

**Written Testimony Presented to the
HOUSE SUBCOMMITTEE ON NATIONAL SECURITY, EMERGING THREATS AND
INTERNATIONAL RELATIONS SUBCOMMITTEE**

**Christopher Shays, Connecticut
Chairman**

**By the
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**At the hearing on
Examining the Status of Gulf War Research and Investigations on Gulf War Illnesses**

June 1, 2004

INTRODUCTION

Thank you, Mr. Chairman for this opportunity to speak to the subcommittee.

Since the conclusion of the Persian Gulf War in 1991, there have been complaints among some veterans of diverse symptoms that include mood changes, concentration problems, muscle and joint pains, skin rashes, chronic fatigue, sleep disturbances, chronic digestive problems and loss of sexual drive. Available medical records do not indicate the soldiers in the Persian Gulf War reported clinical symptoms consistent with exposure to nerve gases. However, when a munitions dump in Kamisiyah, Iraq was blown up during the war, fragments of the destroyed missiles were found to have polyethylene liners, suggesting that the missiles may have contained chemical warfare agents. This information stimulated the theory that some veterans of the Persian Gulf War were unknowingly exposed to sub-clinical levels of nerve gases.

Potential long term effects of a single or even several exposures to sub-clinical levels of nerve gas have not been well studied. The Lovelace Respiratory Research Institute received funding, through a competitive process sponsored by the Department of Defense, to study the effects of single and repeated exposures to sarin at a level that did not produce acute symptoms of nerve gas poisoning.

The soldiers in the Persian Gulf War, as in any war, were under stress. The release of cytokines, such as IL-1 and IL—6, into the brain during the stress response are reported to cause mood alterations, suppressed appetite and libido, sleep stimulation and a febrile response. These

symptoms are not unlike some of the symptoms reported by veterans. Therefore it seems reasonable to think that the stress of war may have exacerbated whatever effects were elicited by chemical agents.

THE EXPERIMENT

The Lovelace studies were designed to use inhalation exposures of rats under normal and heat-stressed conditions to determine the interactive effect of heat stress and sub-clinical levels of sarin 1) on the levels of cytokines and apoptotic cells in the brains of rats, 2) on the immune system of the rats and 3) on cholinergic muscarinic receptors in the brains of the heat-stressed and non-stressed rats.

Rats were exposed to one tenth and one-twentieth the acutely toxic level of sarin for one hour a day for one, five or 10 days and observed for alterations at one day and 30 days after the exposures. Half of the rats were kept at normal room temperature and half were kept at 90 degrees F. None of the rats showed symptoms of acute nerve gas poisoning.

There were many negative findings, which indicated that the exposures were indeed below the level that causes acute symptomology. The sarin did not affect body weight, respiratory parameters, activity measurements, or control of body temperature. There were no brain lesions as observed by standard histopathology and there was no increase in apoptotic cells in the brain.

In the repeatedly exposed rats, there was an induction of brain cytokines known to be associated with mood alterations, appetite suppression, libido suppression and sleep stimulation.

Two other major findings were observed.

FINDING ONE

The repeatedly exposed rats, even without heat stress, showed a reduced ability to mount an effective immune response. White blood cells in the rats did not respond well to antigens. Tests were made to determine if this observation was caused by increased corticosteroids in the blood due to the stress of the exposures. The opposite was found. The rats had unusually low levels of blood corticosteroids. The reduced immune response could be prevented, however, by treating the rats with a ganglionic blocker, indicating that the effects of the sarin were on the autonomic nervous system.

FINDING TWO

The brains of the rats repeatedly exposed to low levels of sarin under heat stress conditions showed alterations in the densities of muscarinic acetyl choline receptor sites in areas of the brain responsible for memory and cognitive function. Of great interest was the fact that in some cases these changes persisted for 30 days and in some cases the alterations were delayed and did not appear until 30 days after the exposures.

RESEARCH GAPS OF HIGH PRIORITY

These initial studies raise many questions. What are the behavioral problems associated with alterations in the density of receptor sites in the brain? What is the temporal pattern of the response? How long will the effects last? When did delayed effects first occur and how long will they last? What interventions could be used to prevent the delayed effects?

What is the mechanism by which sarin causes immunosuppression? Does sarin increase the susceptibility for microbial infections? How can the immune system be restored to normal function?

Is it possible that low blood corticosteroids could be used as a marker for subclinical exposure to a nerve gas?

At the present time, the DoD has funded us to do additional research on the effects on the immune system. We are still seeking additional funding to continue studies on the effects on brain receptor sites and subsequent behavioral changes.

Thank you for the opportunity to tell you about our studies on the effects of sub-clinical exposures to sarin. The information should be useful in the future for development of prevention and therapeutic measures for both our military exposed during hostile actions and for civilians exposed in terrorist attacks.

REPORTABLE OUTCOMES FROM THESE STUDIES

Abstracts:

1. R. F. Henderson, E. B. Barr, C. R. Clark, M. L. Sopori, C. A. Conn, Y. Tesfaigzi, T. H. March and D. B. Mash, "Effects of Inhalation Exposure to Low Levels of Sarin in Fischer 344 Rats," Society of Toxicology meeting in San Francisco on March 27, 2001.
2. R. F. Henderson, E. B. Barr, C. R. Clark, M. Sopori, C. A. Conn, Y. Tesfaigzi, T. March and D. B. Mash, "Effects of Inhalation Exposure to Low Levels of Sarin in Fischer 344 Rats," Conference on Illnesses Among Gulf War Veterans: A Decade of Scientific Research, Alexandria VA, January 24-26, 2001.
3. Sopori MB, Henderson RF. Neuroimmune Effects of Subclinical Doses of Sarin: Sarin Suppresses T Cell Responsiveness through the CNS. Presented at the Conference on Illness among Gulf War Veterans: A Decade of Scientific Research, Alexandria, VA, January, 2001.
4. Conn CA, Dokladny K, Menache MG, Barr EB, Kozak W, Kozak A, Wachulec M, Rudolph K, Kluger MJ and Henderson RF. Effects of Acute Inhalation Exposure to Low Levels of Sarin on Temperature and Activity of Rats. Presented at the Federation of American Societies of Experimental Biology, Orlando, FL, May, 2001.
5. Henderson, RF, CA Conn, EB Barr. , TH March , JR Krone , ML Sopori, Y Tesfaigzi. M Wachulec and DB Mash. Effect of low level sarin exposure on physiological parameters in rats. Presented at the 39th annual meeting of the Society of Toxicology, March 19-23, 2000 in Philadelphia, PA.
6. Henderson, RF, EB Barr. ML Sopori, S Singh, CA Conn, Y Tesfaigzi, TH March and JR Krone. Effects of inhalation exposure to low levels of sarin in Fisher 344 rats. Presented at the 96th International Conference of the American Thoracic Society, Toronto, Canada, May 5-10, 2000.
7. Henderson, RF, EB Barr. M Sopori, S Singh, R Kalra, C Conn, Y Tesfaigzi, T March and DB Mash. Effect of inhalation exposure to low levels of sarin in Fischer 344 rats. Presented at the USAMRICD Bioscience 2000 meeting, June 4-9, 2000, Baltimore, MD.
8. Hobbs, CH, Henderson RF, Kluger, MJ and Barr EB. Effect of Exposure to Low Levels of Sarin During Heat Stress on Brain Cytokines. Abstract presented at the Conference on Federally Sponsored Gulf War Veterans' Illnesses Research. Pentagon City, June, 1999.

Publications:

Henderson RF, Barr EB, Sopori MB, Singh S, Kalra R, Conn CA, Tesfaigzi Y, March T and Mash DB. Effects of inhalation exposure to low levels of sarin in Fischer 344 rats. Published as part of the proceedings of the Bioscience 2000 meeting, Baltimore, MD, June, 2000.

Henderson, RF, Barr, EB, Blackwell, W, Clark, CR, Conn, CA, Kalra, R, March. T, Sopori, M, Tesfaigzi, Y, Menache, M and Mash DB. Response of rats to low levels of sarin. *Toxicol. Appl. Pharmacol.* 184: 67-76, 2002..

Kalra, R., Singh, S, Boroujerdi, SR, Langley, R, Blackwell, W., Henderson, RF and Sopori, ML. Subclinical doses of the nerve gas sarin impair T cell responses through the autonomic nervous system. *Toxicol Appl. Pharmacol.* 184: 82-87, 2002.

Conn, CA, Dokladny K, Menache MG, Barr EB, Kozak W, Kozak A, Wachulec M, Rudolph K, Kluger MJ and Henderson RF. Effects of sarin on temperature and activity of rats as a model for Gulf War syndrome neuroregulatory functions *Toxicol. appl. Pharmacol.* 184: 77-81, 2002.