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Gulf War (GW)Veterans and/or their sexual partners have been experiencing burning, pain and swelling of the urogenital tract after exposure to semen since returning from the Persian Gulf which has been referred to as "Burning Semen Syndrome" (BSS). The objectives of this research project are 1) to identify the prevalence of BSS; 2) evaluate GW veterans and their sexual partners with BSS; 3) to determine if the underlying mechanism(s) of BSS is immunologic, infectious and/or toxicologic in nature; 4) to determine if the onset of BSS is related to chemical and/or biologic exposures encountered by GW veterans during their tour of duty in the Persian Gulf and; 5) to identify potential treatment(s) for BSS. The second year of this project has concentrated on obtaining completed questionnaires from the GW veteran and their spouse or sexual partner, obtaining screening laboratory tests to exclude an obvious underlying cause for their symptoms (ie. sexually transmitted diseases), establishing the respective prevalence of BSS and localized/systemic seminal plasma protein hypersensitivity among the GW and civilian populations, obtaining body fluids from GW and civilian couples (sera and semen) to further investigate immunologic and infectious etiologies for their symptoms and finally, identification of control populations.

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Principal Investigator 10/20/98
Date

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I. Introduction:

Gulf War (GW)Veterans and/or their sexual partners have been experiencing burning, pain and swelling of the urogenital tract after exposure to semen since returning from the Persian Gulf. This phenomenon has been referred to as "Burning Semen Syndrome" (BSS). The objectives of this research project are 1) to identify the prevalence of BSS; 2) evaluate GW veterans and their sexual partners with BSS; 3) to determine if the underlying mechanism(s) of BSS is immunologic, infectious and/or toxicologic in nature; 4) to determine if the onset of BSS is related to chemical and/or biologic exposures encountered by GW veterans during their tour of duty in the Persian Gulf and; 5) to identify potential treatment(s) for BSS. This report will discuss the progress of each of these objectives over the past two years.

Prior to discussing the progress of our research, it is important to review the background of this problem. Seminal plasma protein reactions in civilian populations of women have been previously well described.[1-4] Women who experience post-coital anaphylaxis have been demonstrated to produce specific IgE antibodies to seminal plasma proteins.[2] These women have been successfully desensitized using relevant homologous seminal plasma protein antigens obtained from their sexual partner.[2-4] Subsequently, women experiencing localized vaginal inflammation, characterized by burning and pain and occurring immediately after contact with their sexual partner's semen, were also successfully treated with seminal plasma protein desensitization in the majority of cases. This suggested that some post-coital localized vaginal reactions may be IgE-mediated.[1] A questionnaire survey distributed to 1,073 women who suspected they might have symptoms consistent with localized and/or systemic seminal plasma protein hypersensitivity revealed that 12% fulfilled the diagnostic criteria for this disorder. This survey indicates that seminal plasma protein hypersensitivity reactions are more common than previously reported.

The initial hypothesis of this project postulated that BSS occurred secondary to specific IgE antibody responses to one or more seminal plasma proteins. This hypothesis was based on observations that civilian women diagnosed and successfully treated for localized vaginal seminal plasma protein hypersensitivity, experienced similar reactions.[1] Therefore, our clinical experience investigating seminal plasma protein hypersensitivity in civilian female populations provided the foundation for the current investigation of GW veterans and their sexual partners with BSS.

The first year activities focused on identifying the scope of this problem. This required establishing contacts with: 1) GW veterans with and without BSS; 2) GW screening physicians at local and remote Veterans Administration Hospitals; 3) veterans organizations such as the American Legion, AmVets, and Veterans of Foreign Wars and; 4) other advocates of GW veterans. A significant amount of time was devoted to publicizing this project to the news media in order to inform the general public and GW veterans about BSS. Several magazines (ie. Men's Health, Science News, Playboy...) and newspapers published reports on BSS. Major radio and television news wires (i.e. Reuters, NBC) aired stories regarding BSS. This media exposure successfully heightened the public's awareness of BSS and our investigation of this problem in GW veterans. Many GW veterans with symptoms suggestive of BSS subsequently expressed interest in participating in this project. The most effective means of identifying this population to

date has been through our internet web page.

The focus of the project during the second year has been to 1) build our data base by obtaining completed questionnaires from the GW veteran and their sexual partners (if they had one); 2) obtain screening laboratory tests to exclude an obvious underlying cause for their symptoms (ie. sexually transmitted diseases, chronic vaginal candidiasis); 3) establish the prevalence of BSS and localized/systemic seminal plasma protein hypersensitivity among the GW and civilian populations, respectively; 4) obtain body fluids from GW and civilian couples (sera and semen) to further investigate immunologic and/or infectious etiologies for their symptoms; 5) identify cohort GW control populations and; 6) correlate symptoms to exposure using an existing geographical information modeling system.

II. Body:

A. Experimental Methods/Procedures

Questionnaires:

A web page was established on the Internet to identify GW veterans deployed to the Persian Gulf with and without BSS (see Appendix I). The web page includes two questionnaires (see Appendix II) to be completed by the GW veteran and his sexual partner. These questionnaires can be easily transmitted back to our site by E-mail. Questionnaires #1 and #2 were also mailed to the 120 GW veterans who were previously screened at the Cincinnati VAH Gulf War clinic for general health problems or any individual with symptoms consistent with BSS who learned about this project through word of mouth. All individuals who responded to the screening questionnaires were sent more detailed questionnaires to further elucidate details about their symptoms and GW exposures (see Appendix III). Separate questionnaires were designed for the male and female. This questionnaire packet also included screening surveys for post-traumatic stress disorder (PTSD). The questionnaire inquiring about BSS symptoms was modified from a standard questionnaire previously used to evaluate women with seminal plasma protein hypersensitivity reactions. Follow-up phone calls to encourage questionnaire completion and their prompt return have been made on a regular basis.

We have attempted to have questionnaire #2 distributed by local gynecologist/obstetrician offices to their patients in order to determine a more accurate prevalence of seminal plasma protein hypersensitivity among civilian women. We anticipated over 1,000 responses to this questionnaire from women in the Greater Cincinnati area. Thus far the response rate to this questionnaire has been very low which we believe either reflects the low prevalence of this problem in the general population or an uneasiness by patients with the subject matter in the questionnaire.

Concomitantly, the Cincinnati VAH has been selected as one of 11 centers participating in a multicenter project designed to randomly evaluate 1,000 GW families in the Greater Cincinnati area. When completed, approximately 11,000 couples will have completed questionnaire surveys. As a co-investigator of this project, I was successful in lobbying for the addition of specific questions regarding BSS to be included in this survey (see Appendix IV). The information collected from these questionnaire responses should provide a fairly accurate prevalence of BSS among GW couples.

Clinical Evaluation of GW veterans:

Gulf War veterans and their sexual partners who consent to participate in this project are required to undergo screening blood tests and cultures to exclude bacterial, fungal and viral infections or other medical disorders (ie. diabetes mellitus, chronic yeast infections, prostatitis...) which could be causing or contributing to their symptoms (see Appendix V). All GW veterans and their sexual partners are skin tested using the "prick" method to assess their allergic status. Skin testing is performed to box elder (tree), fescue (grass), short ragweed, *Alternaria* (outdoor mold), *Mucor* (indoor mold), cat, and dust mite in addition to a positive histamine and negative saline control. A fresh ejaculate is collected from each male at the time of the initial evaluation. A small portion of the ejaculate is used for prick skin testing of the male and female in order to determine if either elicits an immediate hypersensitivity reaction. The remaining portion of the sample is sent for semen cultures. All females undergo a pelvic examination which includes a pap smear, vaginal and/or cervical cultures. Finally, serum and an additional semen specimen is obtained from the male and serum from the female to screen for specific IgG, IgA and IgE antibodies to the male's seminal plasma proteins and to other unrelated male seminal plasma proteins by ELISA.

Processing of Semen

Semen specimen are all specimens are allowed to liquify at room temperature for 1 hr, and the pH is checked. The specimen is transferred to a high-speed centrifuge tube and an equal volume of phosphate-buffered saline (for specimens that are to be used for treatment) or Tris-buffered saline (for specimens to be used for analytical purposes) is added. The specimen is centrifuged at 30,000 X G for 1 hr at 4°C in a JA-14 rotor in a Beckman J2-21M high speed centrifuge. The supernatant fluid, whole seminal plasma, (which is usually a pale straw color and completely clear) is removed, leaving approximately 1 ml of fluid to avoid removing any pelleted material. To the pellet is added 1 ml of PBS or TBS (see above) and the pellet is allowed to soak in the fluid overnight at 4°C. The next day the pellet is vortexed to liquify the material and is immediately frozen at -75°C. In those cases where the seminal plasma is to be used for treatment, the fluid is dialyzed against PBS with three changes of the outer dialysis fluid. The seminal plasma is then aliquoted and also frozen at -75°C. **Previous comparison studies evaluating SDS-PAGE protein patterns of fresh whole seminal plasma to pooled ejaculates collected and stored over several days revealed no differences.**

Direct Competitive ELISA:

IgG, IgA and IgE ELISA is performed using whole seminal plasma obtained from the GW male subject and asymptomatic civilian male controls. A Costar flat-bottom, 96-well polystyrene plate (Corning) is coated with 100 µl of seminal plasma protein previously diluted to concentration of 10 µg/ml with 0.15 mol/L NaCl. The plate is incubated for two hours at room temperature with 0.15 mol/L tween-phosphate buffer saline to block for unreacted sites. Both the GW veteran and their sexual partner's serum is diluted 1:5 and added in triplicate to the microtiter wells. The plate is allowed to incubate for 24 hours at room temperature. For IgG and IgA antibody detection, alkaline phosphatase conjugated goat anti-human IgG and IgA (Sigma) respectively, are diluted 1:2000 and added to each well. After the plate incubates for one hour at room temperature, 100 µl of 1 mg/ml p-nitrophenyl phosphate substrate is added to each well. The enzyme reaction is

allowed to proceed for 30 minutes and then stopped with KOH. The optical density of each well is measured using a microplate ELISA reader at 405 nm. For IgE antibody detection, goat anti-human IgE (Kirkegard and Perry) diluted 1:1000 is added is each well and incubated for one hour at room temperature. The plate is then washed and alkaline-phosphatase labeled rabbit anti-goat IgG diluted to 1:2000 is added to each well. After the plate incubates for one hour at room temperature, the optical density is determined as described for IgG and IgA isotype specific antibodies.

Column Chromatography of Seminal Plasma

Ten ml of seminal plasma is chromatographed on a Sephacryl S-200 HR [High Resolution] Hi-Prep 26/60 column (Amersham Pharmacia Biotech) using PBS, pH 7.4, as the running buffer. The column is controlled with a computerized FPLC unit, and the absorbance of the effluent is monitored at 280 nm. Fractions of 5 ml are collected and fraction pools are made of the peaks according to the readout in the UV chromatogram. Separate columns are used for civilians and Persian Gulf War veterans, but the column is cleaned with 0.25M NaOH-1M NaCl between patient specimens. Molecular weights are estimated by comparison with a set of known molecular weight standards (also from Pharmacia), which are run approximately monthly when the columns are in use.

Affinity Chromatography

The globulin fraction of serum from GW and spouses is precipitated with 40% saturated ammonium sulfate, using two separate precipitations. The globulins are dissolved in PBS, pH 7.4 and held at -20°C until used. Immediately prior to affinity chromatography the globulins are dissolved in coupling buffer (0.2 M $NaHCO_3$-0.5 M NaCl, pH 8.3) by running through a PD-10 column (Amersham Pharmacia Biotech), which contains Sephadex G-25, equilibrated in the buffer. The concentration of globulins is adjusted to 10 mg/ml, as determined using the Pierce BCA Protein Assay. 1 ml of globulin (10 mg) is coupled to the matrix of a Hi-Trap NHS [N-hydroxysuccinamide]-activated column, 1 ml size at room temperature for 30 min, and the excess material is washed out and unbound coupling sites blocked with successive washes with 0.5 M ethanolamine-0.5 M NaCl alternating with 0.1 M Na acetate-0.5 M NaCl. The coupling efficiency of this method is 95%.

Whole seminal plasma (WSP) is transferred to adsorption buffer (0.075 M Tris-HCl, pH 8.0) in a PD-10 column, and run through the coupled column at a flow rate of 0.2 ml per minute. Those proteins of the WSP which are not bound to specific antibody in the coupled globulins are collected in a separate tube, and the column is rinsed with 15 ml of adsorption buffer. The specifically adsorbed protein(s) are then eluted with 25 ml of elution buffer (0.1 M glycine-HCl, 0.5 M NaCl, pH 2.7), and 5 ml fractions are collected. The fractions are concentrated on an Amicon minicon-CS15 concentrator (15,000 MW cut-off).

Gel Electrophoresis

Gel electrophoresis is performed on a Pharmacia Phast Electrophoresis unit, using 6/4 or 8/1 gel combs. Staining of the gels is also performed on the Phast Unit using the staining module. Most gels are silver stained to take advantage of the high sensitivity of this type of stain. With the small volume and relatively low concentrations of proteins used in this study, Coomassie blue or Amido black staining does not possess the required sensitivity.

Immunoblotting

Whole seminal plasma, electrophoresed on a 12.5% acrylamide gel, was transferred to polyvinylene difluoride (PVDF) membranes using the Pharmacia Phast system. This membrane allows better retention of low molecular weight proteins <50kd. Immunoblots were blocked using non-fat dry milk at 37° C for 2.5 hours, followed by the addition of either the male or female sera for incubated for one hour at room temperature. After washing with tris buffered saline containing 0.5% Tween-20, anti-human IgG alkaline phosphatase conjugate was added and incubation was allowed for 1 hour at room temperature. After washing NBT/BCIP substrate was added and incubated at room temperature for 30 minutes. The membranes were washed using distilled water and air dried at room temperature.

Polymerase Chain Reaction (PCR) and Southern Blotting for Ureaplasma urealyticum DNA

PCR was performed on DNA isolated from the seminal pellet for the presence of DNA of *Ureaplasma urealyticum.* The DNA was extracted using a procedure for extraction of DNA from sperm provided by the Qiagen Corporation, using their QIAamp Tissue Kit (Cat. No. 29304). The sequences for the 20-mer PCR primers for the urease gene of *U. urealyticum* (termed UU1 and UU2) were obtained from Krieger, et al. (J. Clin. Microbiol. 34:3120-3128, 1996) and prepared by a commercial supplier. Control DNA from two strains of *U. urealyticum,* 9R and 27817, was supplied by Dr. George Kenny (University of Washington). The PCR procedure was also taken from Krieger, et al. (above).

Amplified DNA was separated on a 2% Nu-Sieve agarose gel and stained with ethidium bromide for visualization. The DNA was blotted through to a nylon membrane (Magnagraph) using a neutral Southern blot procedure in a S&S TurboBlotter downward transfer apparatus. The DNA was detected with a 20-mer probe 3'-tailed with biotin-dCMP and developed using the Life Technologies Photogene™ assay kit for chemiluminescent detection of the biotin probe.

Cell Proliferation Assays

Cell proliferation assays were performed on peripherial blood mononuclear cells isolated from blood of both partners. Cells are islolated in Accuspin® tubes using Histopaque®-1077 (both from Sigma Diagnostics). The isolated PBMC's are quantitated by the Clinical Hematology Laboratory at University Hospital. 1 X 10^6 cells are placed in the wells of a 96-well cell culture plate (Costar), in 100 µl of complete medium (RPMI-1640 contain in 10% fetal bovine serum). 100 µl of whole seminal plasma at dilutions of 1:10 and 1:100 are added to the cells, and controls of no additive (medium alone) and phytohemaglutinin (PHA) at 10 µg/ml are also added. The plate is sealed and incubated at 37°C for 5 days. The proliferation of the cells is quantitated using the 5-Bromo-2'-deoxy-uridine Labeling and Detection Kit III from Boehringer Mannheim (Catalog No. 1444611).

B. Results

Questionnaires:

Table I summarizes demographic data of GW veterans who returned either questionnaires #1, #2 and/or the more detailed questionnaire #3 for the first two years. The geographic distribution of GW respondents is well represented throughout the United States. Responses to template screening

questionnaire #1 from GW veterans are summarized Appendix II and responses from their sexual partners for questionnaire #2 are summarized in Table II. **It is evident from Table I that the percentage of respondents completing and returning questionnaires has more than doubled over the past year. All of the questionnaire respondents to this point have been male GW veterans. A total of 162 GW veterans responded to at least one of the screening questionnaires. There has been an increase of 109 GW males completing screening questionnaire #1. Of the 151 subjects who completed questionnaire 1 thus far, 75% indicated they were interested in participating in this project (see Appendix II). The vast majority of these individuals (92%) reported the onset of BSS after returning from the GW whereas only 8% had this problem prior to deployment. Forty-four percent of these respondents experienced BSS symptoms with their first sexual encounter after returning from the GW. Only 46% of the GW veterans indicated that their symptoms are prevented with the use of a condom.** Interestingly, only 43% of subjects have sought medical attention for their symptoms.

Questionnaire #2 is the same questionnaire which has been used to screen civilian populations of women with localized and/or systemic seminal plasma hypersensitivity. This questionnaire has been validated as reliable in detecting women with probable local and/or systemic seminal plasma hypersensitivity reactions.[3] **This questionnaire was completed by the sexual partners of 52 GW veterans. Table II summarizes and compares questionnaire #2 responses obtained from the sexual partners of GW veterans (N=52) to a group of civilian females seeking medical attention for symptoms suggestive of localized and/or systemic seminal plasma protein hypersensitivity (N=34) and a population of women previously reported in the literature diagnosed with seminal plasma protein hypersensitivity.[10] Chi square analysis was used to statistically compare these three groups. Many women from the GW group and civilian groups reported systemic and localized symptoms. However, civilian females indicated that condoms prevented their symptoms 77% of the time whereas only 39% of the GW group experienced symptomatic relief with a condom ($p < .01$). A significant difference was also observed between the civilian groups and GW group with respect to a personal and family history of atopy ($p < .01$ and $p < .001$, respectively). It is also interesting to note that the GW women had a fewer number of sexual partners with whom they experienced BSS symptoms compared to civilian women ($p < .05$).**

Figure 1 is a general map illustrating the Gulf War theater. All GW respondents indicated in their questionnaires that they had been deployed to either Saudi Arabia, Kuwait or Iraq. 71% indicated they were in multiple locations whereas 29% were stationed in one specific area. We have been working with Dr. Jack Heller, the Senior Scientist at the U.S. Army Center for Health Promotion and Preventive Medicine located at Aberdeen Proving Grounds, Maryland to generate modeled exposure data for each of our GW veterans using an established geographical information survey. **Initial delays in receiving exposure data were encountered because the database was originally set up to identify exposure of GW units, not individual soldiers. The database has since been modified. The database provides information on modeled pollutants of concern which primarily includes oil fire particulates and sampled pollutants of concern which includes both oil fire and all other particulate exposures. The data base provides the day, month and year the GW veteran entered and left the theater, whether they were near the Khamisiyah plume, modeled data estimating the number of days exposed to oil fire particulate and associated risks for cancer and/or other health problems calculated from this data. It also provides the number**

of actual sampling days of all particulates that were performed while the GW veteran was still in the theater and the associated risks for cancer and/or other health problems calculated from this data. Reports on 16 study participants evaluated thus far indicates that five GW veterans were in close proximity to the Khamisiyah "plume" and all were exposed to oil fire pollutants. However, none of these study participants had an exposure level believed by the Environmental Protection Agency to result in adverse health effects ($>10^{-4}$). The greatest exposure level experienced by one of our GW veterans was 10^{-11}. We have recently submitted the social security numbers of the other GW veterans enrolled in this project to obtain similar exposure reports. The exposure data for these 16 GW veterans is included in Appendix VI. The GIS data base is now capable of providing modeled exposure risks for individual pollutants (ie. Volatile organic compounds such as benzene, polycyclic aromatic hydrocarbons, sulfur dioxide and metals) as well as provide an exposure trail for each GW veteran from the time they entered and left the GW theater. This information should help to validate the exposure history recorded in their questionnaire by each GW veteran while they were in the Persian Gulf.

Table III summarizes the responses of questionnaire #3 designed to obtain more detailed information regarding GW male veterans and their sexual partners. **Completed questionnaires were received from 42 males and 36 females which is more than double the response from one year ago.** The average age of the males and their female sexual partners was 35 and 33 years old, respectively which has essentially remained unchanged after two years. Of particular interest, 92% of GW veterans reported chemical exposures, 31% reported exposure to depleted uranium, 58% reported exposure to biological agents, 74% ingested pyridostigmine bromide of which 1/3 had side effects, 50% were exposed to pesticides and 63% received vaccinations. **We will correlate these exposure histories to data provided by the GIS database after we have received final reports on each GW veteran. Forty-five percent of GW veterans were previously evaluated for PTSD and 26% of these individuals were undergoing active treatment.** Only 26% reported they were in good or better health. Burning semen syndrome symptoms have been reported by 58% of GW veterans and by 94% of their sexual partners. There has been fairly good correspondence between responses to the same questions asked of the GW veteran and their sexual partner. One discrepancy in questionnaire responses by the male and female pertained to the question asking if the onset of their reaction occurred with their first sexual encounter after the male returned from the GW. Females responded "Yes" 44% of the time whereas males responded only "26%" of the time. The explanation of this discrepancy is most likely due to the woman's reluctance to report these symptoms to their sexual partner.

The Mississippi Post-Traumatic Stress Disorder (MPTSD) and Combat Exposure Scale (CES) questionnaires were used to screen for PTSD. In general, GW veterans have been reluctant to complete these surveys for various reasons. Some have previously completed these questionnaires as part of their previous work-up for PTSD, some were offended by the questions asked and some were insulted that we would even suggest that the etiology of their symptoms has any psychological bearing. Table IV summarizes the results of all PTSD questionnaires returned by GW veterans from year 1. **Based on the responses from those veterans completing the MPTSD, 44% were negative for PTSD, 26% were possible for PTSD and 29% were probable for PTSD. Currently, 26% of GW respondents are undergoing therapy for PTSD.**

Results of Clinical Evaluation of GW Couples with BSS after Year 1 Pilot Project:

A pilot study was completed during year 1 of this project to test the questionnaires and ensure that the evaluation of the GW couples was well coordinated. The pilot study included interviews and evaluations of five GW veterans and their sexual partners with BSS at the Cincinnati Veterans Administration Hospital (VAH). One additional GW veteran was evaluated but his wife refused to participate. The interview included answering the above questionnaires, completing a PTSD questionnaire packet, obtaining blood samples from both the male and female to exclude underlying concomitant disorders such as sexually transmitted diseases (see Appendix V), a pap smear with vaginal/cervical cultures of the female and a fresh semen ejaculate for skin testing and cultures from the male. Both males and females were prick skin tested to common seasonal and perennial allergens to determine their atopic status and to the male's whole semen. Four of the six GW veterans had evidence of atopy defined as a skin reaction eliciting ≥ 3 mm wheal with erythema to one or more allergens. Four of six GW veterans and two of five female sexual partners elicited at least one positive skin test reaction to an aeroallergen.

Pertinent positive results of screening laboratory tests for the GW male and sexual partner are summarized in Table V with rows one through six referring to the pilot study group and rows 7-8 representing test results of two new GW couples. Three of five women evaluated grew *Ureaplasma urealyticum* from their cervical culture. Two of these women also exhibited positive ANA titers and one had an increased sedimentation rate. One woman grew *Streptococcus* Group B from her cervical culture and had a chronic vaginal yeast infection. Both the males and females exhibited varying antibody titers to either HSV, CMV or mycoplasma. There did not appear to be a correlation between symptoms and PTSD in the small number of subjects evaluated thus far.

We have offered treatment with Doxycycline, an antibiotic effective against mycoplasma infections, to the three women with positive cervical cultures for *Ureaplasma urealyticum*. Two GW couples (male and female) took a four week course of this antibiotic but did not experience improvement in their clinical symptoms. The female from the third GW couple (who were not married) refused to take the medication as she had become estranged from her GW sexual partner. Follow-up cervical cultures for *Ureaplasma urealyticum* have not been obtained for the two women who took the antibiotic.

None of the GW veterans or their sexual partners who participated in the pilot study elicited a positive skin test reaction to their whole seminal plasma. However, several GW veterans and/or their sexual partners have been documented to elicit IgG and/or IgE antibody responses to seminal plasma proteins (Table VI). These results are discussed in more detail in the antibody result section below. Specific antibody responses were present in only the male of some couples, only the female of some couples and in both the male and female of some couples. The results of the initial pilot study revealed that: 1) the operational procedures for initial screening interviews and laboratory evaluations of the GW veterans and their sexual partners was very labor intensive, requiring frequent phone calls and written correspondence to maximize compliance with the protocol; 2) the questionnaire responses regarding BSS by the GW veterans and their sexual partners was variable and their response rate seemed to proportionately decrease as the questionnaires became more detailed; 3) there was a very poor completion rate of the PTSD questionnaire packets, however, there did not seem to be a correlation between BSS and PTSD among the participants in this pilot study; 4) none of the six GW veterans or their sexual partners elicited positive skin test responses to their semen, however, several

did elicit significant levels of IgG and/or IgE antibodies to seminal plasma proteins in their sera; 5) three of the five women evaluated grew *Ureaplasma urealyticum* in their cervical cultures, two had positive ANA titers and one had a high sedimentation rate.

Since this initial pilot study, we have been successful in obtaining completed questionnaires from a larger number of GW couples with BSS. **However, we have had a very difficult time collecting screening laboratory data from GW couples to exclude obvious underlying causes for their symptoms such as sexually transmitted diseases. We have recently established a better mechanism for getting this information with assistance from the VAH central office. Permission has been granted for the GW veteran and their sexual partner to have screening testing conducted at the regional VAH. However, it took approximately six months of inquiry and persistent lobbying until GW couples finally received permission to obtain these screening tests which are outlined in Appendix V.**

ELISA for specific IgG and IgE antibodies to seminal plasma proteins:

Specific IgG and IgE ELISAs have been performed for 12 GW veterans and ten GW sexual partners (two did not have sexual partners); data is also available for 12 civilian couples with symptoms suggestive of seminal plasma hypersensitivity (Table VI). Positive controls included women who had been previously found to consistently elicit specific IgG and IgE antibody responses to seminal plasma proteins after repeated screening of many seminal plasma specimens. Negative controls included subjects who elicited specific antibody responses after repeated screening to a number of whole seminal plasma specimens. A positive antibody response is defined as an optical density greater than the mean optical density of negative control subjects ± 3 standard deviations. Each run was performed in triplicate and repeated at least once to ensure reproducibility of the results. A heterogeneous antibody response by the male and female to their respective seminal plasma proteins was observed (Table VI). **Figure 2 presents a comparison of IgG and IgE antibody responses elicited to whole seminal plasma proteins by GW veterans, civilian males and their respective sexual partners. There were no significant differences between IgG and IgE antibody responses elicited by GW and civilian males or between the GW female sexual partners and civilian females (Mann-Whitney Rank Sum Test, $\rho > .05$).**

SDS-PAGE and Western blotting:

Figure 3 illustrates the SDS-PAGE of whole seminal plasma obtained from GW and civilian men. In general, a very similar protein pattern has been observed for all subjects. We are currently performing SDS-PAGE followed by western immunoblotting on all of the seminal plasma protein fractions isolated by column chromatography to detect specific IgG and/or IgE responses to one or more of these proteins. **Figure 4 is a representative SDS-PAGE gel of a GW veteran's whole seminal plasma before and after fractionation and figure 5 is the western blot for specific IgG antibody of this gel. Specific IgG was found for proteins with molecular weights of 45kd, 50 kd, 80kd and approximately 180kd. These proteins are currently being further analyzed by mass spectroscopy. Figure 6 is a representative gel of whole seminal plasma and fractionated proteins from a civilian male whose sexual partner had systemic symptoms consistent with seminal plasma protein hypersensitivity. Figure 7 is the western blot for IgG antibody of this gel. Specific IgG antibodies were identified to several proteins (8-12 proteins) ranging from**

molecular weights of <10kd to 200kd. These proteins are also being further analyzed mass spectroscopy. SDS-PAGE gels with specific IgG and IgE immunoblots are being prepared using the whole seminal plasma and fractionated proteins obtained from each GW veteran and civilian male participating in this study.

Column Chromatography

Whole seminal plasma from GW males and civilian males fractionated by column chromatography thus far are illustrated in figures 8-17. Figure 8 includes the spectrographic patterns of 10 GW veterans. These patterns are all very similar. Figure 9 illustrates the spectrographic patterns of civilian males whole seminal plasma. Distinct differences in protein peaks were noted among this group. Civilian males elicited 3-8 peaks consistently whereas GW veterans have no fewer than 7-9 peaks. When the spectrographic patterns of GW veteran's whole seminal plasma are compared on the basis of whether or not their they or their sexual partner elicits specific IgE antibody to one or more of their proteins, no differences are noted (see Figures 10, 11, 12 and 13). However, when the spectrographic patterns of civilian males are compared on the basis of whether their sexual partners elicit specific IgE antibody responses to one or more of their seminal plasma proteins, distinct differences are seen (see Figures 14 and 15). The civilian men whose sexual partners elicit specific IgE antibody responses to seminal plasma protein have a fewer number of peaks, in particular a notable absence of the lower molecular weight peaks. These trends are not observed for civilian men when their spectrographic patterns are compared on the basis of whether they make specific IgE antibodies to their own seminal plasma proteins (see Figures 16 and 17).

Affinity column chromatography has not been initiated at this point to the extent that reportable data is available. Using serum from the GW veteran's sexual partner who elicited positive antibody responses by ELISA preliminary experiments have been successful in eluting off specific bands from whole seminal plasma using this methodology. This technique will be used in year 3 to further characterize those proteins to which GW veterans or their sexual partners elicit specific antibody responses.

PCR and Southern blotting:

We have performed preliminary analysis using a PCR technique to detect the presence of *Ureaplasma urealyticum* in the semen of GW veterans. The interest in pursuing this assay is twofold: 1) initial laboratory screening of GW couples revealed a number of females with positive cervical cultures for this organism and; 2) there has been a significant amount of controversy whether mycoplasma organisms are causing or contributing to some or all of the clinical symptoms characterized as GW syndrome. Obtaining culture data from a larger population of GW female sexual partners should help to clarify this issue. Figure 18 illustrates preliminary PCR results of GW veterans and civilian controls after probing DNA isolated from their semen with a specific Ureaplasma urealyticum urease primer. Distinct bands for urease were not observed for the two GW veterans evaluated at this time. However, a larger number of specimens are required for screening to determine the relationship of this organism and BSS. Thus far, the two women treated with Doxycycline for one month had no improvement in their symptoms. This aspect of our laboratory investigation is of secondary importance as the main objective of this project is to identify potential immune responses

associated with BSS symptoms.

Future Investigation:

We have been performing cell proliferation experiments using whole seminal plasma from the GW male and fresh peripheral blood mononuclear cells from the GW male and their sexual partner. Proliferation was not observed in three subjects studied thus far. Future cell proliferation experiments will be performed using fractionated seminal plasma proteins to which the GW veteran and/or their sexual partner have elicited a specific antibody response. The objective of these experiments will be to investigate the role of lymphocytes in regulating specific antibody responses associated with BSS. Previous work has demonstrated that seminal plasma proteins may have an inhibitory effect on lymphocyte proliferation.[3,4]

Presentations and publications to date:

An abstract was presented at the Society of Toxicology meeting held in Cincinnati, March 1997, pertaining to BSS in GW veterans. A second abstract was presented at the American Academy of Allergy, Asthma and Immunology (AAAAI) in Washington D.C. in 3/98.[7,8] **Finally, a third abstract reporting our most recent data has been submitted to the AAAAI for 3/99. A manuscript is in preparation which will report our early finding of antibody responses to seminal plasma proteins in GW couples with BSS.**

III. Conclusions:

Overall, there continues to be a significant response from GW veterans complaining of BSS as the total number of respondents continues to increase. Questionnaire responses indicate that the majority of BSS cases began after the GW veterans returned from the Persian Gulf. The female sexual partner is experiencing the burning sensation in the majority of cases but over half of the GW veterans also experience burning during ejaculation or after contact with their own semen. Initial assessment of a small group of GW veterans and their sexual partners has indicated that several of the participants have underlying bacterial infections which could be causing or contributing to their symptoms. Some of the subjects exhibit non-specific laboratory abnormalities suggestive of an underlying inflammatory condition which could be consistent with a chronic infection. We have been delayed in obtaining screening laboratory tests and vaginal cultures in larger number of GW couples because a mechanism which paid for this assessment was not in place at VAHs or military hospitals. However, we have been successful in getting approval for this testing through the VAHs. The Cincinnati VAH is now permitted to evaluate women of GW veterans with BSS. Many of our GW couples are currently undergoing the testing outlined in Appendix V. We will also continue to screen all male semen samples for *Ureaplasma urealyticum* by PCR.[6,9] This organism is related to the mycoplasma family of organisms and is difficult to grow in routine culture because semen is rich in bacteriostatic and enzymatic proteins that inhibit growth of bacterial organisms. Therefore, DNA determination is the only practical way of detecting the presence of this specific organism.[6,9]

Only one of the subjects (a GW veteran's sexual partner) evaluated thus far have exhibited a positive skin test to whole semen however, several have elicited specific IgG and/or IgE antibody responses by ELISA. This may reflect the poor sensitivity of skin testing to whole seminal plasma as

the relevant protein(s) may be too low in concentration to detect by this technique. **We are currently performing ELISA for IgG and IgE antibody on the seminal plasma protein fractions prepared for all GW veterans who have provided us with specimens. These protein fractions are being further analyzed by SDS-PAGE, immunoblotting, mass spectroscopy and eventually by affinity chromatography.**

In order to determine whether these antibody responses have any bearing on the clinical responses manifested as BSS, we plan to attempt to desensitize as many female with specific antibody responses to their sexual partner's seminal plasma proteins as possible. Our experience with civilian women diagnosed with localized and/or systemic seminal plasma hypersensitivity reactions who have been desensitized has been very favorable in reducing or preventing their symptoms, presumably by inducing tolerance.[1,4] Successful clinical treatment will provide direct evidence that the specific immune responses identified *in vitro* are responsible for the female's BSS symptoms. We have attempted to desensitize the sexual partner of one GW veteran where both the male and female elicited specific antibody responses. This treatment was unsuccessful. In our experience, desensitization has not been successful when the male has specific antibody to his own seminal plasma proteins. This may represent an autoantibody and will be the subject of further investigation as our population grows. Subsequent pharmacologic treatment of this female for chronic vaginal candidiasis with an antifungal agent was successful in alleviate all of their symptoms.

An essential part of this project is to identify and evaluate cohort control populations for comparison with the deployed GW symptomatic veterans for BSS (i.e. GW veterans deployed to Persian Gulf without BSS symptoms). This will involve recruiting subjects from nearby military installations (i.e. Wright Patterson Air Force Base in Dayton and local and regional national guard installations). All subjects (GW couples with BSS and control groups) will be asked to complete questionnaires #1-3, PTSD packets and undergo screening tests outline in Appendix V. We have begun to make contacts with nearby military installations. Field trips to several facilities will be arranged in the next year to obtain a normal GW male control population.

To complete this project we have submitted a revised budget for 1999 which was approved as a reformulation of the existing budget over an additional six months. Our progress has been slowed by delays in obtaining screening laboratory testing in remote VAH facilities not to mention our own VAH.. Hiring a project administrative coordinator has greatly facilitated the progress of this study. Ms. Adrienne Perez has been very diligent in maintaining constant contact with the GW couples and in assisting them throughout their evaluation.

Finally, the design of this project has been modified over the past two years in response to the magnitude and complexity of this problem. The initial protocol was prepared based on certain assumptions which later proved incorrect. The modifications have included a greater emphasis on epidemiology of BSS and localized/systemic seminal plasma hypersensitivity disorders in the GW and civilian populations, respectively. We are working closely with a statistician who is assisting us with analysis of our database. Certain procedures such as culposcopy, vaginal biopsies and lavage for cytokine analysis will not be pursued at this time as the number of women who are likely to be recruited for these procedures would be too small to provide meaningful information that would enhance our understanding of BSS. We believe the experiments outlined above will provide the most useful information about BSS.

In summary, it appears a significant number of GW couples have antibodies to seminal plasma proteins. The focus over the next year will be to determine whether these antibody responses are related to their clinical symptoms. We will also pursue infectious etiologies and other immune responses that may be involved in causing BSS. Finally, epidemiological information about the prevalence of BSS among GW couples compared to the normal civilian population should be available over the next two years.

IV. References:

1) Bernstein JA, Herd Z, Bernstein DI, Korbee L, Bernstein IL. Evaluation and Treatment of Localized Vaginal Immunoglobulin E-Mediated Hypersensitivity to Human Seminal Plasma. Obstet Gynecol 1993;82:667-73.

2) Bernstein JA, Sugumaran R, Bernstein DI, Bernstein IL. Prevalence of Human Seminal Plasma Hypersensitivity Among Symptomatic Women. Ann Allergy Asthma Immunol 1997; 78:54-8.

3) Bernstein IL, Englander BE, Gallagher JS, Nathan P, Marcus ZH. Localized and Systemic Hypersensitivity Reactions to Human Seminal Plasma Fluid. Annals of Int Med 1981;94:459-465.

4) Friedman SA, Bernstein IL, Enrione M, Marcus ZH. Successful Long-Term Immunotherapy for Human Seminal Plasma Anaphylaxis. JAMA 1984;251:2684-87.

5) Fair WR, Couch J, Wehner N. Prostatic Antibacterial Factor. Urology 1976;7:169-77.

6) Krieger JN, Riley DE, Roberts MC, Berger RE. Prokaryotic DNA Sequences in Patients with Chronic Idiopathic Prostatitis. J Clin Microbio 1966; 34:3120-28.

7) Bernstein JA, Martin RLM, Lummus ZL. Localized Human Seminal Plasma Hypersensitivity: A Potential Model For Gulf War "Burning Semen Syndrome". Fundamental and Applied Toxicology 1997;37:201.

8) Bernstein JA. Evaluation of Persian Gulf War Veterans and Their Sexual Partners with Burning Semen Syndrome. J Allergy Asthma and Clin Immunol 1997; (submitted as abstract).

9) Blanchard A. *Ureaplasma urealyticum* urease genes; use of a UGA tryptophan codon. Mol Microbio 1990;4:669-676.

10) Presti ME, Druce HM. Hypersensitivity Reactions to Human Seminal Plasma. Annals of Allergy 1989; 63:477-482.

Table I. Summary of response and demographic data for second year report

Gulf War Veterans and Partners

Total number of study participants to date – GW Veterans	162
Number of participants not participating further due to lack of interest	53
Number of participants excluded (HIV+, spousal contact-Veteran's whereabouts unknown	2
Number completing Questionnaire #1	151
Number completing Questionnaire #2 – Females	52
Number completing Questionnaire #2 – Males	21
Number completing Questionnaire #3 – Females	36
Number completing Questionnaire #3 – Males	42
Number completing PTSD Surveys	
Combat Exposure Scale	40
Mississippi PTSD Rating Scale	34

Civilian Seminal Plasma Hypersensitivity Patients

Total number of participants to date: Positive Control Group	38
Number of participants not currently pursuing treatment	16
Number of participants post treatment	7
Number of participants pursuing treatment	15
Number of respondents to Questionnaire 2 (female)	34
Number of respondents to Questionnaire 3 (male)	11
Number of respondents to Questionnaire 3 (female)	11

Geographic Distribution of GW Veteran Participants

3 Alabama	1 Arizona	3 Arkansas
5 California	2 Colorado	3 Florida
6 Georgia	1 Hawaii	1 Idaho
1 Illinois	3 Indiana	1 Iowa
8 Kentucky	1 Louisiana	2 Maryland
3 Massachusetts	3 Minnesota	3 Missouri
2 Montana	3 New Hampshire	2 New Mexico
3 New York	5 North Carolina	20 Ohio
5 Oklahoma	1 Oregon	5 Pennsylvania
1 Rhode Island	1 South Carolina	8 Texas
2 Tennessee	2 Utah	3 Virginia
4 Washington	1 West Virginia	1 Wisconsin
2 Canada	1 Michigan	2 New Jersey

Table II. Comparison of GW Females with BSS to Civilian Females with SPH

ITEM	Presti et al. 1989 N=32	Civilian Comparison Group N=34	Partners of GW Veterans N=52	ρ *
Age of Onset:				
<20	.03	.15	.12	
20-30	.56	.56	.54	
31-40	.16	.15	.23	
41-50	.03	.09	.08	
>50	.03	0	.02	
Reactions:				
Dermatitis/Urticaria/Pruritus	.84	.71	.79	
Edema	.47	–	–	
Dyspnea	.22	.41	.23	
Local Pain	.56	.91	.90	
Anaphylaxis	.22	.12	.02	
Atopy:				
Yes	.59	.62	.37	=0.009
No	.31	.32	.63	
Unknown	.09	.06	0	
Multiple Partners:				
Yes	.22	.24	.12	=0.015
No	.25	.76	.85	
Unknown	.53	0	.04	
Predisposing Conditions:				
First Intercourse	.41	.35	.35	
History of :				
Pregnancy, Gyn/Urological Surgery	.63	.29	.27	
Unknown	.31	.32	.52	
Onset of Symptoms:				
0-60 minutes	.47	.94	.83	
>60 minutes	.22	.06	.15	
Unknown	.31	0	.02	
Family History of Atopy:				
Yes	.38	.68	.37	<0.001
No	.13	.32	.60	
Unknown	.50	.00	.04	
Prevented by Condom:				
Yes	.63	.77	.39	=0.009
No	0	.18	.21	
Unknown	.38	.06	.40	

* Chi square analysis excluding "Unknown" responses

Table III: Summary of questionnaire #3 gulf war couple responses

Responses	Male=42	Female=36
Average age	35	33
Average length of tour	5.4 months	------
Location while in Persian Gulf	Iraq, Kuwait, Saudi Arabia	------
Reported chemical exposures	92 %	------
Average length of exposure	Varied	------
Diagnosis of Leishmaniasis	5 %	------
Treatment for Leishmaniasis	0 %	------
Uranium exposure	31 %	------
Exposure to biological agents	58 %	------
Ingestion of Pyridostigmine Bromide	74 %	------
Side effects from Pyridostigmine Bromide	37 %	------
Exposure to pesticides	50 %	------
Received vaccinations	63 %	------
Diagnosis/Evaluation of Post-traumatic Stress Disorder	45%	------
Treatment of Post-traumatic Stress Disorder	26 %	------
Involvement in decontamination operations	29 %	------
Current state of health	26 % good or better	59 % good or better
Sexually transmitted disease	13 %	9 %

Reaction to semen	58 %	94 %
Sexual partner has reaction	87 %	------
Onset of reaction with first sexual encounter after returning from GW	26 %	44 %
Time onset of symptoms occur	"Minutes" for all responding	"Minutes" for 84 %
Length of time symptoms persist	"Minutes" for 24 %	"Minutes" for 16 % "Days" for 28 %
Systemic symptoms	55 %	63 %
Condoms eliminate reactions	40 %	34 %
History of vasectomy	16 %	------
History of infertility problems	5 %	------
History of Allergies	29 %	38 %
Food Allergies	16 %	16 %
Drug Allergies	21 %	44 %
Same sexual partner pre/post GW	53 %	75 %
Recurrent vaginal yeast infections	------	53 %
Current use of oral contraceptives	------	13 %

Table IV. Summary of PTSD findings

Mississippi PTSD Rating Scale (MPTSD)

MPTSD is an inventory of statements about how one views oneself and experiences life situations.

Total number of respondents	34
Average score	99.4
Standard deviation of scores	30.9
Number of respondents negative for PTSD*	15
Number of respondents possible for PTSD*	9
Number of respondents probable for PTSD*	10
Number of respondents evaluated and/or diagnosed with PTSD (Questionnaire #3)	19
Number of respondents currently under treatment for PTSD (Questionnaire # 3)	11

*** MPTSD scores ≤95 are negative, MPTSD scores = 96 - 115 are possible, and MPTSD scores ≥116 are probable.**

Table V: Summary of Pertinent Positive Laboratory Results of GW Veterans and Their Sexual Partners Evaluated in the First Year Pilot Study.

Subject	Laboratory Test Result	Male (GW Veteran)	Female
1 (-) PTSD	ANA Serum Mycoplasma IgG Ab Serum HSV-1 IgG Ab Serum CMV IgG Ab Cervical Urea.urealyticum	Positive (1:40) Positive	Positive 1:160 speckled Positive Positive Positive Positive
2 poss. PTSD	ANA Serum Mycoplasma IgG Ab Serum HSV-1 IgG Ab Serum CMV IgG Cervical Urea. urealyticum Urine Group B strep.	Positive Positive Positive	Positive 1:80 Positive Positive Positive Positive Positive (10-50,000 cfu/ml)
3 (-) PTSD	Serum Mycoplasma IgG Ab Serum CMV IgG Ab Cervical pap smear	Positive Positive	Positive Positive for Candida yeast
4 (-) PTSD	WSR Bands on differential Serum HSV-1 IgG Cervical Urea. urealyticum		68 mm/hr (nl=0-20) 14% (nl=0-6) Positive Positive
5 poss. PTSD	Serum HSV-1 IgG Ab Serum HSV-2 IgG Ab Cervical culture Cervical pap smear	Positive	Positive Positive Moderate Strep Group B Many inflammatory cells
6 (+) PTSD	Serum HSV-1 IgG Ab	Positive	Not available (wife did not participate in evaluation)
7 (+) PTSD	Cervical Cytologic Material		Acute Inflammation
8 (-) PTSD	Serum CMV IgG	Positive	Positive

Table VI. Individual Results of Specific IgG & IgE Antibody Responses in Gulf War and Civilian Couples

Couple		IgG	IgE	Age	Sx Type	Other Problems
GW Veterans & their Partners						
1080	M	**.19 (+)**	**.12 (+)**	27	None	Isolated
1080	F	**.13 (+)**	**.12 (+)**	28	Local	Isolated
1165	M	**.27 (+)**	.10 (-)	28	None	Isolated
1165	F	**1.06 (+)**	**.62 (+)**	27	Local	Isolated
1135	M	**.88 (+)**	**.35 (+)**	38	Local	Multiple
1135	F	**.40 (+)**	.01 (-)	37	Local	Unknown
1055	M	**.12 (+)**	.09 (-)	30	Local	Multiple
1055	F	.04 (-)	.08 (-)	42	Local	Isolated
1030	M	.06 (-)	.06 (-)	28	Systemic	Multiple
1030	F	**.86 (+)**	.04 (-)	27	Local	Isolated
1175	M	**.49 (+)**	**.36 (+)**	29	Local	Multiple
1175	F	**.28 (+)**	**.45 (+)**	24	Systemic	Multiple
2034	M	.05 (-)	.11 (-)	42	None	Multiple
2034	F	**.18 (+)**	**.16 (+)**	42	Systemic	Isolated
1115	M	.06 (-)	.07 (-)	40	Local	Multiple
1115	F	**.13 (+)**	**.12 (+)**	39	Systemic	Multiple
2041	M	.03 (-)	.06 (-)	41	Systemic	Multiple
1125	M	.03 (-)	.03 (-)	50	Systemic	Multiple
1025	M	.03 (-)	.03 (-)	34	Systemic	Multiple
1025	F	.02 (-)	.01 (-)	35	Systemic	Multiple
1090	M	.06 (-)	.02 (-)	36	None	Isolated
1090	F	.04 (-)	.03 (-)	33	Local	Isolated
Civilians/Spouses						
3141	M	.10 (-)	.06 (-)	46	None	Isolated
3141	F	**.12 (+)**	.07 (-)	44	Systemic	Isolated
3115	M	**.15 (+)**	**.25 (+)**	39	None	Isolated
3115	F	**1.15 (+)**	**.78 (+)**	34	Systemic	Isolated
3009	M	**.65 (+)**	**.21 (+)**	27	None	Isolated
3009	F	**.57 (+)**	**.14 (+)**	23	Local	Isolated
3122	M	.02 (-)	.01 (-)	29	None	Isolated
3122	F	**.38 (+)**	**.43 (+)**	23	Local	Isolated
3121	M	**.46 (+)**	**.32 (+)**	40	None	Isolated
3121	F	.02 (-)	.09 (-)	31	Local	Isolated
3117	M	.04 (-)	.04 (-)		None	Isolated
3117	F	.09 (-)	.06 (-)	28	Local	Isolated
3140	M	**.20 (+)**	**.12 (+)**	41	None	Isolated
3140	F	.06 (-)	.04 (-)	42	Local	Isolated
3001	M	.05 (-)	-0.01 (-)	32	None	Isolated
3001	F	.02 (-)	.01 (-)	29	Systemic	Isolated
3125	M	.01 (-)	.00 (-)	60	None	Isolated
3125	F	.01 (-)	.01 (-)	53	Systemic	Isolated
3124	M	.02 (-)	.01 (-)	53	None	Isolated
3124	F	.05 (-)	.01 (-)	51	Systemic	Isolated
3139	M	.04 (-)	.03 (-)		None	Isolated
3139	F	**.63 (+)**	**.17 (+)**	29	Local	Isolated
3142	M	.02 (-)	.02 (-)	35	None	Isolated
3142	F	**1.33 (+)**	**3.02 (+)**	27	Local	Isolated

GW Males (n=12) mean age = 33.6
Civ. Males (n=12) mean age = 40.2
GW Females (n=10) mean age = 33.4
Civ. Females (n=12) mean age = 34.5

Figure 1. Geographical map of the Persian Gulf War theater.

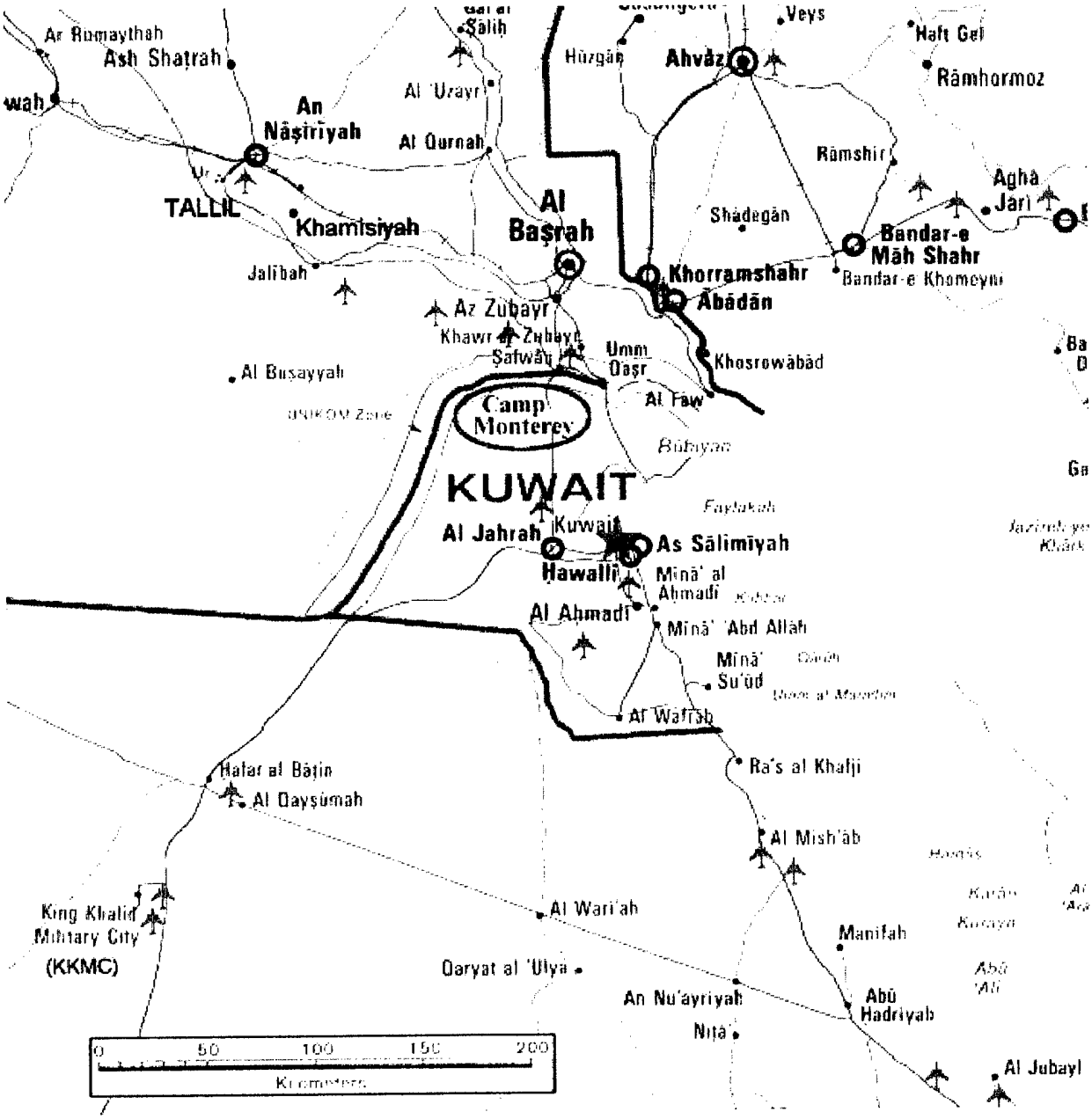


Figure 2. Comparison of IgG and IgE Antibody Responses to Whole SPP in Gulf War & Civilian Couples

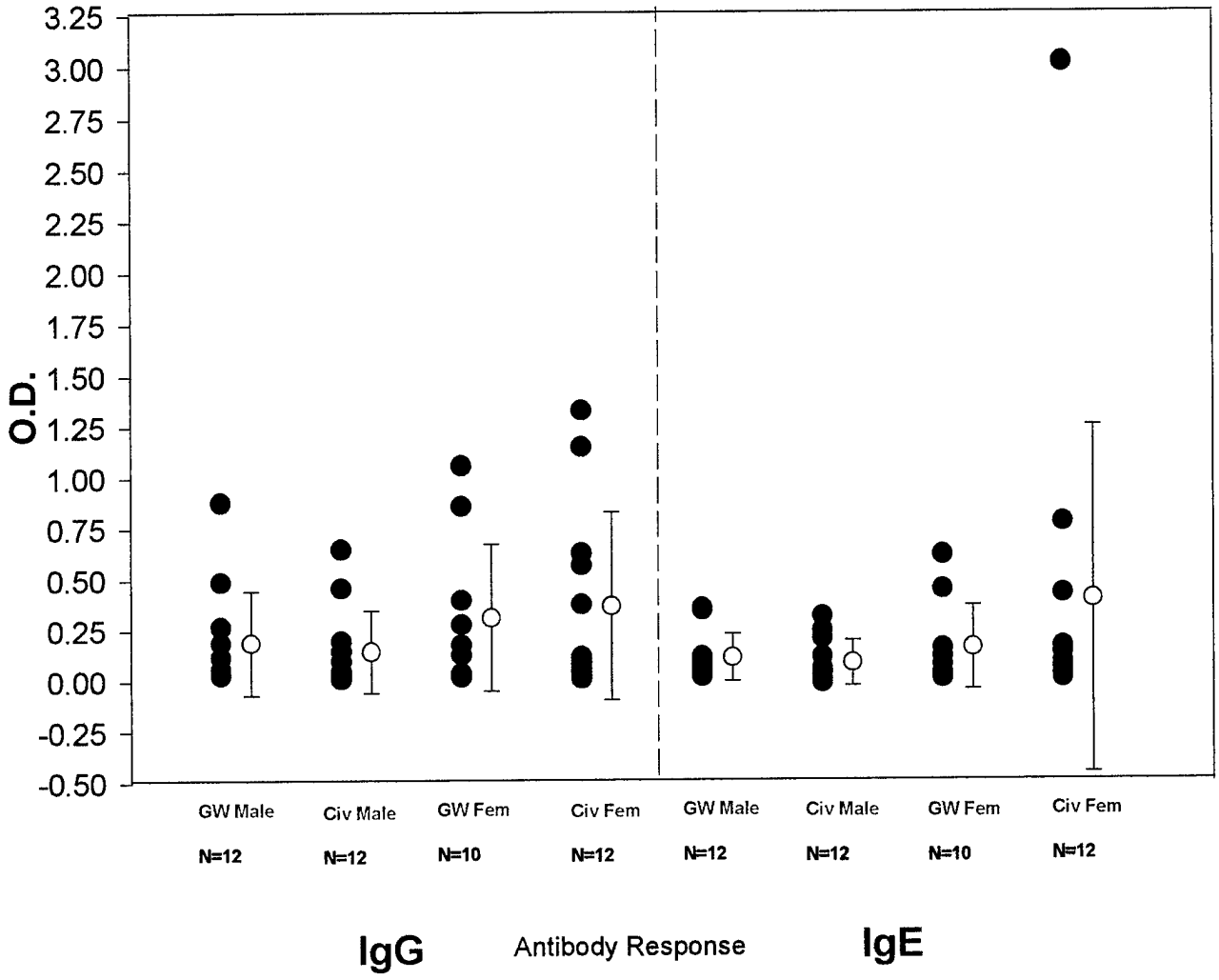


Figure 3. Gel electrophoresis and silver staining of whole seminal plasma from GW veterans and civilian controls. From left to right: Lanes 1-6 and 8-11 are GW specimens; Lanes 7 and 12 are civilian specimens.

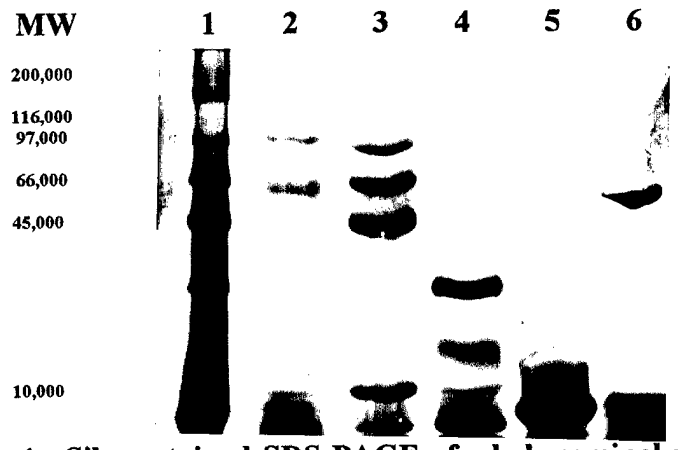


Figure 4. Silver stained SDS-PAGE of whole seminal plasma and seminal plasma protein fractions from a GW veteran. Lane 1 is whole seminal plasma, lane 2 is fraction 1a, lane 3 is fraction 1b, lane 4 is fraction 2, lane 5 is fraction 3 and lane 6 is fraction 4.

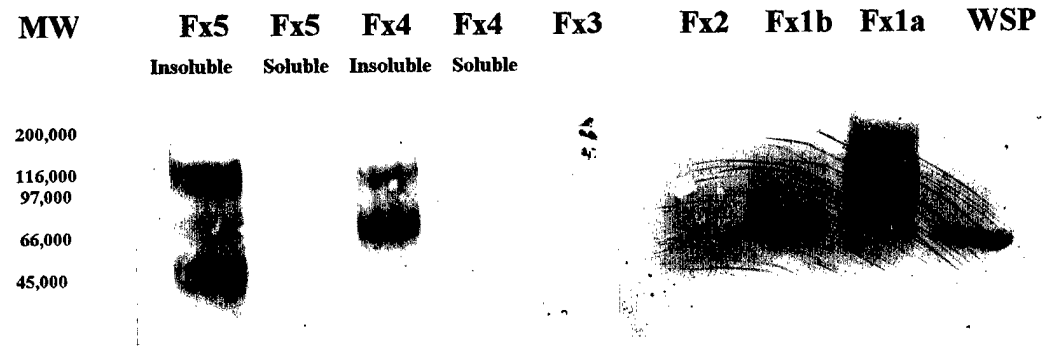


Figure 5. IgG immunoblot of SDS-PAGE gel of GW veteran in figure 4. PVDF membrane incubated with the GW veteran's serum. IgG immunoblotting using the serum of the GW veteran's sexual partner showed similar protein bands for whole seminal plasma and fraction 1b but the other protein bands observed for the GW veteran were not observed (blot not shown).

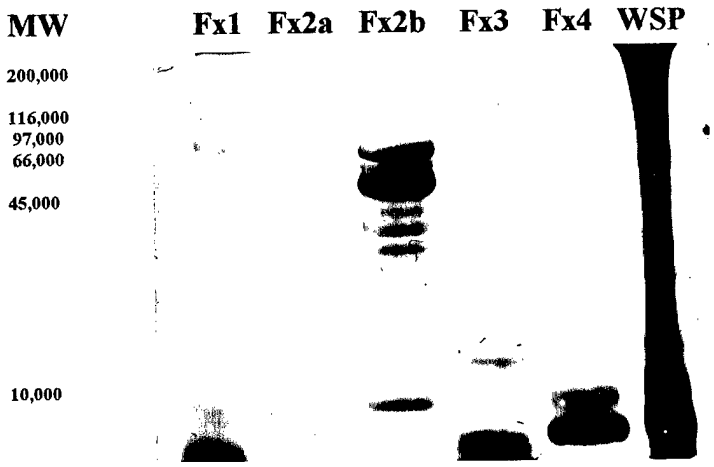


Figure 6. SDS-PAGE of whole seminal plasma and seminal plasma protein fractions from a civilian male whose sexual partner was diagnosed with systemic seminal plasma hypersensitivity.

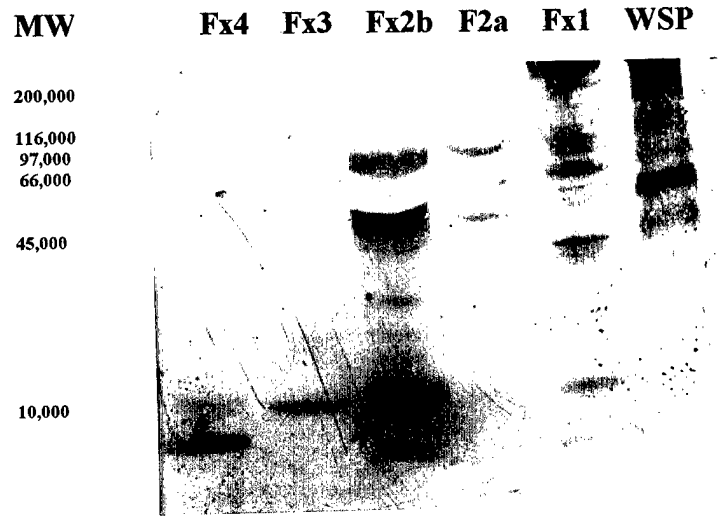


Figure 7 . IgG immunoblot to SDS-PAGE gel in figure 6 using serum of civilian female diagnosed with systemic seminal plasma hypersensitivity and successfully desensitized to fractions 3 and 4.

Figure 8. Chromatography of Whole Seminal Plasma from GW Veterans with Burning Semen Syndrome

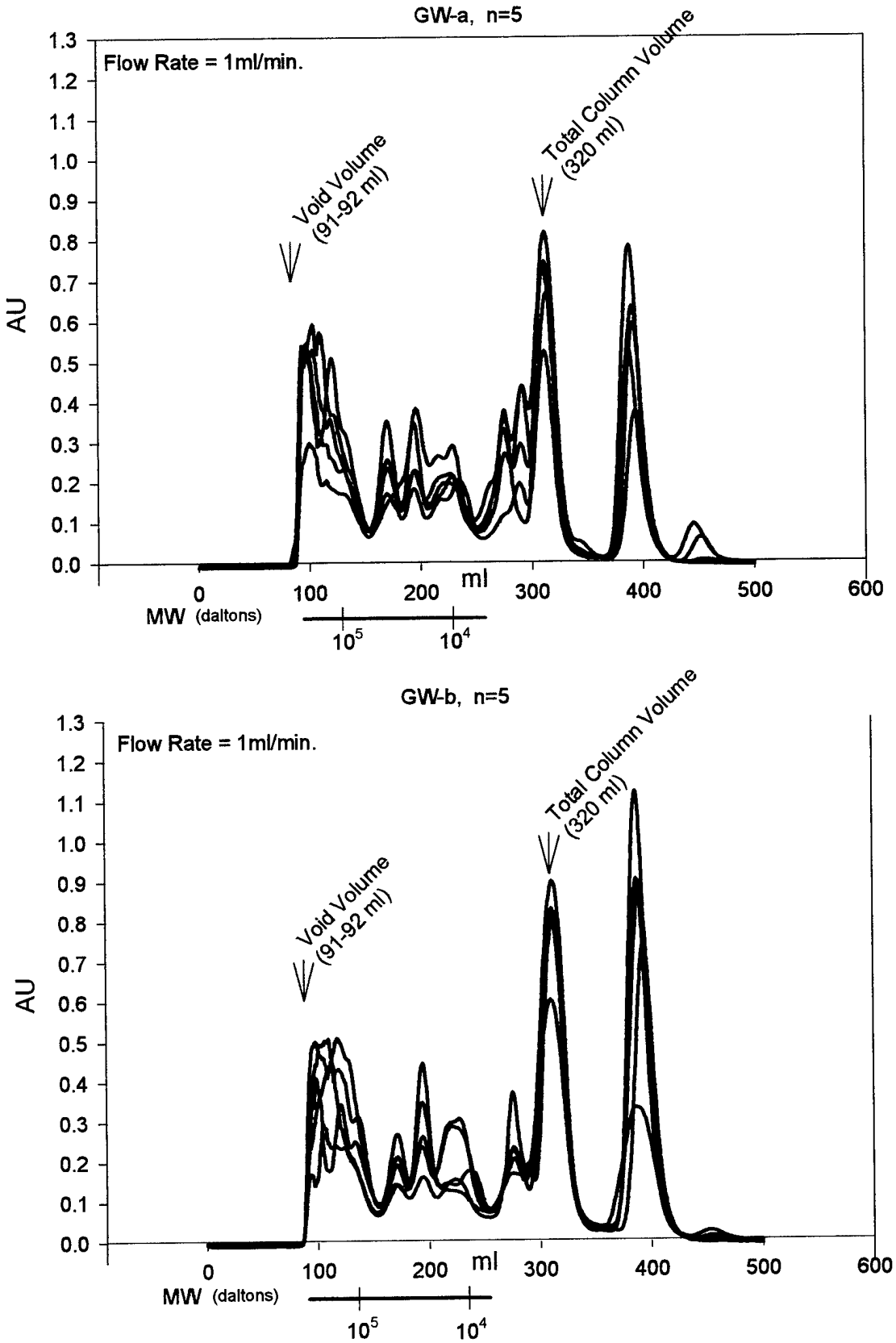


Figure 9. Chromatography of Whole Seminal Plasma from Civilians with Seminal Plasma Hypersensitivity

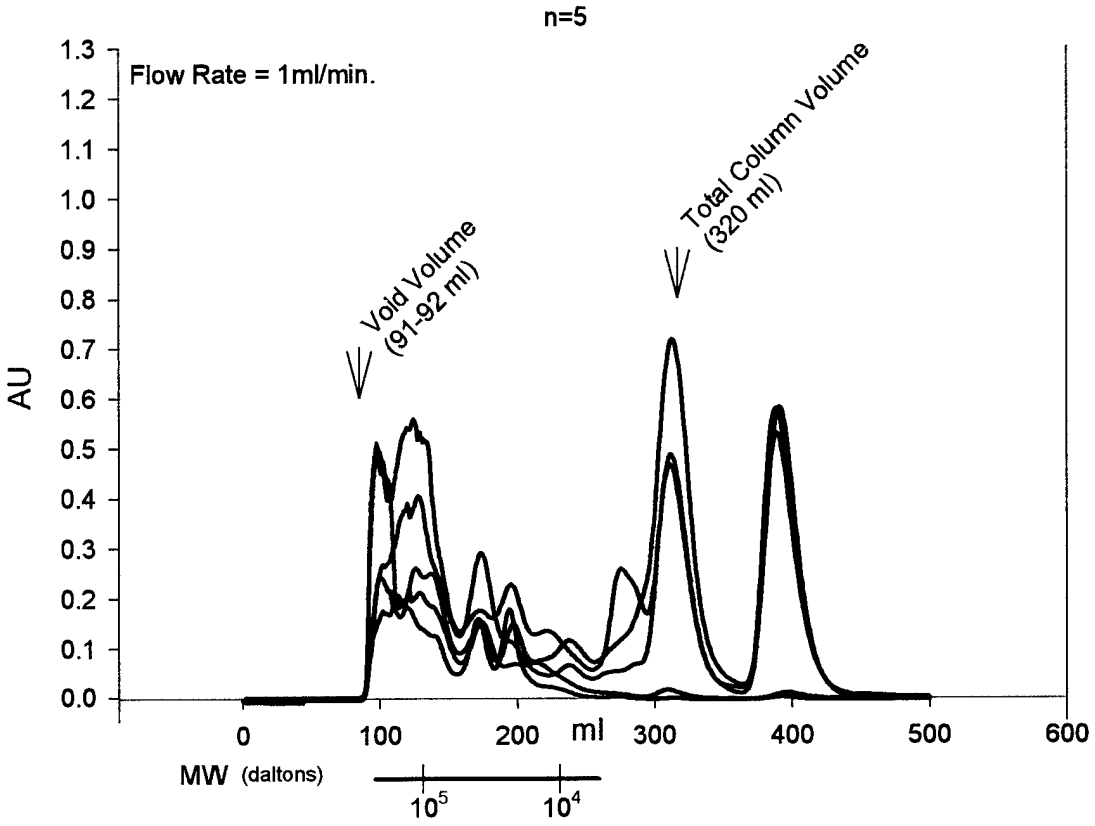


Figure 10. Chromatography of Whole Seminal Plasma from GW Veterans Whose Sexual Partners have Specific IgE Antibody to their Seminal Plasma Proteins

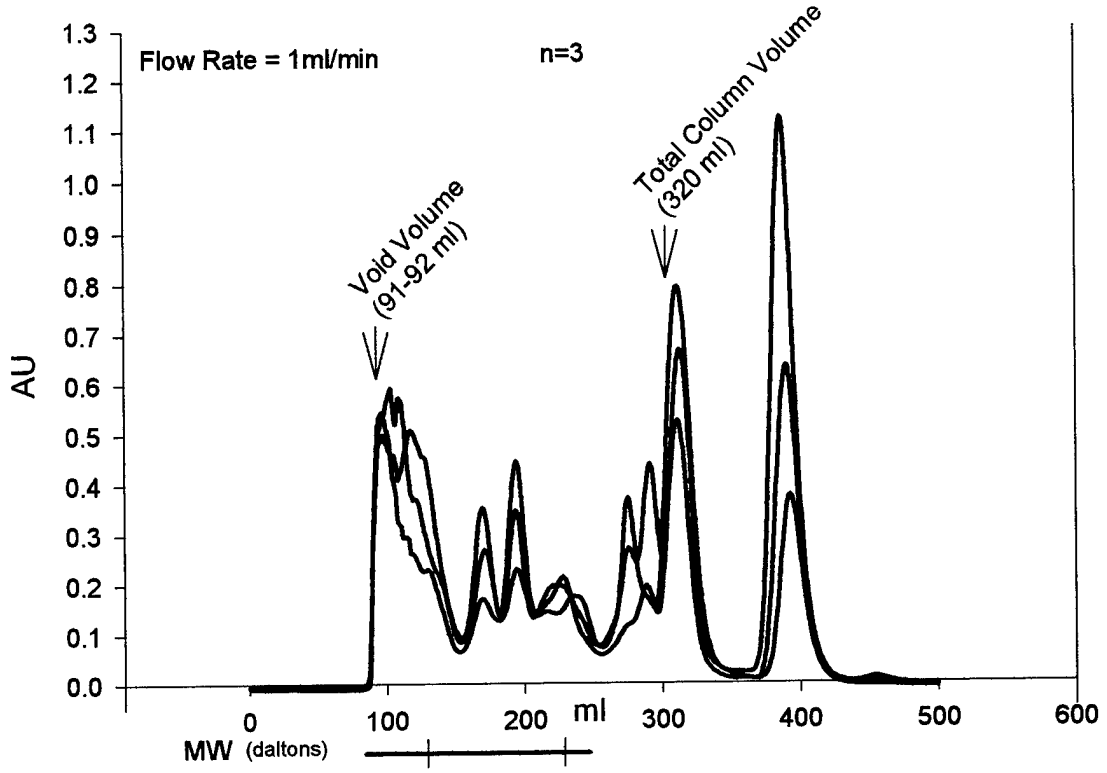


Figure 11. Chromatography of Whole Seminal Plasma from GW Veterans Whose Sexual Partners do not have Specific IgE Antibody to their Seminal Plasma Proteins

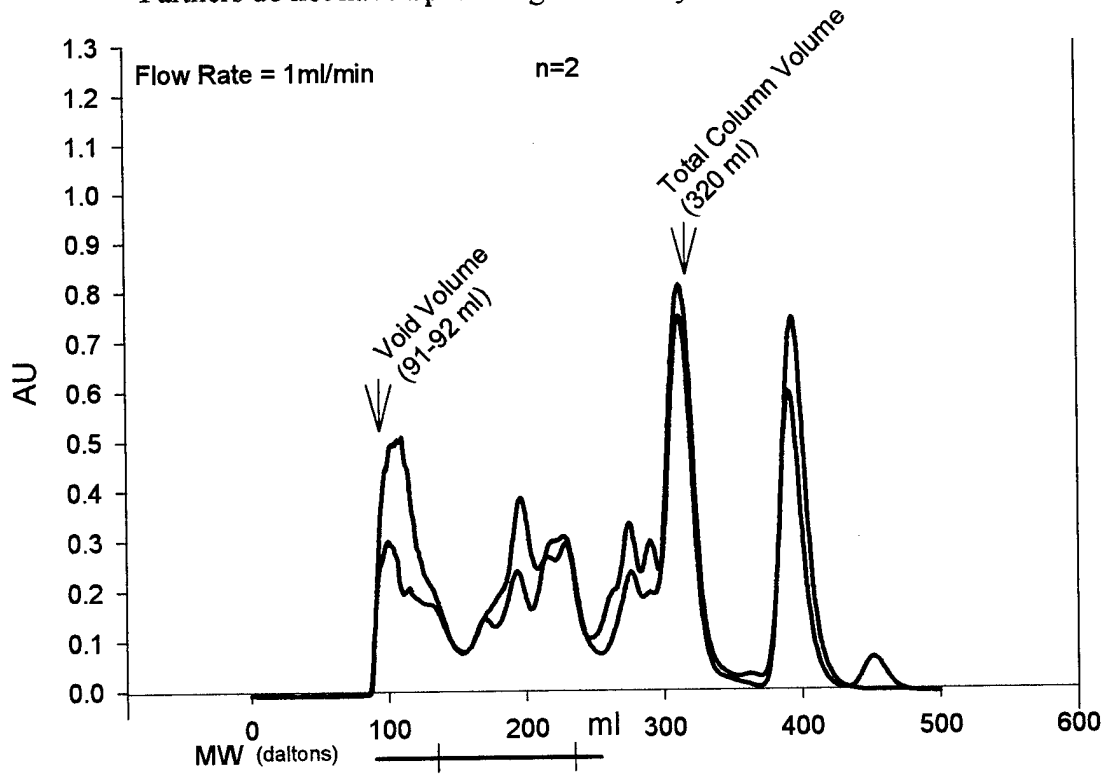


Figure 12. Chromatography of Whole Seminal Plasma from a GW Veteran Who has Specific IgE Antibody to his Own Seminal Plasma Proteins

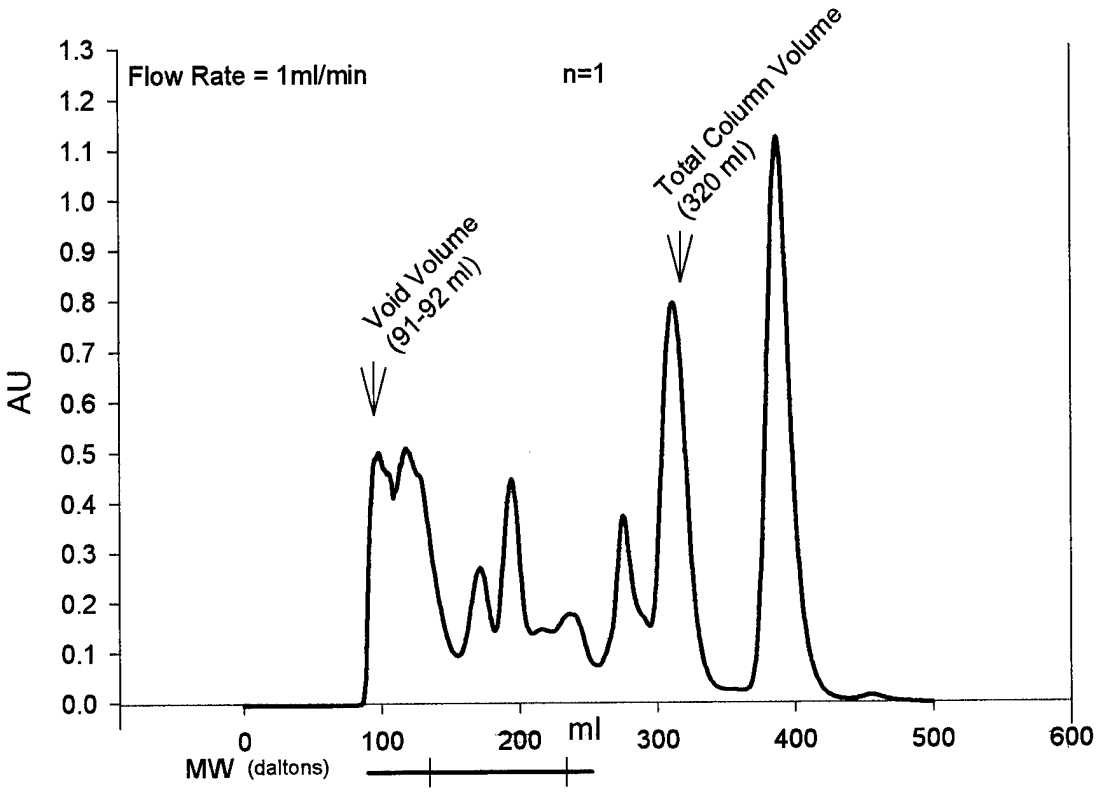


Figure 13. Chromatography of Whole Seminal Plasma from GW Veterans Who do not have Specific IgE Antibody to their Own Seminal Plasma Proteins

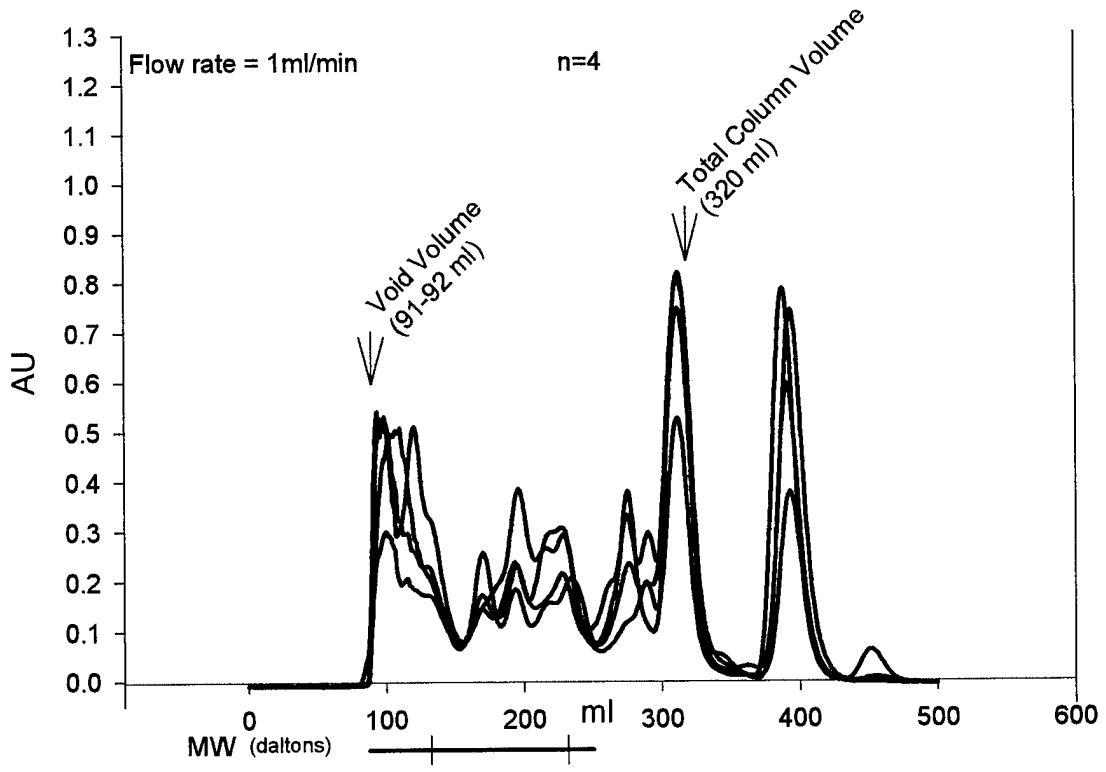


Figure 14. Chromatography of Whole Seminal Plasma from Civilian Males Whose Sexual Partners have Specific IgE Antibody their Seminal Plasma Proteins

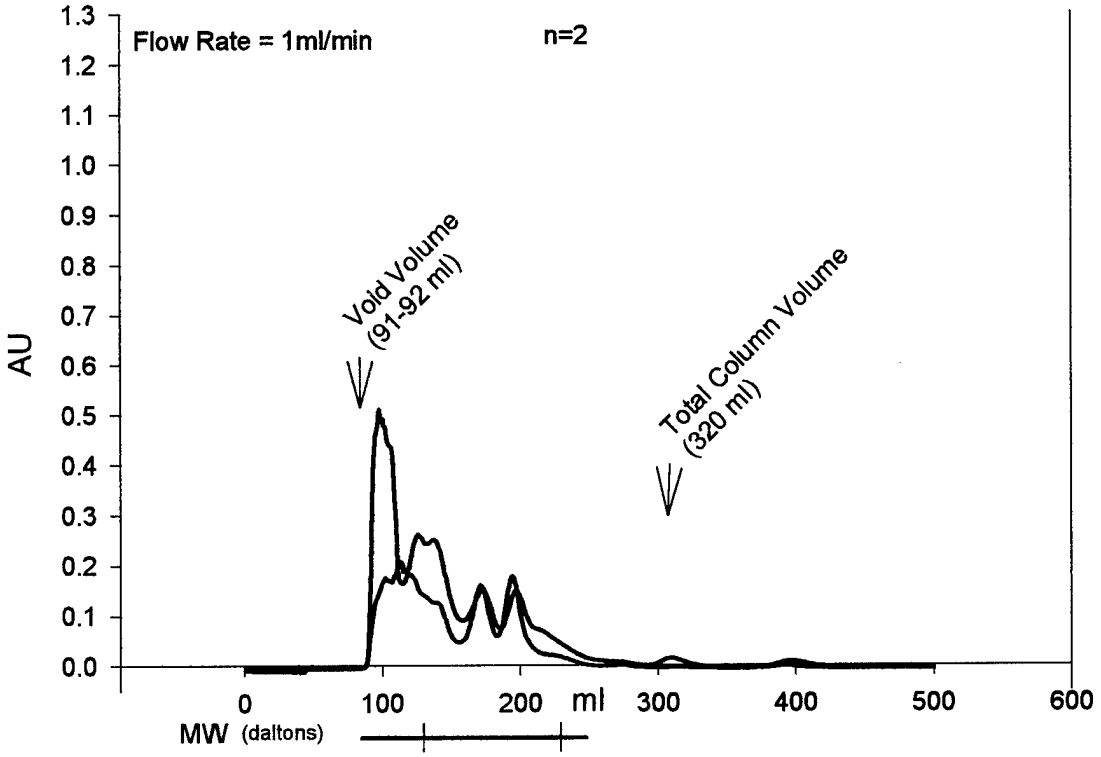


Figure 15. Chromatography of Whole Seminal Plasma from Civilian Males Whose Sexual Partners do not have Specific IgE Antibody to their Seminal Plasma Proteins

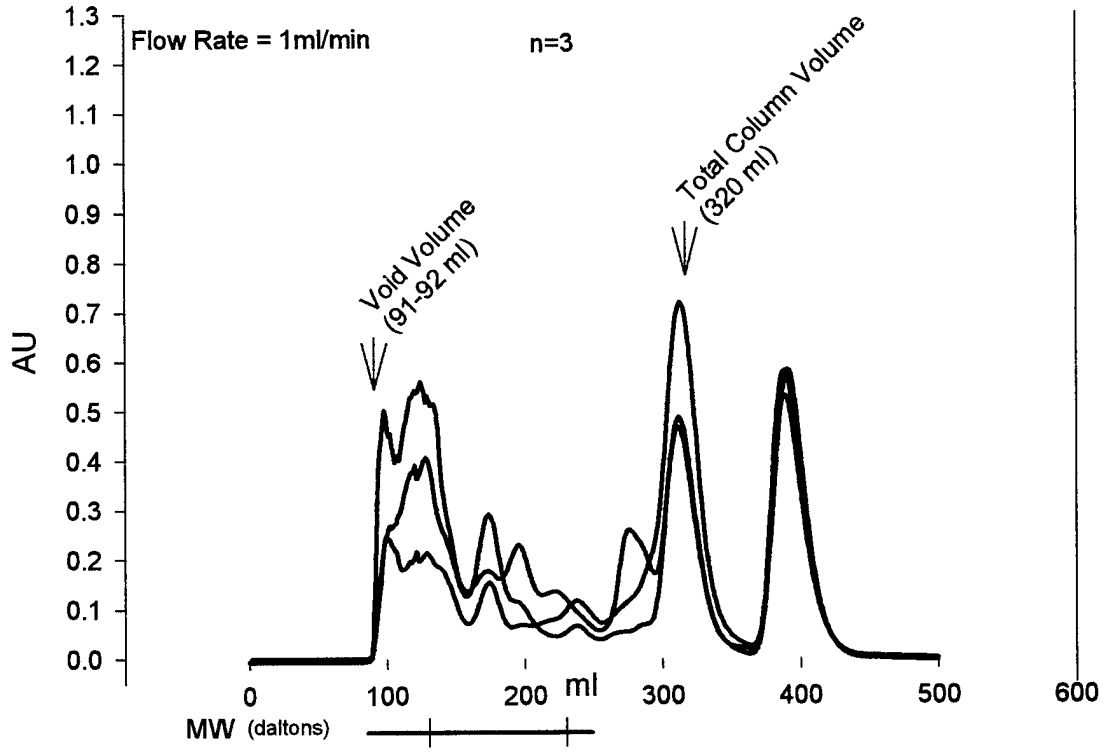


Figure 16. Chromatography of Whole Seminal Plasma from a Civilian Male Who has Specific IgE Antibody to his Own Seminal Plasma Proteins

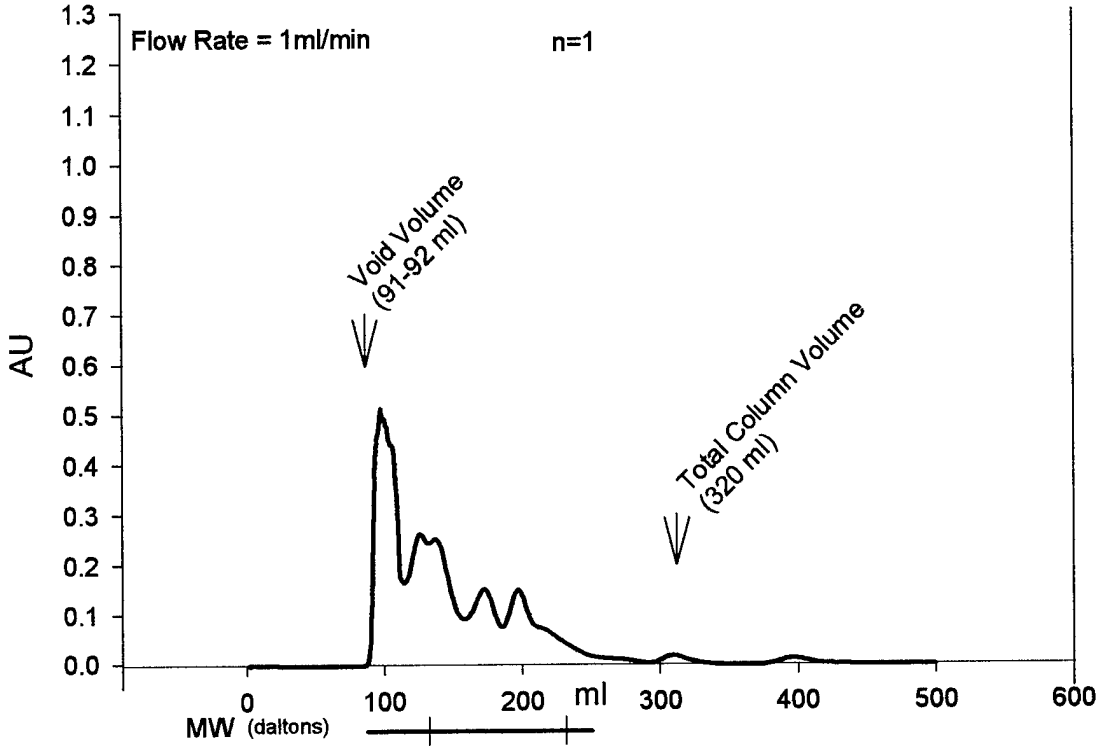
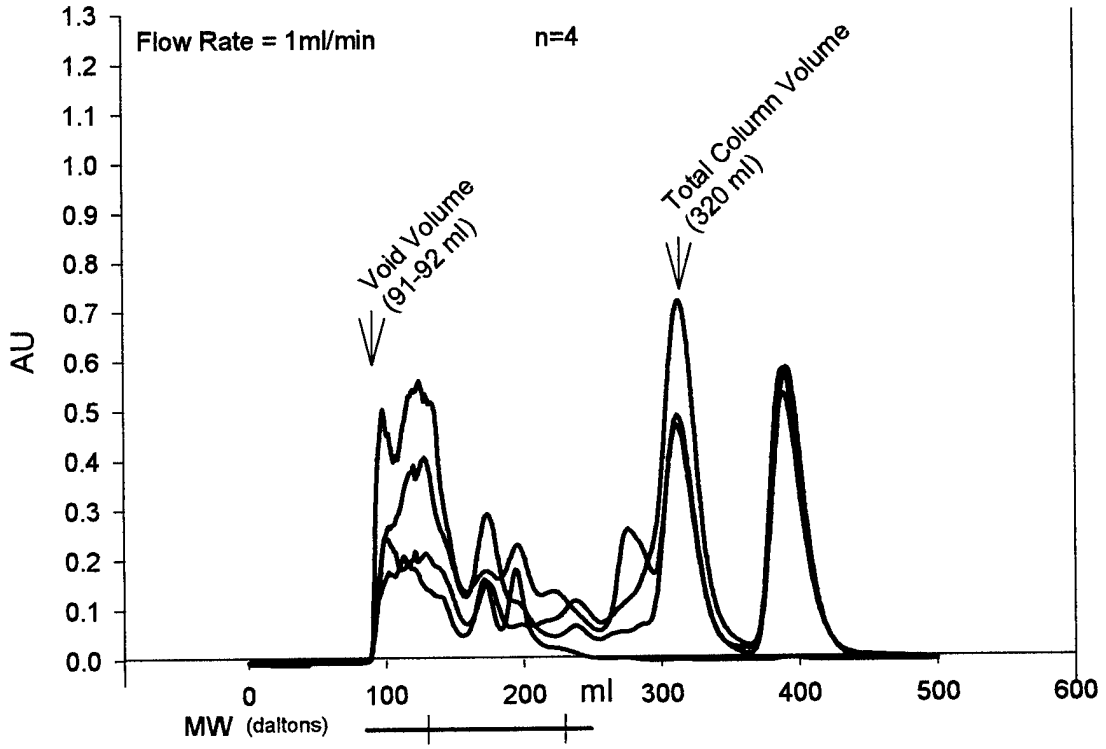


Figure 17. Chromatography of Whole Seminal Plasma from Civilian Males Who do not have Specific IgE Antibody to their Own Seminal Plasma Proteins



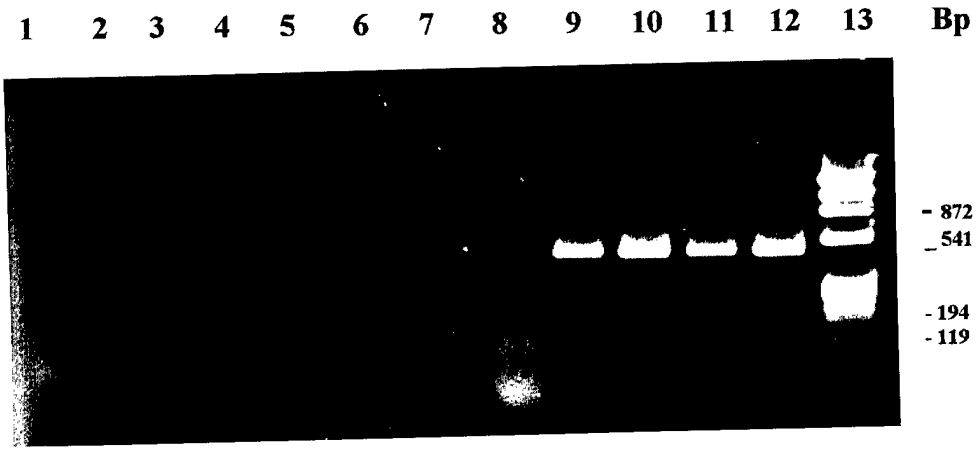


Figure 18. Agarose gel electrophoresis of PCR products from GW veterans. DNA was extracted from the seminal pellets of semen obtained from GW veterans with BSS and PCR was performed using primers specific for the urease gene of *Ureaplasma urealyticum*. Bands of correct size (541 base pairs) are seen in lanes 2 and 3 only. From left to right: Lanes 1 and 13- molecular weight standards (0.174 RD DNA Hae III); lanes 2 through 8 - DNA of 7 GW veterans; lane 9 - control *Ureaplasma urealyticum* DNA strain 9r; lane 10 - control *Ureaplasma urealyticum* DNA strain 27817; lane 11 - normal donor DNA; lane 12 - no DNA.

V. Appendices

I. Web Page
II. Questionnaires #1 and #2
III. Questionnaire #3 and PTSD Packet
IV. VA Cooperative Study
V. Laboratory Evaluation Tests
VI. GW Veteran Exposure Data

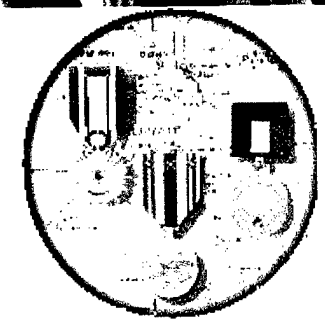
Appendix I.

Burning Semen Syndrome

About This Web Site

My CV

Survey



Appendix II.
Questionnaire #1

University of Cincinnati
Medical Center

College of Medicine
Department of Internal Medicine

Division of Immunology
University of Cincinnati
PO Box 670563
Cincinnati OH 45267-0563

231 Bethesda Avenue (Rm 7562)
Phone (513) 558-4701
Fax (513) 558-3799

QUESTIONNAIRE FOR GULF WAR "BURNING SEMEN SYNDROME"
N=151

1. Do you experience a burning sensation during or after ejaculation?
   Yes ___60%_____ No_____

2. Do you experience a burning sensation if you come in contact with your semen?
   Yes ___39%_____ No_____

3. Does your sexual partner experience a burning sensation of her skin or vagina when she comes in contact with your semen?
   Yes ___85%_____ No _____

4. Did this problem exist prior to serving in the Persian Gulf War?
   Yes ___8%_____ No _____

5. If no, did this problem begin immediately after returning from the Persian Gulf War after the first sexual encounter with your spouse or sexual partner?
   Yes ___44%_____ No _____

6. Does this burning sensation go away when you use a condom during sexual intercourse?
   Yes ___46%____ No _____

7. If you experience this problem, have you sought medical attention?
   Yes ___43%____ No _____

8. Have you been treated for any sexually transmitted diseases since returning from the Gulf War, such as gonorrhea, syphilis, cytomegalovirus, herpes virus, papilloma virus, hepatitis or human immunodeficiency virus?
   _____13%_____
   _____

9. If you and your sexual partner have experienced burning after contact with semen, would you be interested in participating in a study which investigates this problem further?
   Yes ___75%____ No _____

10. If yes, please write your name, age, wife or sexual partner's name and age along with your address, day phone, work phone and FAX if you have one.
    Name _____Age _____
    Wife or partner's name _____Age _____
    Address_____
    Phone(day)_____(work)_____Fax_____

Thank you for answering this questionnaire. If you have answered yes to these questions, I will be contacting you in the near future with further details about participation in a study investigating burning semen syndrome.

Patient Care • Education • Research • Community Service
An affirmative action/equal opportunity institution

Appendix II.
Questionnaire #2

NAME:_____

ADDRESS:_____

PHONE:_____

QUESTIONAIRE ABOUT POSSIBLE ALLERGY TO SEMEN

1. How long have you had the problem? A._____months B._____years

2. Do you have the problem exclusively with your current sexual partner?
   A._____YES B._____NO

3. If not, how many times have you experienced a reaction with other sexual partners? ____

4. Did you have the reaction on your first intercourse? A._____YES B._____NO

5. If the answer above is no, how many years after your first intercourse did the firstreaction occur?
   _____

6. Prior to the first reaction did you have:
   A. _____ a recent pregnancy
   B. _____ recent gynecologic operation
   C. _____ other gynecologic problem

7. How soon after intercourse do your reactions occur?
   A. _____ Minutes B. _____ Hours C. _____ Days

8. How long after intercourse do your reactions last?
   A. _____ Minutes B. _____ Hours C. _____ Days

9. Do you have the following symptoms?

Generalized itching	A._____YES	_____NO
Hives	B._____YES	_____NO
Chest tightness	C._____YES	_____NO
Shortness of breath	D._____YES	_____NO
Cough	E._____YES	_____NO
Wheezing	F._____YES	_____NO
Dizziness	G._____YES	_____NO
Faintness	H._____YES	_____NO
Complete collapse (shock)	I. _____YES	_____NO
Unconsciousness	J._____YES	_____NO

10. If your symptoms are localized only to the vaginal tissue and surrounding areas, do you have symptoms of:

Deep pain	A. _____YES	_____NO
Burning	B. _____YES	_____NO
Redness	C. _____YES	_____NO
Rash	D. _____YES	_____NO
Blisters	E. _____YES	_____NO

11. Does the use of condoms prevent the reaction? A._____YES B._____NO

12. How old are you now? _____

13. How old were you when the reaction first began? _____

14. Do you have other types of allergies such as asthma, hayfever, hives or eczema?
A. _____YES B. _____NO

15. Do you have allergy to foods? A. _____YES B. _____NO

16. If so, which one (s)? _____

17. Do you have allergy to drugs? A. _____YES B. _____NO

18. If so, which one (s)? _____

19. Does anyone in your family have a history of hayfever, asthma, eczema, or hives?
A. _____YES B. _____NO

20. Have you been treated for this condition before? A. _____YES B. _____NO

21. If so, what types of treatment have you had? _____

_____

22. Have you had any prior evaluation about the possible allergic aspects of your problem?
A. _____YES B. _____NO

23. Have you had any vaginitis due to Candida? A. _____YES B. _____NO

24. Do you wish to be evaluated by our medical group? A. _____YES B. _____NO

25. What is the name and address of the physician who had been treating you most recently for your problem?

NAME: _____

ADDRESS: _____

_____

PHONE:_____

(Q-SP. ltr)

QUESTIONNAIRE FOR POSSIBLE ALLERGY TO SEMEN: FOR MALES

NAME:_____

ADDRESS:_____

PHONE: ( )-_____

WHEN AND WHERE IS THE BEST TIME TO CONTACT YOU DURING THE WEEK?

_____

DATE OF BIRTH:_____ **AGE:**_____

CURRENT MILITARY STATUS:_____

1) WERE YOU STATIONED IN THE PERSIAN GULF?_____**YES** _____**NO; IF NO GO ON TO QUESTION 16. IF YES, FOR HOW LONG?**_____

_____

2) WHERE WERE YOU STATIONED WHILE IN THE PERSIAN GULF?_____

_____

3) WHAT WERE YOUR RESPONSIBILITIES OR JOBS WHILE IN THE PERSIAN GULF?_____

_____

4) WERE YOU EXPOSED TO CHEMICAL, DIESEL, PETROLEUM OR OTHER FUMES WHILE IN THE PERSIAN GULF?_____**YES** _____**NO IF SO, WHICH FUMES AND FOR HOW LONG WERE YOU EXPOSED?**_____

_____

5) DID YOU CONTRACT LEISHMANIASIS WHILE IN THE PERSIAN GULF?

_____**YES**_____**NO; IF YES, HOW WAS THIS TREATED AND FOR HOW LONG?**

_____

6) DID YOU HAVE CLOSE CONTACT WITH URANIUM WHILE IN THE PERSIAN GULF?_____YES _____NO; IF YES, WHEN AND FOR HOW LONG?_____

_____

7) WERE YOU IN THE VICINITY OF SCUD MISSILE ATTACKS WHERE YOU MAY HAVE COME IN CONTACT WITH BIOLOGICAL OR CHEMICAL WARFARE AGENTS?_____YES _____NO; IF YES, WHEN AND WHERE WERE YOU EXPOSED?

_____

_____

8) WHILE IN THE PERSIAN GULF DID YOU EVER TAKE PYRIDOSTIGMINE BROMIDE IN ANTICIPATION YOU MIGHT BE EXPOSED TO CHEMICAL WARFARE AGENTS?_____YES _____NO; IF SO, HOW MANY TABLETS DID YOU TAKE OF THIS MEDICATION AND FOR HOW LONG?_____

_____

9) DID YOU EXPERIENCE ANY SIDE EFFECTS FROM THIS MEDICATION? _____YES _____NO; IF SO, WHAT SIDE EFFECTS DID YOU EXPERIENCE AND HOW LONG DID THEY LAST?_____

_____

10) WERE YOU DIRECTLY EXPOSED TO ANY PESTICIDES WHILE IN THE PERSIAN GULF? _____YES _____ NO; IF YES, WHEN AND FOR HOW LONG WAS YOUR EXPOSURE?_____

_____

11) WERE YOU VACCINATED TO ANTHRAX AND BOTULINUM TOXIN PRIOR TO GOING TO THE GULF WAR?_____YES _____NO; WHAT OTHER VACCINATIONS, IF YES, DID YOU RECEIVE THEM PRIOR TO GOING TO THE GULF WAR?_____

_____

12) HAVE YOU EVER BEEN EVALUATED, DIAGNOSED OR TREATED FOR POST TRAUMATIC STRESS DISORDER (PTSD) SINCE RETURNING FROM THE PERSIAN GULF?_____YES _____NO; IF YES, ARE YOU CURRENTLY RECEIVING PSYCHOTHERAPY AND/OR MEDICATION FOR PTSD?_____YES _____NO; PLEASE LIST ALL MEDICATIONS YOU ARE TAKING FOR PTSD.

_____

13) WHAT WAS YOUR GENERAL STATE OF HEALTH PRIOR TO GOING TO THE GULF WAR?_____

_____

14) WERE YOU INVOLVED IN ANY DECONTAMINATION OPERATIONS AFTER THE WAR?_____YES _____NO; IF YES, PLEASE DESCRIBE YOUR INVOLVEMENT_____

_____

_____

15) DESCRIBE YOUR CURRENT STATE OF HEALTH SINCE RETURNING FROM THE PERSIAN GULF_____

_____

_____

16) DESCRIBE YOUR CURRENT STATE OF HEALTH (NON-VETERANS ONLY).

_____

_____

_____

_____

17) HAVE YOU EVER BEEN DIAGNOSED AND/OR TREATED FOR ONE OR MORE OF THE FOLLOWING SEXUALLY TRANSMITTED DISEASES?

A) GONORRHEA _____YES _____NO

B) SYPHILIS _____YES _____ NO

C) HERPES SIMPLEX VIRUS I OR II _____YES _____ NO

D) CYTOMEGALOVIRUS (CMV) _____YES _____ NO

E) HUMAN IMMUNODEFICIENCY VIRUS (HIV) _____YES _____ NO

F) HUMAN PAPILLOMA VIRUS (HPV) _____YES _____ NO

G) HEPATITIS B OR C VIRUS _____YES _____ NO

18) WERE THESE SEXUALLY TRANSMITTED DISEASES DIAGNOSED BEFORE OR AFTER SERVING IN THE GULF WAR? _____BEFORE _____AFTER

_____NOT APPLICABLE

19) DO YOU HAVE BURNING, REDNESS OR PAIN AFTER CONTACT WITH YOUR SEMEN?_____YES _____NO; IF SO, HOW LONG HAS THIS BEEN OCCURRING?_____

20) DOES YOUR SEXUAL PARTNER HAVE BURNING, REDNESS OR PAIN OF HER SKIN OR VAGINA AFTER CONTACT WITH YOUR SEMEN? _____YES _____NO; IF SO, HOW LONG HAS THIS BEEN OCCURRING?_____WKS_____MOS_____YRS

21) HAS THIS OCCURRED WITH OTHER SEXUAL PARTNERS? _____YES _____NO; IF YES, HOW MANY SEXUAL PARTNERS HAVE YOU EXPERIENCED THESE SYMPTOMS WITH?_____

22) DID YOU HAVE THIS REACTION PRIOR TO GOING TO THE PERSIAN GULF? _____YES _____NO _____NOT APPLICABLE

23) DID YOU HAVE THIS REACTION WITH YOUR FIRST INTERCOURSE AFTER RETURNING FROM THE PERSIAN GULF?_____YES _____NO _____NOT APPLICABLE; IF NO, HOW LONG AFTER RETURNING FROM THE PERSIAN GULF DID IT TAKE BEFORE YOU OR YOUR SEXUAL PARTNER STARTED TO EXPERIENCE THESE SYMPTOMS? _____DAYS _____WKS _____MOS _____ YRS

24) HOW SOON AFTER CONTACT WITH SEMEN DO THESE SYMPTOMS OCCUR?

(FOR FEMALE) _____MINS _____HRS _____DAYS

(FOR YOURSELF) _____MINS _____HRS _____DAYS

25) HOW LONG AFTER CONTACT WITH SEMEN DO THESE SYMPTOMS LAST?

(FOR FEMALE) _____MINS _____HRS _____ DAYS

(FOR YOURSELF) _____MINS _____HRS _____ DAYS

26) DO YOU HAVE ANY OF THE FOLLOWING SYMPTOMS AFTER CONTACT WITH YOUR SEMEN?

GENERALIZED ITCHING _____YES _____NO

HIVES _____YES _____NO

CHEST TIGHTNESS _____YES _____NO

SHORTNESS OF BREATH _____YES _____NO

COUGH _____YES _____NO

WHEEZING _____YES _____NO

DIZZINESS _____YES _____NO

FAINTNESS _____YES _____NO

COMPLETE COLLAPSE(SHOCK) _____YES _____NO

UNCONSCIOUSNESS _____YES _____NO

27) DOES USE OF A CONDOM PREVENT SYMPTOMS IN YOUR SEXUAL PARTNER? _____YES _____NO

28) HAVE YOU EVER HAD PROSTATITIS, A URINARY TRACT INFECTION OR OTHER URINARY TRACT DISORDER? _____YES _____NO

29) HAVE YOU HAD A VASECTOMY? _____YES _____NO; IF YES, WHAT YEAR?

_____

30) HAVE YOU EVER BEEN EVALUATED FOR AN INFERTILITY PROBLEM?

_____YES_____NO; IF YES, PLEASE EXPLAIN_____

_____

_____

31) DO YOU HAVE ANY PHYSICIAN DIAGNOSED HISTORY OF HAYFEVER, ASTHMA, HIVES AND/OR ECZEMA?_____YES _____NO; IF YES, PLEASE SPECIFY_____

_____

32) DO YOU HAVE ANY FOOD ALLERGIES? _____YES _____NO; IF YES, TO WHICH FOODS AND WHAT KIND OF REACTION(S) DO YOU EXPERIENCE?

_____

_____

33) DO YOU HAVE ANY DRUG ALLERGIES SUCH AS TO PENICILLIN OR SULFA DRUGS?_____YES _____NO; IF YES, PLEASE SPECIFY WHICH DRUGS, THE KIND OF REACTION(S) EXPERIENCED, AND HOW OLD YOU WERE AT THE TIME_____

_____

34) DO YOU TAKE ANY PRESCRIPTION OR OVER THE COUNTER MEDICATIONS ON AN AS NEEDED OR REGULAR BASIS? _____YES _____NO; IF YES, PLEASE SPECIFY_____

_____

_____

35) DOES ANYONE IN YOUR FAMILY HAVE A HISTORY OF HAYFEVER, ASTHMA, HIVES AND/OR ECZEMA?_____

_____

36) HAVE YOU PURSUED MEDICAL TREATMENT FOR THIS PROBLEM?

_____YES _____NO; IF YES, PLEASE EXPLAIN. _____

_____

_____

37) ARE YOU CURRENTLY WITH THE SAME SEXUAL PARTNER YOU HAD PRIOR TO GOING TO THE PERSIAN GULF? _____YES _____NO _____NOT APPLICABLE; IF NO; PLEASE EXPLAIN. _____

_____

_____

38) ARE YOU CURRENTLY HAVING REGULAR SEXUAL RELATIONS WITH YOUR SEXUAL PARTNER? _____YES _____NO

39) WOULD YOU BE WILLING TO PARTICIPATE IN A STUDY INVESTIGATING "BURNING SEMEN SYNDROME" WHICH WOULD REQUIRE A VISIT TO CINCINNATI, OHIO (OR A VA OR MILITARY HOSPITAL NEAR YOU) FOR A FEW DAYS IN THE NEXT SEVERAL MONTHS? (IF YOU ARE TRAVELING A FAR DISTANCE, FUNDS ARE AVAILABLE TO PARTIALLY COVER SOME TRAVEL EXPENSES FOR VETERANS AND THEIR PARTNER.) _____YES_____NO; IF NO, PLEASE EXPLAIN WHY NOT. _____

_____

_____

PLEASE USE THE SPACE BELOW AND THE BACK OF THIS QUESTIONNAIRE TO PROVIDE ANY ADDITIONAL INFORMATION THAT MAY BE RELEVANT TO YOUR PROBLEM. THANK YOU FOR ANSWERING THIS QUESTIONNAIRE. WE WILL BE CONTACTING YOU IN THE NEAR FUTURE FOR MORE INFORMATION_____

_____

_____

QUESTIONNAIRE FOR POSSIBLE ALLERGY TO SEMEN: FOR FEMALES

NAME:_____

ADDRESS:_____

PHONE: ( )-_____

WHEN AND WHERE IS THE BEST TIME TO CONTACT YOU DURING THE WEEK?

_____

DATE OF BIRTH:_____ **AGE:**_____

CURRENT MILITARY STATUS:_____

1) WERE YOU STATIONED IN THE PERSIAN GULF?_____**YES**_____**NO; IF NO GO TO QUESTION 15; IF YES, FOR HOW LONG?**_____

2) IF YES, WHERE WERE YOU STATIONED WHILE IN THE PERSIAN GULF?____

_____

3) WHAT WERE YOUR RESPONSIBILITIES OR JOBS WHILE IN THE PERSIAN GULF?_____

_____

4) WERE YOU EXPOSED TO CHEMICAL, DIESEL, PETROLEUM OR OTHER FUMES WHILE IN THE PERSIAN GULF? A._____**YES B.**_____**NO IF SO, WHICH FUMES AND FOR HOW LONG WERE YOU EXPOSED?**_____

_____

5) DID YOU CONTRACT LEISHMANIASIS WHILE IN THE PERSIAN GULF?

_____**YES**_____**NO; IF YES, HOW WAS THIS TREATED AND FOR HOW LONG?**

_____

6) DID YOU HAVE CLOSE CONTACT WITH URANIUM WHILE IN THE PERSIAN GULF?_____YES _____NO; IF YES, PLEASE EXPLAIN?_____

_____

7) WERE YOU IN THE VICINITY OF SCUD MISSILE ATTACKS WHERE YOU MAY HAVE COME IN CONTACT WITH BIOLOGICAL OR CHEMICAL WARFARE AGENTS?_____YES _____NO; IF YES, PLEASE EXPLAIN?_____

_____

8) WHILE IN THE PERSIAN GULF DID YOU EVER TAKE PYRIDOSTIGMINE BROMIDE IN ANTICIPATION THAT YOU MIGHT BE EXPOSED TO CHEMICAL WARFARE AGENTS? _____YES _____NO; IF YES, HOW MANY TABLETS DID YOU TAKE OF THIS MEDICATION AND FOR HOW LONG?_____

9) DID YOU EXPERIENCE ANY SIDE EFFECTS FROM THIS MEDICATION? _____YES _____NO; IF YES, WHAT SIDE EFFECTS DID YOU EXPERIENCE AND HOW LONG DID THEY LAST?_____

_____

10) WERE YOU DIRECTLY EXPOSED TO ANY PESTICIDES WHILE IN THE PERSIAN GULF? _____YES _____ NO; IF YES, PLEASE EXPLAIN?_____

_____

11) WERE YOU VACCINATED TO ANTHRAX AND BOTULINUM TOXIN PRIOR TO GOING TO THE GULF WAR?_____YES _____NO; WHAT OTHER VACCINATIONS, IF ANY, DID YOU RECEIVE PRIOR TO GOING TO THE GULF WAR?_____

_____

12) HAVE YOU EVER BEEN EVALUATED, DIAGNOSED OR TREATED FOR POST TRAUMATIC STRESS DISORDER (PTSD) SINCE RETURNING FROM THE PERSIAN GULF?_____YES _____NO; IF YES, ARE YOU CURRENTLY RECEIVING PSYCHOTHERAPY AND/OR MEDICATION FOR PTSD?_____YES _____NO.

IF YES, PLEASE LIST ANY MEDICATIONS YOU ARE TAKING FOR PTSD.

_____

_____

13) WERE YOU INVOLVED IN ANY DECONTAMINATION OPERATIONS AFTER THE WAR?_____YES _____NO; IF YES, PLEASE DESCRIBE YOUR INVOLVEMENT.

_____

_____

14) WHAT WAS YOUR GENERAL STATE OF HEALTH PRIOR TO GOING TO THE GULF WAR?_____

_____

15) DESCRIBE YOUR CURRENT STATE OF HEALTH._____

_____

_____

_____

_____

_____

_____

_____

16) HAVE YOU EVER BEEN DIAGNOSED AND/OR TREATED FOR ONE OR MORE OF THE FOLLOWING SEXUALLY TRANSMITTED DISEASES?

A) GONORRHEA _____YES _____NO

B) SYPHILIS _____YES _____NO

C) HERPES SIMPLEX VIRUS I OR II _____YES _____NO

D) CYTOMEGALOVIRUS (CMV) _____YES _____NO

E) HUMAN IMMUNODEFICIENCY VIRUS (HIV) _____YES _____NO

F) HUMAN PAPILLOMA VIRUS (HPV) _____YES _____NO

G) HEPATITIS B OR C VIRUS _____YES _____NO

17) WERE THESE SEXUALLY TRANSMITTED DISEASES DIAGNOSED BEFORE OR AFTER SERVING IN THE GULF WAR? _____BEFORE _____AFTER

_____NOT APPLICABLE (GO TO QUESTION 18)

18) WERE THESE SEXUALLY TRANSMITTED DISEASES DIAGNOSED BEFORE OR AFTER YOUR SEXUAL PARTNER SERVED IN THE GULF WAR?

_____BEFORE_____AFTER _____NOT APPLICABLE (GO TO QUESTION 19)

19) DO YOU HAVE BURNING, REDNESS OR PAIN AFTER CONTACT WITH YOUR SEXUAL PARTNER'S SEMEN?_____YES _____NO; IF YES, HOW LONG HAS THIS BEEN OCCURRING?_____

20) HAVE YOU EXPERIENCED BURNING, REDNESS OR PAIN OF YOUR SKIN OR VAGINA AFTER CONTACT WITH SEXUAL PARTNERS OTHER THAN YOUR CURRENT PARTNER? _____YES _____NO; IF YOU HAVE ORAL SEX, DO YOU GET BURNING OR OTHER SYMPTOMS IN YOU MOUTH, THROAT OR STOMACH? _____YES_____NO_____NOT APPLICABLE

21) HOW LONG HAVE THESE SYMPTOMS BEEN OCCURRING? _____WKS _____MOS _____YRS

22) HOW MANY OTHER SEXUAL PARTNERS HAVE YOU EXPERIENCED THESE SYMPTOMS WITH? _____

23) DID YOU HAVE THESE REACTIONS PRIOR TO GOING TO THE PERSIAN GULF?_____YES _____NO_____NOT APPLICABLE (GO TO QUESTION 24)

24) DID YOU HAVE THESE REACTIONS PRIOR TO YOUR SEXUAL PARTNER GOING TO THE PERSIAN GULF? _____YES _____NO_____NOT APPLICABLE (GO TO QUESTION 25)

25) DID YOU HAVE THIS REACTION WITH YOUR FIRST INTERCOURSE AFTER RETURNING FROM THE PERSIAN GULF?____YES____NO ____NOT APPLICABLE (GO TO QUESTION 26)

26) DID YOU HAVE THIS REACTION WITH FIRST INTERCOURSE AFTER YOUR SEXUAL PARTNER RETURNED FROM THE PERSIAN GULF? ____YES ____NO_____NOT APPLICABLE (GO TO QUESTION 27)

27) HOW LONG AFTER RETURNING FROM THE PERSIAN GULF DID IT TAKE BEFORE YOU STARTED TO EXPERIENCE THESE SYMPTOMS? _____DAYS _____WKS _____MOS _____ YRS_____NOT APPLICABLE (GO TO QUESTION 28)

28) HOW LONG AFTER YOUR SEXUAL PARTNER RETURNED FROM THE PERSIAN GULF DID IT TAKE BEFORE YOU STARTED TO EXPERIENCE THESE SYMPTOMS? _____DAYS_____WKS_____MOS_____YRS_____NOT APPLICABLE (GO TO QUESTION 29)

29) HOW SOON AFTER CONTACT WITH SEMEN DO THESE SYMPTOMS OCCUR? _____MINS _____HRS _____DAYS

30) HOW LONG AFTER CONTACT WITH SEMEN DO THESE SYMPTOMS LAST?

_____MINS _____HRS _____ DAYS

31) PRIOR TO YOUR FIRST REACTION, DID YOU HAVE A RECENT PREGNANCY, GYNECOLOGIC OPERATION OR OTHER PROCEDURE? _____YES _____NO; IF YES, PLEASE SPECIFY_____

_____

32) WHICH OF THE FOLLOWING SYMPTOMS AFTER CONTACT WITH SEMEN DO YOU EXPERIENCE?

GENERALIZED ITCHING	_____YES _____NO
HIVES	_____YES _____NO
CHEST TIGHTNESS	_____YES _____NO
SHORTNESS OF BREATH	_____YES _____NO
COUGH	_____YES _____NO
WHEEZING	_____YES _____NO
DIZZINESS	_____YES _____NO
FAINTNESS	_____YES _____NO
COMPLETE COLLAPSE(SHOCK)	_____YES _____NO
UNCONSCIOUSNESS	_____YES _____NO
BURNING	_____YES _____NO
VAGINAL ITCHING	_____YES _____NO
VAGINAL SWELLING	_____YES _____NO

BLISTERS _____YES _____NO

DEEP PAIN _____ YES _____NO

RASH OTHER THAN HIVES _____YES _____NO

OTHER REACTIONS (PLEASE DESCRIBE)_____

_____

33) DOES USE OF A CONDOM COMPLETELY PREVENT SYMPTOMS?

_____YES _____NO

34) DO YOU HAVE ANY PHYSICIAN DIAGNOSED HISTORY OF HAYFEVER, ASTHMA, HIVES AND/OR ECZEMA?_____YES _____NO; IF YES, PLEASE SPECIFY_____

_____

35) DO YOU HAVE ANY FOOD ALLERGIES? _____YES _____NO; IF YES, WHICH FOODS AND WHAT KIND OF REACTION(S) DO YOU EXPERIENCE?_____

_____

36) DO YOU HAVE ANY DRUG ALLERGIES SUCH AS TO PENICILLIN OR SULFA DRUGS?_____YES _____NO; IF YES, PLEASE SPECIFY WHICH DRUGS, THE KIND OF REACTION(S) EXPERIENCED AND HOW OLD YOU WERE AT THE TIME THE REACTION OCCURRED_____

_____

_____

37) DO YOU HAVE RECURRENT VAGINAL YEAST INFECTIONS? _____YES ____NO; IF YES, HOW FREQUENT ARE THEY?_____

_____

38) DO YOU HAVE DIABETES?_____YES _____NO

39) HAVE YOU EVER TAKEN ORAL CONTRACEPTIVES? _____YES_____NO

40) ARE YOU CURRENTLY USING ORAL CONTRACEPTIVES?_____YES _____NO;

IF YES; WHICH BRAND AND FOR HOW LONG?_____

_____

41) DO YOU TAKE ANY PRESCRIPTION OR OVER THE COUNTER

MEDICATIONS ON AN AS NEEDED OR REGULAR BASIS? _____YES _____NO;

IF YES, PLEASE SPECIFY_____

_____

42) DOES ANYONE IN YOUR FAMILY HAVE A HISTORY OF HAYFEVER,

ASTHMA, HIVES AND/OR ECZEMA?_____

43) ARE YOU CURRENTLY WITH THE SAME SEXUAL PARTNER THAT YOU

WERE WITH FIVE YEARS AGO?_____YES _____NO; IF NO; PLEASE EXPLAIN

_____

_____

44) ARE YOU CURRENTLY HAVING REGULAR SEXUAL RELATIONS WITH

YOUR SEXUAL PARTNER? _____YES _____NO

45) HAVE YOU PURSUED MEDICAL TREATMENT FOR THIS PROBLEM?

_____YES _____NO; IF YES, PLEASE EXPLAIN_____

_____

_____

_____

46) WOULD YOU BE WILLING TO PARTICIPATE IN A STUDY INVESTIGATING "BURNING SEMEN SYNDROME" WHICH MAY ENTAIL COMING TO CINCINNATI, OHIO (OR A VA OR MILITARY HOSPITAL NEAR YOU) FOR A FEW DAYS IN THE NEXT SEVERAL MONTHS? (IF YOU ARE TRAVELING A FAR DISTANCE, FUNDS ARE AVAILABLE TO PARTIALLY COVER TRAVEL EXPENSES FOR VETERANS AND THEIR PARTNER.) _____YES _____NO; IF NO, EXPLAIN WHY

_____

_____

_____

PLEASE USE THE SPACE BELOW OR THE BACK OF THIS QUESTIONNAIRE TO PROVIDE ANY INFORMATION THAT MAY BE RELEVANT TO YOUR PROBLEM. THANK YOU FOR ANSWERING THIS QUESTIONNAIRE. WE WILL BE IN CONTACT WITH YOU IN THE NEAR FUTURE TO DISCUSS FURTHER EVALUATION OF YOUR PROBLEM IF YOU ARE AGREEABLE.

_____

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Appendix III.
PTSD Surveys

SURVEYS TO BE COMPLETED BY PERSIAN GULF WAR VETERANS

NAME:_____

ADDRESS:_____

PHONE:_____

DATE OF BIRTH:_____ AGE:_____

Please complete the attached surveys so that we may have a more complete view of your Persian Gulf War experience and your general well being. Please call the program coordinator should you have any questions.

Thank you.

COMBAT EXPOSURE SCALE

Please circle one answer for each item.

1. Did you ever go on combat patrols or have other very dangerous duty? (drive in convoys, in a combat zone, patrol rivers, helicopter assaults, perimeter guard duty, etc.)

1	2	3	4	5
NO	1-3 TIMES	4-12 TIMES	13-50 TIMES	MORE THAN 50 TIMES

2. Were you ever under enemy fire?

1	2	3	4	5
NEVER	< 1 MONTH	1-3 MONTHS	4-6 MONTHS	MORE THAN 6 MONTHS

3. Were you ever surrounded by the enemy?

1	2	3	4
NO	1-2 TIMES	3-12 TIMES	MORE THAN 12 TIMES

4. What percentage of the men in your unit were killed (KIA), wounded, or missing in action (MIA)?

1	2	3	4
NO ONE	1-25%	26-50%	MORE THAN 50%

5. How often did you fire rounds at the enemy?

1	2	3	4	5
NEVER	1-2 TIMES	3-12 TIMES	13-50 TIMES	51 OR MORE

6. How often did you see someone hit be incoming or outgoing rounds? (at the moment it happened or very soon afterwards, enemy or American)

1	2	3	4	5
NEVER	1-2 TIMES	3-12 TIMES	13-50 TIMES	51 OR MORE

7. How often were you in danger of being injured or killed? (i.e., pinned down, ambushed, near miss, an incident where you thought you were not going to make it, a really close call, etc.)

1	2	3	4	5
NEVER	1-2 TIMES	3-12 TIMES	13-50 TIMES	51 OR MORE

8. Were you involved in handling dead bodies?

1	2	3	4
NO	1-2 TIMES	3-12 TIMES	MORE THAN 12 TIMES

Please answer the following questions about atrocities that you may have heard of, witnessed, or participated in during your military experience. Circle the answer that is most appropriate to your experience.

1. Torturing prisoners of war:
   (a) no experience
   (b) heard about it
   (c) witnessed it
   (d) participated in it

2. Torturing civilians:
   (a) no experience
   (b) heard about it
   (c) witnessed it
   (d) participated in it

3. Killing prisoners of war:
   (a) no experience
   (b) heard about it
   (c) witnessed it
   (d) participated in it

4. Killing civilians:
   (a) no experience
   (b) heard about it
   (c) witnessed it
   (d) participated in it

5. Mutilating corpses:
   (a) no experience
   (b) heard about it
   (c) witnessed it
   (d) participated in it

6. Killing children:
   (a) no experience
   (b) heard about it
   (c) witnessed it
   (d) participated in it

MISSISSIPPI PTSD RATING SCALE

Please circle the number that best describes how you feel about each statement.

1. In the past, I had more close friends than I have now.

01	02	03	04	05
NOT AT ALL TRUE	SLIGHTLY TRUE	SOMEWHAT TRUE	VERY TRUE	EXTREMELY TRUE

2. I do not feel guilt over things that I did in the past.

01	02	03	04	05
NEVER TRUE	RARELY TRUE	SOMETIMES TRUE	USUALLY TRUE	ALWAYS TRUE

3. If someone pushes me too far, I am likely to become violent.

01	02	03	04	05
VERY UNLIKELY	UNLIKELY	SOMEWHAT UNLIKELY	VERY LIKELY	EXTREMELY LIKELY

4. If something happens that reminds me of the past, I become very distressed and upset.

01	02	03	04	05
NEVER	RARELY	SOMETIMES	FREQUENTLY	VERY FREQUENTLY

5. The people who know me best are afraid of me.

01	02	03	04	05
NEVER TRUE	RARELY TRUE	SOMETIMES TRUE	FREQUENTLY TRUE	VERY FREQUENTLY TRUE

6. I am able to get emotionally close to others.

01	02	03	04	05
NEVER	RARELY	SOMETIMES	FREQUENTLY	VERY FREQUENTLY

7. I have nightmares of experiences in my past that really happened.

01	02	03	04	05
NEVER	RARELY	SOMETIMES	FREQUENTLY	VERY FREQUENTLY

8. When I think of some of the things I have done in the past, I wish I were dead.

01	02	03	04	05
NEVER TRUE	RARELY TRUE	SOMETIMES TRUE	FREQUENTLY TRUE	VERY FREQUENTLY TRUE

9. It seems as if I have no feelings.

01	02	03	04	05
NOT AT ALL TRUE	RARELY TRUE	SOMETIMES TRUE	FREQUENTLY TRUE	VERY FREQUENTLY TRUE

10. Lately, I have felt like killing myself.

01	02	03	04	05
NOT AT ALL TRUE	SLIGHTLY TRUE	SOMEWHAT TRUE	VERY TRUE	EXTREMELY TRUE

11. I fall asleep, stay asleep and only awaken when the alarm goes off.

01	02	03	04	05
NEVER	RARELY	SOMETIMES	FREQUENTLY	VERY FREQUENTLY

12. I wonder why I am still alive when others have died.

01	02	03	04	05
NEVER	RARELY	SOMETIMES	FREQUENTLY	VERY FREQUENTLY

13. Being in certain situations make me feel as though I am back in the past.

01	02	03	04	05
NEVER	RARELY	SOMETIMES	FREQUENTLY	VERY FREQUENTLY

14. My dreams at night are so real that I waken in a cold sweat and force myself to stay awake.

01	02	03	04	05
NEVER	RARELY	SOMETIMES	FREQUENTLY	VERY FREQUENTLY

15. I feel like I can not go on.

01	02	03	04	05
NOT AT ALL TRUE	RARELY TRUE	SOMETIMES TRUE	VERY TRUE	ALMOST ALWAYS TRUE

16. I do not laugh or cry at the same things other people do.

01	02	03	04	05
NOT AT ALL TRUE	RARELY TRUE	SOMETIMES TRUE	VERY TRUE	EXTREMELY TRUE

17. I still enjoy doing many things that I used to enjoy.

01	02	03	04	05
NEVER TRUE	RARELY TRUE	SOMETIMES TRUE	USUALLY TRUE	ALWAYS TRUE

18. Daydreams are very real and frightening.

01	02	03	04	05
NOT AT ALL TRUE	RARELY TRUE	SOMETIMES TRUE	FREQUENTLY TRUE	VERY FREQUENTLY TRUE

19. I have found it easy to keep a job.

01	02	03	04	05
NOT AT ALL TRUE	SLIGHTLY TRUE	SOMEWHAT TRUE	VERY TRUE	EXTREMELY TRUE

20. I have trouble concentrating on tasks.

01	02	03	04	05
NEVER TRUE	RARELY TRUE	SOMETIMES TRUE	FREQUENTLY TRUE	VERY FREQUENTLY TRUE

21. I have cried for no good reason.

01	02	03	04	05
NEVER	RARELY	SOMETIMES	FREQUENTLY	VERY FREQUENTLY

22. I enjoy the company of others.

01	02	03	04	05
NEVER	RARELY	SOMETIMES	FREQUENTLY	VERY FREQUENTLY

23. I am frightened by my urges.

01	02	03	04	05
NEVER	RARELY	SOMETIMES	FREQUENTLY	VERY FREQUENTLY

24. I fall asleep easily at night.

01	02	03	04	05
NEVER	RARELY	SOMETIMES	FREQUENTLY	VERY FREQUENTLY

25. Unexpected noises make me jump.

01	02	03	04	05
NEVER	RARELY	SOMETIMES	FREQUENTLY	VERY FREQUENTLY

26. No one understands how I feel, not even my family.

01	02	03	04	05
NOT AT ALL TRUE	RARELY TRUE	SOMEWHAT TRUE	VERY TRUE	EXTREMELY TRUE

27. I am an easy-going, even-tempered person.

01	02	03	04	05
NEVER	RARELY	SOMETIMES	USUALLY	VERY MUCH SO

28. I feel there are certain things that I have done that I can never tell anyone, because no one would ever understand.

01	02	03	04	05
NOT AT ALL TRUE	SLIGHTLY TRUE	SOMEWHAT TRUE	TRUE	VERY TRUE

29. There have been times when I used alcohol (or other drugs) to help me sleep or to make me forget about things that happened in the past.

01	02	03	04	05
NEVER	INFREQUENTLY	SOMETIMES	FREQUENTLY	VERY FREQUENTLY

30. I feel comfortable when I am in a crowd.

01	02	03	04	05
NEVER	RARELY	SOMETIMES	USUALLY	ALWAYS

31. I lose my cool and explode over minor everyday things.

01	02	03	04	05
NEVER	RARELY	SOMETIMES	FREQUENTLY	VERY FREQUENTLY

32. I am afraid to go to sleep at night.

01	02	03	04	05
NEVER	RARELY	SOMETIMES	FREQUENTLY	ALMOST ALWAYS

33. I try to stay away from anything that will remind me of things which happened in my past.

01	02	03	04	05
NEVER	RARELY	SOMETIMES	FREQUENTLY	ALMOST ALWAYS

34. My memory is as good as it ever was.

01	02	03	04	05
NOT AT ALL TRUE	RARELY TRUE	SOMETIMES TRUE	USUALLY TRUE	ALMOST ALWAYS TRUE

35. I have a hard time expressing my feelings, even to the people I care about.

01	02	03	04	05
NOT AT ALL TRUE	RARELY TRUE	SOMETIMES TRUE	FREQUENTLY TRUE	ALMOST ALWAYS TRUE

36. At times I suddenly act or feel as though something that happened in the past were happening all over again.

01	02	03	04	05
NOT AT ALL TRUE	RARELY TRUE	SOMETIMES TRUE	FREQUENTLY TRUE	ALMOST ALWAYS TRUE

37. I am unable to remember some important things that happened in the past.

01	02	03	04	05
NOT AT ALL TRUE	RARELY TRUE	SOMETIMES TRUE	USUALLY TRUE	ALMOST ALWAYS TRUE

38. I feel "super alert" or "on guard" much of the time.

01	02	03	04	05
NOT AT ALL TRUE	RARELY TRUE	SOMETIMES TRUE	FREQUENTLY TRUE	ALMOST ALWAYS TRUE

39. If something happens that reminds me of the past, I get so anxious or panicky that my heart pounds hard; I have trouble getting my breath, I sweat, tremble or shake; or feel dizzy, tingly, or faint.

01	02	03	04	05
NEVER	RARELY	SOMETIMES	FREQUENTLY	VERY FREQUENTLY

VA COOPERATIVE STUDY 458
National Health Survey of Gulf War Era Veterans and Their Families

ADULT HISTORY (FORM 05)

HOSPITAL CODE [ ][ ][ ] FAMILY ID [ ][ ][ ] PERSON ID [ ][ ]

FORM [0][5] RECORD [ ][ ] DATE OF EXAM [ ][ ] MONTH [ ][ ] DAY [ ][ ][ ][ ] YEAR

7. Genitourinary

At any time during the past year have you had:	Yes	No	Don't Know	Refused
a. Unexplained frequent urination?	1	2	8	9
b. A loss of control of your bladder?	1	2	8	9
c. Repeated interruption of your sleep because of a need to urinate?	1	2	8	9
d. Difficulty starting to urinate?	1	2	8	9
e. A weak, dribbling urinary stream?	1	2	8	9
f. A full bladder but were unable to urinate?	1	2	8	9
g. Blood in your urine?	1	2	8	9
h. A discharge from your penis? **(Males only)**	1	2	8	9
i. Any sores, growths, or warts on your penis? **(Males only)**	1	2	8	9
j. A swelling of your testicles or scrotum? **(Males only)**	1	2	8	9
k. Persistent difficulty in getting a satisfactory erection for sexual purposes? **(Males only)**	1	2	8	9
l. Any persistent difficulty in getting a satisfactory ejaculation? **(Males only)**	1	2	8	9
m. Do you experience a burning sensation during or after ejaculation? **(Males only)**	1	2	8	9
n. Do you experience a burning sensation if you come in contact with your semen? **(Males only)**	1	2	8	9
o. Does your sexual partner experience a burning sensation of her skin or vagina when she comes in contact with your semen? **(Males only)**	1	2	8	9
p. Did this problem exist prior to serving in the Gulf War? **(Males only)**	1	2	8	9
q. If no, did this problem begin immediately after returning from the Gulf War after the first sexual encounter with your spouse or sexual partner? **(Males only)**	1	2	8	9
r. Does this burning sensation go away when you use a condom during sexual intercourse? **(Males only)**	1	2	8	9

Laboratory Orders for Persian Gulf War Veterans & Spouses "Burning Semen Syndrome" Study

Male (Semen Cultures)	Female (Vaginal/Cervical Cultures)
	***Pap Smear**
***Candida - Culture and KOH prep**	***Candida - Culture and KOH prep**
Gardinerella - KOH prep/wet mount	Gardinerella - KOH prep/wet mount
Trichomonas - KOH prep/wet mount	Trichomonas - KOH prep/wet mount
Chlamydia - viral transport medium	Chlamydia - viral transport medium
***Mycoplasma - mycoplasma medium**	***Mycoplasma - mycoplasma medium**
Gonorrhea - thayer-martin agar plate	Gonorrhea - thayer martin agar plate
HSV I and II - viral transport medium	HSV I and II - viral transport medium
CMV - viral transport medium	CMV - viral transport medium
	HPV - DNA probe B211

Serologic Assessment (both Male and Female)	
CBC with differential	Renal, bone, liver panels
***ANA**	TSH
C_3, C_4 (Complement 3 & 4)	***WSR**
Urinalysis	Routine Urine Culture
***RPR**	HSV I and II
CMV	***HIV**
Hepatitis B Surface Antigen	Hepatitis C Antibody
Hepatitis B Core IgM	

Male only
PSA (prostate specific antigen)

*** IN ADDITION**

From each male and female, collect **4 tubes of 10 ml of blood in a Serum Separator tube** (e.g. Vacutainer red top tube with separator gel and clot activator). Leave at room temperature until blood clots, about 1 hour, then centrifuge and remove serum. Serum can be stored a 4°C (refrigerator) up to 3 days prior to shipping. For each male and female, also **draw 1 tube of 5 ml of blood in a whole blood EDTA tube** (lavender top). Whole blood specimens should be kept at room temperature until shipped. Specimens should be shipped with ice packs during warm weather and should arrive in our lab within 24 hours of being drawn. DO NOT FREEZE THE SPECIMENS.

The tubes should be packed to prevent leakage or breakage (e.g. sealed plastic ziplock bag or biohazard bag in a Styrofoam tube container) and shipped by overnight carrier, preferably FedEx.** FedEx shipping charges will be covered by our Laboratory. Call for the account number. SHIP SPECIMENS USING ICE PACKS. DO **NOT** USE DRY ICE.

SHIP TO: Dr. Jonathan Bernstein (513) 558-3941 or 513-558-4701
Allergy Laboratory (atten: A. Perez)
University of Cincinnati, ML 563
Medical Sciences Building, Room 7457
231 Bethesda Avenue
Cincinnati, OH 45267-0563

* **These tests and specimens are considered essential.**

** **Specimen collection and shipping supplies are available upon request.**

Laboratory Orders for Persian Gulf War Veterans & Spouses
"Burning Semen Syndrome" Study

For both male and female, a skin prick test should be performed to the following allergens:

HISTAMINE	**SALINE**	**DUST MITE**
RAGWEED, SHORT	**CAT**	**DOG**
FESCUE	**BOX ELDER**	**OAK**
WILLOW	**ALTERNARIA**	**CLADOSPORIUM**
PENICILLIUM	**ASPERIGILLUS FUMIGATUS**	
MUCOR	****SEMEN**	

The **PRICK TESTS** are to be interpreted 20 minutes after application.
CRITERIA FOR GRADING:

0 NO REACTION
1+ ERYTHEMA ONLY
2+ ERYTHEMA PLUS WHEAL < 3mm
3+ ERYTHEMA PLUS WHEAL ≥ 3mm
4+ WHEAL ≥ 3mm WITH PSEUDOPODS

** This requires obtaining a fresh ejaculate from the spouse and letting it sit at room temperature to liquefy for 30 minutes. The specimen should then be spun down to remove the spermatozoa (the precipitant). A prick test should then be performed using the seminal plasma supernatant which contains the seminal plasma proteins on both the female and her spouse's forearms. This is to confirm a systemic hypersensitivity response to seminal plasma proteins.

* **If skin prick testing is not available**, the following lab work should be substituted:

1) **Total IgE**
2) **Serologic assessment of specific IgE antibodies to the above allergens (i.e. RAST, Unicap...)**

FINALLY

Please provide the couple with two specimen cups (semen) suitable for shipping. The couple will be providing Dr. Bernstein with both a fresh and pooled semen specimen at another time.

DIRECT ANY QUESTIONS TO: Adrienne S. Perez, M.A.
Program Coordinator
513-558-3941

Modeled Pollutants of Concern

Volatile Organic Compounds		
Benzene	Toluene	m-Xylene
o-Xylene	p-Xylene	Propylbenzene
Ethylbenzene		
Polycyclic Aromatic Hydrocarbons		
Naphthalene		
Criteria Pollutant Gases		
Sulfur Dioxide		
Particulates, Metals, Inorganics		
Total Suspended Particulate	Iron	Nickel
Vanadium		

Sampled Pollutants of Concern

Volatile Organic Compounds		
Benzene	Toluene	m-Xylene
o-Xylene	p-Xylene	Propylbenzene
Ethylbenzene	Heptane	
Polycyclic Aromatic Hydrocarbons		
Acenaphthene	Acenaphthylene	Anthracene
Benzo(a)anthracene	Benzo(a)pyrene	Benzo(b)fluoranthene
Benzo(e)pyrene	Benzo(g,h,i)perylene	Benzo(k)fluoranthene
Biphenyl	Chrysene	Carbazole
Dibenzo(ah)anthracene	Dibenzofuran	2,6-dimethylnapthalene
Fluoranthene	Fluorene	Ideno(1,2,3-cd)pyrene
1-methylnapthalene	2-methylnapthalene	Naphthalene
Phenanthrene	Pyrene	

Sampled Pollutants of Concern, Continued

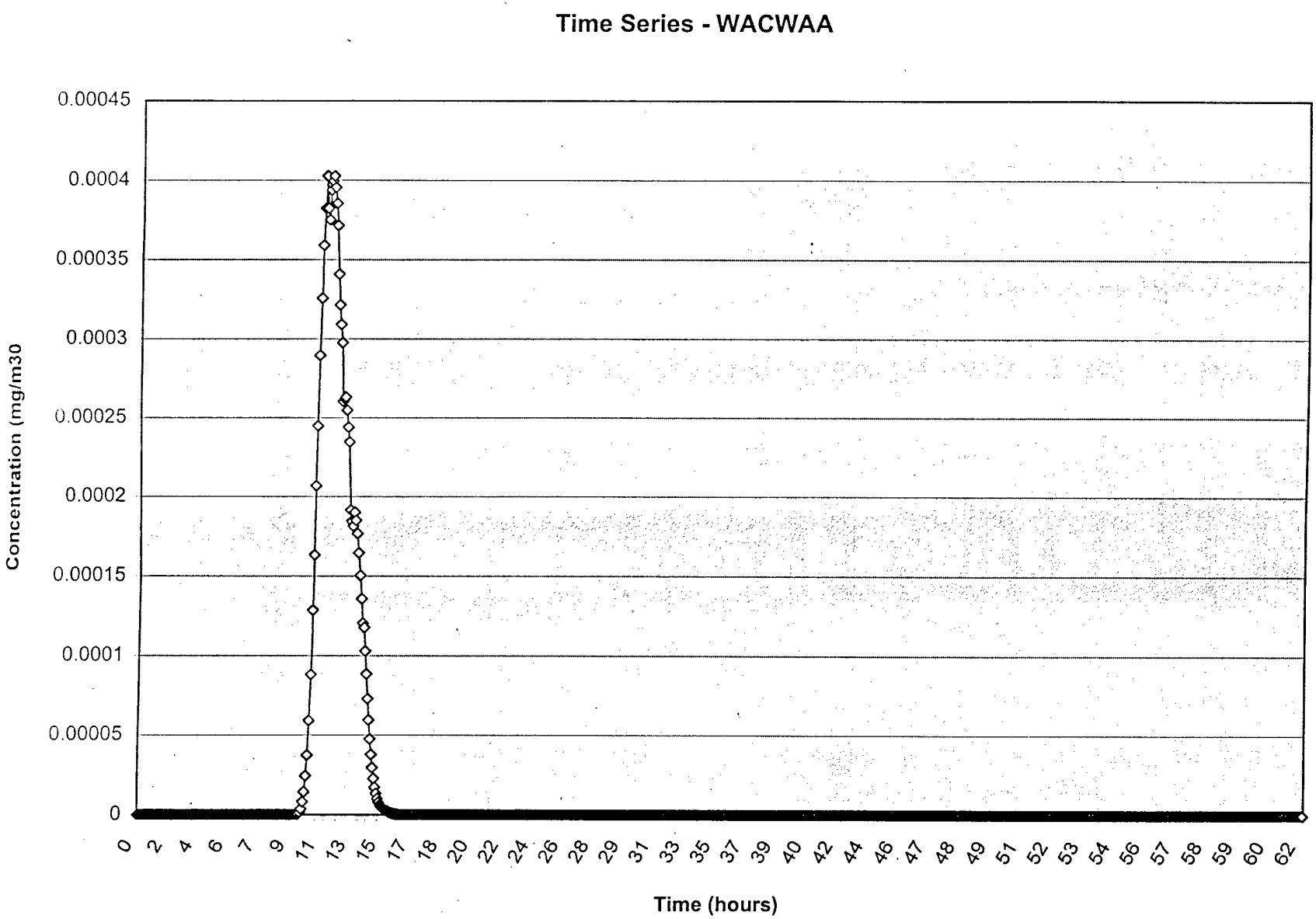
Acid Gases		
Acetic	Formic	Hydrochloric
Nitric	Sulfuric	
Criteria Pollutant Gases		
Nitrogen Dioxide/Nitrogen Oxide	Ozone	Sulfur Dioxide
Particulates, Metals, Inorganics		
Particulate Matter <10um	Total Suspended Particulate	Aluminum
Arsenic	Beryllium	Calcium
Cadmium	Chromium(3)	Chromium(6)
Iron	Mercury	Magnesium
Sodium	Nickel	Lead
Vanadium	Zinc	Sulfates
Nitrates	Chlorides	

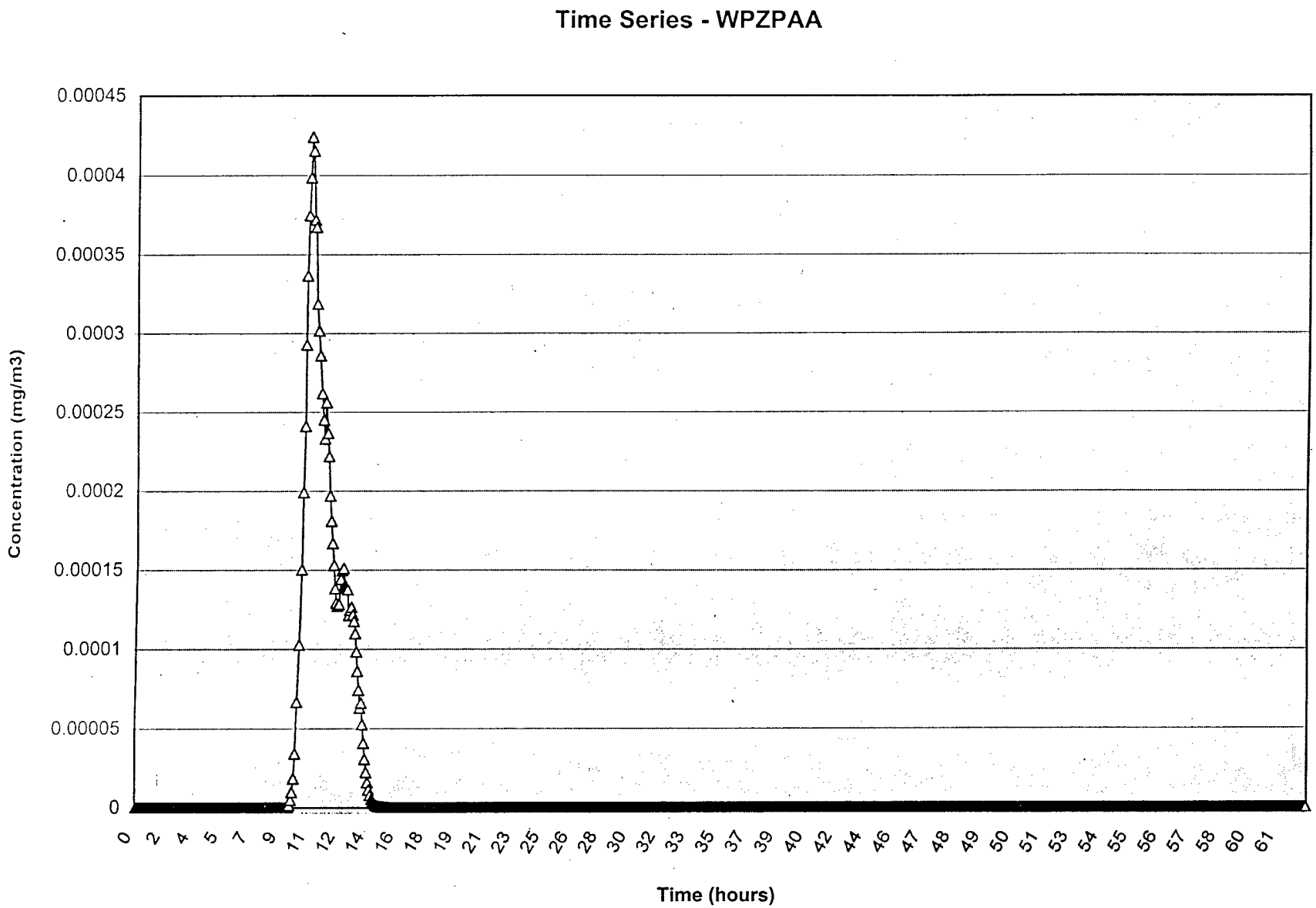
SSN	Date in	Date out	Kham Notify	Kham Epi.	UIC w/ OWF Exp	Mod Days	Max Mod Risk	Min Mod Risk	Max Mod Idx
034-44-3186	91010	91141	Y	Y*	WPZPAA	5	1.94E-13	1.94E-13	0.0001078
133-54-0227	91027	91093	N	N	WA1NT0	3	1.37E-12	1.37E-12	0.00075977
225-21-3110	91053	91090	N	N	WACPT0	1	4.09E-13	4.09E-13	0.000227
233-78-3440			N	N		0	0	0	0
268-78-4212	90346	91144	Y	N	WS2UAA	11	3.49E-12	3.49E-12	0.001941555
278-80-8560	90275	91076	N	N	ADL5	6	5.05E-13	5.05E-13	0.000280683
281-40-3682	90305	91113	N	N	WQ0PAA	23	1.11E-11	1.10E-11	0.006149043
287-62-5501	91001	91151	Y	N	WH54D0	38	1.64E-11	1.64E-11	0.009095672
287-76-4938	90213	91059	N	N	21820	17	4.57E-11	1.40E-11	0.02539142
328-56-8982	91001	91181	N	N	N21247	23	1.74E-11	1.25E-11	0.009647932
401-92-9637	91183	91304	N	N	W7UT15	0	0	0	0
407-84-5421	91001	91140	Y	N	WAPRB0	38	2.60E-11	2.60E-11	0.0144277
424-84-2682	90213	91093	N	N	WABLT0	15	8.30E-11	6.97E-11	0.046131871
449-02-2377			N	N		0	0	0	0
453-90-1358	91060	91120	Y	Y*	WACWAA	27	3.32E-11	2.53E-11	0.01844466
519-94-8549	90213	91059	N	N	21670	15	2.82E-11	1.19E-11	0.01568052
529-19-7478	90274	91166	N	N	11180	50	3.60E-11	3.03E-11	0.019964615
559-23-8761	91008	91134	N	N	WH6JA0	57	4.29E-11	4.20E-11	0.023848471

* - See concentration time series

SSN	Min Mod Idx	Avg Max Mod Idx	Avg Min Mod Idx	Start Date	End Date	Samp Days	Max Samp Risk	Min Samp Risk
034-44-3186	0.0001078	0.00002156	0.00002156			1	1.22E-10	1.22E-10
133-54-0227	0.00075977	2.53E-04	2.53E-04			0	0	0
225-21-3110	0.000227	0.000227	0.000227			0	0	0
233-78-3440	0	0	0			0	0	0
268-78-4212	0.001941555	0.000176505	0.000176505			0	0	0
278-80-8560	0.000280683	4.67805E-05	4.67805E-05			0	0	0
281-40-3682	0.006119325	2.67E-04	2.66E-04			0	0	0
287-62-5501	0.009095672	2.39E-04	2.39E-04			27	1.06E-08	1.06E-08
287-76-4938	0.007761161	1.49E-03	4.57E-04			0	0	0
328-56-8982	0.006955441	4.19E-04	3.02E-04			0	0	0
401-92-9637	0	0	0			0	0	0
407-84-5421	0.0144277	3.80E-04	3.80E-04			5	2.60E-10	2.60E-10
424-84-2682	0.038776971	0.003075458	2.59E-03			0	0	0
449-02-2377	0	0	0			0	0	0
453-90-1358	0.01402783	6.83E-04	5.20E-04			0	0	0
519-94-8549	0.00658741	0.001045368	4.39E-04			0	0	0
529-19-7478	0.016854255	0.000399292	0.000337085			0	0	0
559-23-8761	0.023345371	4.18E-04	4.10E-04			10	5.40705E-08	5.40705E-08

SSN	Max Samp Idx	Min Samp Idx	Avg Max Samp Idx	Avg Min Samp Idx
034-44-3186	0.112475784	0.112475784	0.112475784	0.112475784
133-54-0227	0	0	0	0
225-21-3110	0	0	0	0
233-78-3440	0	0	0	0
268-78-4212	0	0	0	0
278-80-8560	0	0	0	0
281-40-3682	0	0	0	0
287-62-5501	0.420312306	0.420312306	1.56E-02	1.56E-02
287-76-4938	0	0	0	0
328-56-8982	0	0	0	0
401-92-9637	0	0	0	0
407-84-5421	0.074926295	0.074926295	0.014985259	0.014985259
424-84-2682	0	0	0	0
449-02-2377	0	0	0	0
453-90-1358	0	0	0	0
519-94-8549	0	0	0	0
529-19-7478	0	0	0	0
559-23-8761	0.87770005	0.87770005	0.087770005	0.087770005


Time Series - WACWAA
Concentration (mg/m30
0.00045
0.0004
0.00035
0.0003
0.00025
0.0002
0.00015
0.0001
0.00005
0
0 2 4 6 7 9 11 13 15 17 18 20 22 24 26 28 29 31 33 35 37 39 40 42 44 46 47 48 49 51 52 53 54 56 57 58 59 60 62
Time (hours)


Time Series - WPZPAA
Concentration (mg/m3)
0.00045
0.0004
0.00035
0.0003
0.00025
0.0002
0.00015
0.0001
0.00005
0
0 2 4 5 7 9 11 12 14 16 18 19 21 23 25 26 28 30 32 33 35 37 39 40 42 44 46 47 48 49 50 51 53 54 55 56 57 58 60 61
Time (hours)