treatment-Seeking Gulf War Veterans: Pyridostigmine Bromide Use and PTSD. J Psychopath & Behav Assessment, 25: 95-103.

Loss NTE links OP to hyperactivity

- SS: mice: NI & disrupx in Nte (gene for NTE)
- Nte-/-: Die embryo d8. (?defect nl tube closure)
- Nte+/-: 40% decr brain NTE. No change AChE.
- Nte+/-: Hyperactive (incr. locomotor activity)
- Nte+/-: More sensitive to OP exposure: EOPF
- -- Increased death from delayed OP toxicity (EOPF@ 6,10mg/kg)
- -- Lowers locomotion in +/-, Raises in +/+ (EOPF 1mg/kg)
- (85% inh NTE mouse brain at 5mg/kg in vivo):

- * **Winrow, C.J.**, et al., Loss of neuropathy target esterase in mice links organophosphate exposure to hyperactivity. Nat Genet, 2003. **33**(4): p. 477-85.

VACCINE

Vaccine: Macrophagic Myofasciitis

- Design: Review
- Findings; Al-adjuvanted vaccines may produce macrophagic myofasciitis (MMf)
- MMf SX: fatigue and myopathy. 50% meet CFS criteria.
- 1/3 develop an MS-like syndrome
- MMf Genetic Susceptibility: HLA-DRB1*01 (->PMR, RA)
- Vaccine site: persistence of Al adjuvant. Immunologically active lesion.
- Al associated because: EM, microanalytic studies, expts, epi
- WHO: advise study to link focal findings to immunolog active lesions
- "Strikingly similar" to Gulf War sx
- NOTE: Most people with Al-containing vaccines don't get this…
- BG Suggestion: Test HLA type in GWV with MS; ± test for MMf
- * **Gherardi, R.K.**, *[Lessons from macrophagic myofasciitis: towards definition of a vaccine adjuvant-related syndrome].* Rev Neurol (Paris), 2003. **159**(2): p. 162-4.

Depleted Uranium (DU)

DU Effects: Review

- Natural Uranium (U):ubiquitous in soil at 3mg/kg.
- Depleted Uranium (DU):259 tons munitions used, GW
- DU: same chemotoxicity as U: same # protons
- DU: ~40% of the radiotoxicity of U, dif speciation (less % low-half-life isotopes).
- α radiation dominates. (α radiation = pos charged ions w/ 2 neutrons, 2 protons.)
- Penetration range, "typical" 5MeV α radiation: ~4cm in air; 50μM soft tissue

* **Bleise, A.**, P.R. Danesi, and W. Burkart, *Properties, use and health effects of depleted uranium (DU): a general overview.* J Environ Radioact, 2003. 64(2-3): p. 93-112.

DU Effects: Review

- External exposure: thought safe: β,γ radiation.
- Internal exposure: a problem, even w/ short penetration.
- - DU dust: generated when DU hits target, inhalation may ->protracted exposure to lungs, other organ, esp particles < 10µM.
- - Soluble forms: more chemical risk, absorbed from lung to body. Insoluble forms: more radiation risk, stay put.
- - Embedded fragments: 2 orders magnitude incr. in bld/urine several years after exposure.
- - DU resuspension: after deposition on ground, if fine enough
- - DU in water/food: 2-5% ingested DU is absorbed; 90% leaves body within 1wk. Rest distributed - 10% to kidneys, most elim in a few wks. 15% to bone: at 5 & 25 yrs, sev% & 1% (respectively) remains in bone

DU Effects: Review

- Body load in GWV (urine, feces, hair, nail record): not > range for natural U.
- Exception: crews of military vehicles hit by DU
- For these: urine U .01-30.7µg/g creatinine (vs 0.1-0.05 nonexposed)
- "Observable health effects not expected" (with exception as noted).
- CA risk estimates m be based on theoretical considerations. Depends on actual speciation (238U, ~.2%235U)
- BG comments: Doesn't consider possible heavy metal immunological effects (cytokine, etc.)
- * **Bleise, A.**, P.R. Danesi, and W. Burkart, *Properties, use and health effects of depleted uranium (DU): a general overview*. J Environ Radioact, 2003. 64(2-3): p. 93-112.

Illness Characterization

Symptom patterns in Registry GWV

- **Design: mail survey completed by 1161 Registry GWV**
- **84.5% of respondents believed they had med problems attributable to GW service;**
- **5.3% did not answer. (~10% did not believe they did.)**
- **Symptom list: 48 symptoms grouped by organ**

- * **Hallman, W.K.**, *et al.*, *Symptom patterns among Gulf War registry veterans.* Am J Public Health, 2003. **93**(4): p. 624-30.

Symptom patterns in Registry GWV

- Exploratory factor analysis: 4 symptom factors.
  1. Mood/memory/fatigue
  2. Musculoskeletal
  3. Gastrointestinal
  4. Throat/breathing
- K-means cluster analysis: 2 groups
  1. Healthier, 60%: ave 18 sx: 33% mod, 11% severe
  2. Sicker, 40%: ave 37 sx, 40%mod, 35% severe
- Cluster 2 more likely to have ≥1 of 24 medical conditions
  - Includes FM, IBS, MS, CFS, depression, PTSD, bipolar, anxiety d/o, thyroid disase, DM, sterility. Hay fever, TB, eczema/prosriasis appear less frequent.
- * **Hallman, W.K.**, *et al., Symptompatterns among Gulf War registry veterans.* Am J Public Health, 2003. 93(4): p. 624-30.

Seminal Plasma Hypersensitivity - SPH

- Ss: 211 Gulf war males, questionnaire. (No females responded) Desensitization in sev females.
- Design: Survey -> medical testing. Desensitization done in some meeting criteria for seminal plasma hypersensitivity (SPH).
- Survey: 89% reported burning after contact with their own semen, or sex partner with burning after contact with their semen.
- 48% 1st noted on 1st sexual contact after war. < 50% couples had relief of sx with condom, vs 100% gen population.
- * **Bernstein JA**, *et al., Is burning semen syndrom a variant form of seminal plasma hypersensitivity.* Obstetrics and Gynecology 2003 101:93-102.

Seminal Plasma Hypersensitivity

Desensitization

- 67 female partners initially satisfied criteria of condom prevention or didn't answer, 43 from internet and 24 referred by VA GW physicians. 40% had full relief w condom (vs 75% in gen population w sx of SPH)
- Cohort control of 36 women in gen population with sx c/w SPH

Trend but no relation to PB, pesticides; less so vaccine

Assoc w eval & rx PTSD; involved in decontamination ops, p <.05.

Desensitization: 5 GWV, 2 Gen Population

- Using seminal proteins to which skin test reaction
- 3 of 5 GW complete relief, 1 partial. 1 of 2 gen population success.
- Responders -> spec IgE abs to seminal pl protein, nonresponders not.

* **Bernstein JA**, *et al., Is burning semen syndrom a variant form of seminal plasma hypersensitivity.* Obstetrics and Gynecology 2003 101:93-102.

Psychiatric d/o in PGWV: Review

Design: Systematic review

Articles: 2296 abstracts and 409 articles reviewed. Duplicate abstrax.

Abstract: Hypothesis, quality (resp rate, poss selex bias, outcome msrmt bias, data on confounders, adjustment)

Analysis: Summary OR/RR with random effects model with inverse variance due to heterogeneity ("METAN" command with stata), using studies with dichotomous outcomes

Result

PTSD: 11 studies. RR 2.9 (2-4.2). Mostly Unwin, Gray.

Common mental d/o: 11 studies: RR 1.8, 1.6-2.0. Mostly Kang, Unwin.

* **Stimson, N.J.**, 2003, Psychiatric disorder in veterans of the Persian Gulf War of 1991. Brit J

Perceived Exertion in GWV

- Ss: 15 GWV with CFS; 19 healthy GWV
- Intervention: Exercise to exhaustion on cycle ergometer
- Measure: Rating of Perceived Exertion (RPE); also as % of exercise capacity. (In CFS females: not elevated as a fraction of capacity.)
- Result: Higher RPE at each power output, $p < 0.001$;
- Result: Higher RPE/VO2max, too - diff from civilians
- Effect eliminated if adjust for preexisting fatigue

Need larger sample; nonGWV ctrl: look at other parameters

Ss at higher % peak VO2 at gas exchange threshold= point of onset of exercise induced metabolic acidosis (56% v 50.6%)*

*p < .05, CFS vs healthy. **Cook D.B.** 2003. Perceived Exertion in Fatiguing Illness: Gulf War veterans with chronic fatigue syndrome. Medicine & Science in Sports and xercise: 59-74

Olfactory Functioning

- **Ss: 82 GWV, 33 era activated.**
- **GW had more "concerns" about health, cognition, depression.**
- **Pennsylvania Smell Identification Test of hyposmia and anosmia (scratch & sniff): No difference**
- **Emotional distress correlated with self-report health/cognition**
- **Didn't test for adverse response to smell.**

- * **Vasterling**, J.J., *et al.*, *Olfactory functioning in Gulf War-era veterans: relationships to war-zone duty, self-reported hazards exposures, and psychological distress.* J Int Neuropsychol Soc, 2003. 9(3): p. 407-18.

Presentation 8 – Beatrice Golomb

Birth Defects
6-03

B. Golomb, MD, PhD

1994 GAO report

21 potential reproductive toxins in the Gulf

Unclear result of multiple agents: including insecticide, petroleum solutions, sandstorm dust, arthropod-borne pathogens, sarin, mustard gas, prophylactic drugs and meds, vaccines to military.

Review

Previous epi studies show paternal exposure to pesticides, solvents, metals associated with spina bifida, cardiac and renal defects, cleft lip and palate, clubfoot.

Paternal herbicide exposure positively correlated with spina bifida, per IOM report on health effects of Vietnam veterans to herbicide exposure (1996)

IOM 1996. Araneta 1997 Teratology 56: 244-51

Specialized studies

Penman 1996: no link Mississippi Natl Guard

Araneta 1997: no link to Goldenhar syndrome

Araneta 1997: no link Hawaiian GWV

Ishoy 2001: no link Danish GWV

(661 deployed, 215 nondeployed military men)

GWV - Gulf War veterans

1997: Goldenhar syndrome

In response to press suggestions of birth defects -- especially Goldenhar*

Goldenhar = oculoauriculovertebral. Microtia, ear tags, or anotia, & variable eye,face, spine

Military hospital births post GWV to 10-93

34,069 GWV infants; 41,345 NDV infants

7 with Goldenhar: 5 GWV, 2 NDV. All fathers in military at conception and birth

14.7/100,000 live births GWV (5.4-36.4)

4.8/100,000 NDV (0.8-19.5) (NDV=nondeployed veterans)

RR = 3.03, 0.63-20.57, p = 0.26

Araneta 1997 Teratology 56:244-51. Cites Briggs 1995 & Sylvester 95

Hawaiian GWV

Goal: Examine specific birth defects

Sample: Link personal identifiers of GWV (n=684,645) and NDV (1,587,102) and families against live births in Hawaii Dept of Health 1989-93

Subjects: 3717 GWV infants; 13,465 NDV infants

Results:

367 (2.14/100 live births) identified with ≥1 major birth defect dx

Similar prevalence for GWV & NDV prewar and postwar; and GWV infants conceived before & after the war

BUT small # each birth defect category

Araneta 2000 Teratology 62:195-204

Cowan

Subjects: Military hospital liver births (135 military hospitals)
Live births before 10-1-1993 (1991, 1992, 1993) with est conception after return from PG.
For nondeployed: as above but est date conception after 12-31-90.
Each birth, including each of multiple births, was treated as an independent, event
N=30,151 to wives of 29,468 male GWV; 32,638 to wives of 31,646 NDV
N=3847 to 3722 female GWV; and 8825 to 8494 female NDV

Outcome: Birth defects as noted in the medical file. Also, ratio M:F.
Exposure: Days of deployment - no association; interval from return to birth.

Comment: Afr Amer and single more likely to report 'any birth defect'
Navy less likely and army more likely to be deployed.
Adjustment for race/ethnic, marital status, branch of service; age At Delivery!

Adjustment: Age at delivery [should be at conception]; race; ethnicity; marital status
Cowan DN et al. The risk of birth defects among children of Persian Gulf War veterans. NEJM 1997 336:1650-6.

Cowan

	SEVERE BIRTH DEFECTS: RR (95% CI) Crude	Adjusted
Active Duty Men	1.03 (.92-1.15)	NOT GIVEN!
Active Duty Women	1.00 (.90-1.10)	NOT GIVEN!
	ALL BIRTH DEFECTS: RR (95% CI) Crude	**Adjusted**
Active Duty Men	.99 (.93-1.05)	.97 (.91-1.03)
Active Duty Women	1.12 (1.00-1.25)	1.07 (.94-1.22)

Adjustment: Age at delivery [should be at conception]; race; ethnicity; marital status
Cowan DN et al. The risk of birth defects among children of Persian Gulf War veterans. NEJM 1997 336:1650-6.

DoD birth defects registry

- Established Naval Health Research Center, San Diego, in 1998.
- Captures comprehensive data on healthcare utilization to calc prevalence of birth defects in children of military beneficiaries.
- Population-based electronic surveillance supplemented by active case validation.
- Has captured (by publication) data on >90,000 births in military families each year.
- Detailed analyses linking with exposure data, e.g. anthrax vaccination, are under way.

Ryan M.A.K. 2001. Teratology 64:S26-29. The Dept of Defense birth defects registry: Overview of a new surveillance system.

More on DoD birth defects registry

- Compared health record abstraction (active surveillance) with screening electronic medical data (passive surveillance") to detect birth defects among San Diego County military families from 1-1-97 to 6-30-98.
- 171 of 5351 infants (3.2%) identified with major defect. C/w national civilian rates.
- ~80% concordance between "passive" and "active" approaches suggesting use of hybrid with electronic supplemented by active surveillance in a specific region.

Bush R.A. 2001. Military Medicine 166: 2:179. Active surveillance of birth defects aong US Dept of Defense beneficiaries: A feasibility study

Kang Study: Reproductive Outcomes

Subjects: 15,000 GWV; 15,000 era veterans. Stratified random sample. Actually: 3397 GWV; 2646 Era 'had an index pregnancy". 4712 men. 1331 women.

Design: Mailed 16 page Health survey

Included questions on: fetal death (miscarriage <20wk, stillbirth>20wk, other); gestational length; death within 1 year. (Not elective abortions.)

Examined 1 pregnancy per veteran (vs each birth counted separately), the first *ending* after June 30, 1991

Kang H et al 2001. Pregnancy outcomes among US Gulf War veterans: a population-based survey of 30,000 veterans. *Ann Epidemiol 11:504-511.*

Kang Study: Reproductive Outcomes

Outcome:	GWV	ERA	OR	ADJ
Pregnancies				
Male	2739	1934		
Female	632	691		
Live Births				
Male	2236	1689	.64* (.54-.76)	.64* (.55-.76)
Female	471	577	.58 (.44-.76)	.60 (.46-.79)
Stillbirth				
Male	38	16	1.69 (.91-3.16)	1.65 (.91-2.98)
Female	9	7	1.41 (.48-4.22)	1.26 (.46-3.49)
Spont Abortions				
Male	327	148	1.64(1.33-2.02)	1.62 (1.32-1.99)
Female	92	77	1.36 (.97-1.90)	1.35 (.97-1.89)

Adjusted: race, age at outcome, ground vs non troop, active military vs Nat Guard or Reserves; hx smoking; hx prior pregnancy; calendar yr of pregnancy outcome.

Kang H et al 2001. Pregnancy outcomes among US Gulf War veterans: a population-based survey of

Kang Study: Reproductive Outcomes

Outcome:	GWV	ERA	OR	ADJ
Other* (could include elective abortion, currently pregnant)				
Male	138	81	1.21 (.91-1.62)	1.29 (.96-1.72)
Female	60	30	2.31 (1.44-3.73)	2.18 (1.37-3.46)

Adjusted: race, age at outcome, ground vs non troop, active military vs Nat Guard or Reserves; hx smoking; hx prior pregnancy; calendar yr of pregnancy outcome.

Kang H et al 2001. Pregnancy outcomes among US Gulf War veterans: a population-based survey of 30,000 veterans. *Ann Epidemiol 11:504-511.*

Kang Study: Reproductive Outcomes

Outcome:	GWV	ERA	OR	ADJ
Pre-term				
Male	233	150	1.21 (.97-1.51)	1.2 (.97-1.5)
Female	61	69	1.08 (.73-1.59)	.99 (.68-1.45)
Birth Defects				
Male	202	68	2.37 (1.77-3.17)	2.34 (1.76-3.10)
Female	41	21	2.52 (1.43-4.49)	2.85 (1.62-4.99)
Likely Defects				
Male	120	47	1.98 (1.39-2.83)	1.94 (1.37-2.74)
Female	26	13	2.53 (1.23-5.27)	2.97 (1.47-5.99)
Mod to severe				
Male	82	35	1.80 (1.18-2.74)	1.78 (1.19-2.66)
Female	19	10	2.38 (1.04-5.56)	2.8 (1.26-6.25)

Adjusted: race, age at outcome, ground vs non troop, active military vs Nat Guard or Reserves; hx smoking; hx prior pregnancy; calendar yr of pregnancy outcome.

Kang Study: Reproductive Outcomes

Outcome:	GWV	ERA	OR	ADJ
Infant deaths (<1yr), all causes				
Male	12	12	0.75 (.32-1.79)	0.76 (.34-1.72)
Female	3	6	0.61 (.12-2.75)	0.80 (.19-3.38)

Adjusted: race, age at outcome, ground vs non troop, active military vs Nat Guard or Reserves; hx smoking; hx prior pregnancy; calendar yr of pregnancy outcome.

Kang H et al 2001. Pregnancy outcomes among US Gulf War veterans: a population-based survey of 30,000 veterans. *Ann Epidemiol 11:504-511.*

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Adjusted: race, age at outcome, ground vs non troop, active military vs Nat Guard or Reserves; hx smoking; hx prior pregnancy; calendar yr of pregnancy outcome.

Occurrence among 4973 live births: GWV male 2236; Era male 1689; GWV female 471; Era female 577.

Kang H et al 2001. Pregnancy outcomes among US Gulf War veterans: a population-based survey of 30,000 veterans. *Ann Epidemiol 11:504-511.*

Kang Study: Breakdown of defects

Percent infants with defect:	GW%	Era%
Total	5.39	2.65
Isolated anomaly (*X chromosomal & Heritable)	4.1	1.77
Multiple anomalies*	.26	.49
Undescribed isolated cardiac abnormality	.48	.22
Chromosomal	.26	0.0
Congenital malignancy	.04	0.0
Heritable genetic disease	.11	0.0
Other poorly described noncardiac defect	.15	.18

Occurrence among 4973 live births: GWV male 2236; Era male 1689; GWV female 471; Era female 577.

Kang H et al 2001. Pregnancy outcomes among US Gulf War veterans: a population-based survey of 30,000 veterans. *Ann Epidemiol 11:504-511.*

UK reproductive outcomes study

Design: Postal questionnaire, retrospective cohort study of UK GWV vs nondeployed, to 25,085 GWV (24379men); 19,003 era (18439 men).

Ss: 16442 GWV/11517 era, men. 484 GWV/ 377 era, women.

Response rate^: Men: 53% GWV, 42% era. Women 72% GW, 60% era.

Data: All liveborn children: congen defects; serious med conditions ever; death date. Fertility/miscarriage/ectopic pregnancy. Abortions & abnormalities in conceptus. Participant's health. Partner's health. Changes in health since 1991. Info on any partner with whom conceived pregnancies who ever served in Armed Forces.

Missing info: checked by letter or phone. * **Maconochie N**

^Adjusted for undelivered mail. Nonresponder study: response status unrelated to reprod.

UK reproductive outcomes

- ICD 10 code for congen anomalies done blinded.
- Clinical verification of reported conditions: asked for detail of doctor of mother of all reported pregnancies and of children. Asked permission to access medical notes.
- Fetal deaths: Male*: 77 GWV:60 NDV. RR= 0.90
- Miscarriage ≥16 weeks: 175:127 (not divided by sex): RR= 0.97
- Congen malformation,M*: 686:342, RR1.41(p=0.07)
- Congenital malformation, female
- ? If selection bias. Low response rate.

* * Veteran was male
* Maconochie N et al. BMC Public Health 2003, 3:4.

UK birth defects (all): Male veteran

DEFECT	RR	GW 16442	ERA 11517
Metab & sgl gene	1.94	22	8
Musculoskeletal	1.75	194	78
Other, nonc'somal	1.66	45	19
Genital system	1.66	45	19
Urinary system	1.51	103	48
Any congen malfor	1.41	686	342
Digestive system	1.37	72	37
CNS	1.36	58	30
Cranial neural crest	1.36	184	101
Eye,ear,neck,face	1.29	22	12
Circulatory	1.20	126	74
Respiratory	1.06	18	12
Cleft lip/palate	1.05	21	14
Chromosomal	0.86	49	40

UK birth defects (confirmed): Male vet

DEFECT	RR	GW 16442	ERA 11517
Metabolic & single gene	1.23	7	4
Musculoskeletal	1.44	92	45
Other, nonchromosomal	1.03	22	15
Genital system	1.12	19	12
Urinary system	1.49	55	26
Any congenital malformation	1.18	330	196
Digestive system	1.09	31	20
CNS	1.12	27	17
Cranial neural crest	1.13	92	57
Eye,ear,neck,face	1.17	10	6
Circulatory	0.92	60	46
Respiratory	2.10	9	3
Cleft lip/palate	Infin	14	0

UK birth defects (all): Female veteran

Maconochie N et al. BMC Public Health 2003, 3:4.

- **All birth defects (combined, due to small N)**

	RR	GW, n= 705	ERA, n= 564
All	1.69	19	9
Confirmed	1.60	10	5

US birth defects

Design: link military & birth certificate records

Subjects: 11961 GWV infants; 33052 NDV infants

- **684,645 GWV and 1587,102 NDV military records**
- **2,314,908 birth certificate records from states with active ascertainment**

48 diagnoses considered:

- **46 diagnoses routinely obtained by states.**
- **Excluded pulm artery anomalies: require dx'ic echo**
- **Added items of concern to veterans: Goldenhar's, chromosomal, & dextrocardia.**

Araneta, M.R.G. et al. Birth Defects Research 67: 246-260 2003. Prevalence of birth defects among infants of Gulf War veterans in Arkansas, Arizona, California, Georgia, Hawaii, and Iowa, 1989-1993

US birth defects

Design: Link military & birth certificate records from states that conducted active case ascertainment on birth defects from 1989-1993.

Subjects: 11961 GWV infants; 33052 NDV infants

450 GWV mother; 3966 NDV mother

Drawn from:

684,645 GWV and 1587,102 NDV military records

2,314,908 birth certificate records from states with active ascertainment

Araneta, M.R.G. et al. Birth Defects Research 67: 246-260 2003. Prevalence of birth defects among infants of Gulf War veterans in Arkansas, Arizona, California, Georgia, Hawaii, and Iowa, 1989-1993

US birth defects

Mother's Age: Younger in GWV than NDV

25.3 v 25.9 yrs, Vet. Mom*; 25.3vs 26.0* Vet. Dad

Age >35: 3.6 v 6.5%, Vet. Mom; 4.3 vs 5.9%, Vet Dad*

Age 20-24: 48 v 42%, Vet Mom ; 39 v 34%, Vet. Dad*

Father's Age:

↓ in GWV but breakdown not given (NS)

Araneta, M.R.G. et al. Birth Defects Research 67: 246-260 2003. Prevalence of birth defects among infants of Gulf War veterans in Arkansas, Arizona, California, Georgia, Hawaii, and Iowa, 1989-1993

US birth defects

GWV mothers, vs NDV mothers: 450 vs 3966

Less caucasian: 51 vs 60%; More often AA* (38 vs 31%)

Less married: 72 vs 77%*

Less completed high school: 45 vs 47%*

Less active duty: 76 vs 88%*

More Reserve or National Guard: 24 vs 12.4%*

Sl less smoking mothers: 7.2 vs 8.9%, NS

No dif or sl less alcohol, mothers: 1.4 vs 1.5%;
8.9 vs 9.5% prenatal alcohol, born to male GWV vs NDV

Fewer male births: 48.1 vs 50.1%, NS

Fewer multiple births: 1.6 vs 2.5%, NS (young vs sick?)
1.8 vs 2.4% for those born to male GWV *

*p < .05

Araneta, M.R.G. et al. Birth Defects Research 67: 246-260 2003. Prevalence of birth defects among infants of Gulf War veterans in Arkansas, Arizona, California, Georgia, Hawaii, and Iowa, 1989-1993

US birth defects

Prewar: 48 selected birth defects: No dif or trend toward fewer in GWV

- **GWV vs NDV females: 0.7% vs 2.3%, RR 0.31, NS**
- **GWV vs NDV males: 1.56% vs 1.76%, RR 0.88, NS**
- **None of a list of 48 were significantly different for males (despite mult comparisons). Estimates given for females only for the sole case where there was a defect in a GWV.**

*p < .05

Araneta, M.R.G. et al. Birth Defects Research 67: 246-260 2003. Prevalence of birth defects among infants of Gulf War veterans in Arkansas, Arizona, California, Georgia, Hawaii, and Iowa, 1989-1993

US birth defects

Postwar: 48 selected birth defects:

- Females: hypospadias/epispadias 6.4 (1.5-26.8)
- Males: 8 neg trend; 13 positive trend

Males, Effects GWV vs NDV:

Tricuspid insufficiency 2.7 (1.1-6.6) .039

Aortic valve stenosis: 6.0 (1.2-31.0) .026

Males, Effects GWV post- vs pre-deployment: (dif N)

Aortic valve stenosis: 16.3(.09-294) .01? (5 vs 0)

Renal agenesis or hypoplasia: 16.3 (.09-294), .01? (5 vs 0)

Trend to fewer chromosomal anomalies: 0.2 (.03-1.6)

Trend to fewer trisomy 13 specifically: 0.6 (.2-1.9)

None signif better than NDV; or than pre-war

*p < .05. Araneta, M.R.G. et al. Birth Defects Research 67: 246-260 2003. Prevalence of birth defects among infants of Gulf War veterans in Arkansas, Arizona, California, Georgia, Hawaii, and Iowa, 1989-1993

US birth defects

Expect some differences by chance

Difficult to exclude chance as the source of these findings.

*p < .05

Araneta, M.R.G. et al. Birth Defects Research 67: 246-260 2003. Prevalence of birth defects among infants of Gulf War veterans in Arkansas, Arizona, California, Georgia, Hawaii, and Iowa, 1989-1993

Effects of Gestational Chlorpyrifos

Subjects: Pregnant rats exposed; offspring examined

Exposure: Oral chlorpyrifos in corn oil. 0,3,5,7 mg/kg, gestation day 6 to 20. (3mg/kg ->10% CNS ChEi.)*

- Low level ∀: below overt effects (moms or pups)

Outcomes: brain, heart, lung, serum ChE; brain ChAT; liver carboxylase activity. Postnatal day (pnd) 1,3,6,9,12 (sacrificed pups)

Brain ChE inhibition: 26-45%: persist to pnd 6; pnd 9 if hi-dose. (ChE starting to look ?>control by d 12, should look farther)

Liver carboxylesterase inhib, dose dependent, recover with ChE

Nonspecific esterases equally inhibited by all doses.

Delayed reduction in ChAT: 1st noted pnd9, more signif pnd 12).

Richardson et al 2003. J Toxicol Environ Health A 66:275-89. Effect of gestational exposure to chlorpyrifos on postnatal central and peripheral cholinergic neurochemistry

More on AChEi…

AChEi in animals, in many studies, leads to birth defects.

In some studies not.

May depend on agent, timing, duration exposure, and brain regions/outcomes examined

Considered to be "behavioral teratogens"

Appendix B

Public Submission 1 – Albert Donnay

Albert Hamburger Donnay, MHS
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6/16/2003
PUBLIC COMMENT SUBMITTED TO THE
VA RESEARCH ADVISORY COMMITTEE ON GULF WAR VETERANS' ILLNESSES

I request that this committee make it a priority to review the published findings and current status of VA-funded research into the role of:

1) MULTIPLE CHEMICAL SENSITIVITY (MCS) in Gulf War Syndrome, and the degree to which VA has incorporated these research findings into its programs for education of Gulf War veterans and physicians, medical care and compensation.

If you do so, you will find that FIVE years after VA researchers (Kang et al) first announced at the 1998 International CFS Conference in Boston that MCS was the single most common diagnosis among deployed Gulf War (1) veterans (afflicting 15%, over 100,000), the VA has not yet expanded its MCS research and has not yet begun to diagnosis, treat, code or even screen Gulf War veterans for MCS in its Gulf War Registry or other clinical programs. VA also has never explained its neglect of MCS. But more than neglect is involved: Gulf War vets given an MCS diagnosis by non-VA physicians still find that VA denies their requests for MCS treatment and compensation. Please ask the VA why!

In comparison, the VA has recognized both by CFS and Fibromyalgia as Gulf War service related since 1998 (and thus compensable based on disability), even though their COMBINED prevalence among Gulf War veterans is less than that of MCS.

2) CARBON MONOXIDE POISONING (CO) in Gulf War Syndrome, and the degree to which VA has incorporated these research findings into its programs for education of Gulf War veterans and physicians, medical care and compensation.

. If you do so, you will find that the VA has not funded any studies on either the CO levels or CO health effects that Gulf War veterans may have encountered from various types of CO exposure in the Gulf. CO levels in various military occupations could be easily measured by having troops wear small datalogging CO detectors –they cost under $600--while conducting live fire exercises (especially from inside ships and vehicles), driving vehicles, sleeping inside heated but unvented tents, etc. Such CO exposures were ubiquitous in the first Gulf War. The failure to consider CO is astounding given that all the reported symptoms of GWS are all also symptoms of CO poisoning, including MCS and particularly multi-sensory sensitivity (to previously tolerated levels of smell, light, sound, flavors, hot weather, and touch), which is a CO hallmark. Even though oxygen is the standard treatment for CO poisoning, and even though VA has funded studies showing veterans have impaired oxygenation of their brains and blood, VA has never researched or offered O2 treatments to Gulf War vets with CO symptoms. Please ask the VA why!

Reviews written by VA researchers of literature on similar post war syndromes prior also never mention CO, but CO was first associated with shell shock and neurasthenia in The Lancet in 1916 (Feb 12, 331-8), and acknowledged there as a significant battlefield toxin by no less an authority than the director of the British Army's Trench Warfare Department.

Note that of all VA researchers, only Dr. Virginia White of the Boston VA Environmental Hazards Research Center has published on both MCS (in Gulf War veterans) and CO poisoning (in civilians) but only separately, without acknowledging any connection between them. Please ask her why!