

**GAO**

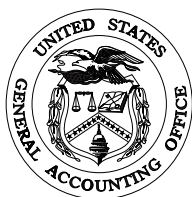
Report to the Honorable  
Jack Metcalf  
House of Representatives

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March 1999

# GULF WAR ILLNESSES

## Questions About the Presence of Squalene Antibodies in Veterans Can Be Resolved



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**National Security and  
International Affairs Division**

B-278779

March 29, 1999

The Honorable Jack Metcalf  
House of Representatives

Dear Mr. Metcalf:

You expressed concern about reports that the blood samples of some ill Gulf War-era veterans contained antibodies for squalene<sup>1</sup>—a component of adjuvant formulations used in some experimental vaccines but not in any licensed vaccines.<sup>2</sup> As requested, we identified whether (1) the Department of Defense (DOD) or the National Institutes of Health (NIH) performed or sponsored research using squalene, (2) DOD considered using adjuvant formulations in vaccines administered to Gulf War-era veterans, and (3) any research has detected the presence of squalene in ill Gulf War-era veterans.

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**Results in Brief**

Prior to and following the Gulf War, DOD and NIH used adjuvant formulations of squalene to perform research on the development of more effective vaccines. DOD officials stated they considered, but decided against, using vaccines with experimental adjuvant formulations during the Gulf War. According to independent researchers, as part of their treatment of sick Gulf War-era veterans, they developed and administered a test, referred to as an assay, that detected antibodies to squalene in the blood of sick Gulf War-era veterans. The researchers stated this assay is similar to a standard assay used in other types of research. As of March 1999, the research had been subjected to peer review, but had not been published. This process is often lengthy, sometimes taking a year or more. According to DOD officials, DOD could develop such an assay inexpensively and test it on a sample of sick Gulf War-era veterans. However, DOD plans to wait until the research is published before deciding whether to conduct testing. Given the researchers' assessment, DOD's comments about the feasibility of developing an assay and that veterans have been waiting for the past

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<sup>1</sup>Squalene is found in shark liver oil, some vegetable oils, and the human liver and can also be manufactured through chemical engineering. Squalane is the hydrogenated form of squalene. When we use the term squalene by itself, it refers to both squalane and squalene.

<sup>2</sup>An adjuvant is a substance incorporated in a vaccine to accelerate, enhance, or prolong a specific immune response. An antigen is a substance that stimulates production of an antibody. Neither squalane or squalene is a complete adjuvant by itself. Both serve as vehicles in which adjuvant formulations and vaccine antigens can be mixed and delivered.

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7 years for answers on the nature and origin of their illnesses, DOD has the opportunity now to expand on the research already performed.

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## Background

Many of the approximately 700,000 veterans of the Gulf War have reported health problems. Some fear that their illnesses might be due to exposure to chemicals, pesticides, and other agents used during the war, including vaccines administered to protect them against biological warfare agents. Questions about vaccine adjuvant formulations were raised to DOD in June 1994. At that time, an immunologist from the private sector notified the Defense Science Board that some symptoms being reported by Gulf War-era veterans were very similar to those of her patients with autoimmune diseases. These patients had a range of symptoms affecting more than one of the body systems and the immunologist believed they were associated with exposure to vaccine adjuvant formulations. In October 1995, DOD, before a meeting of the Presidential Advisory Commission on Gulf War illnesses, dismissed this hypothesis on the grounds that it had administered only vaccines with aluminum salts as adjuvants. In November 1996 and again in 1997, the immunologist notified DOD, based on independent research, that she had found antibodies to squalene in the blood of a few sick veterans who had served in the military during the Gulf War. However, DOD has not responded to these findings. According to the researcher, she continues to be willing to discuss the research with DOD.

To date, aluminum hydroxide is the only adjuvant used in vaccines licensed by the Food and Drug Administration (FDA) in the United States. While widely considered to be safe, this adjuvant provides only a limited boost in the immune response, and researchers have long emphasized the critical need for new, more effective adjuvant formulations. According to the National Institute of Allergy and Infectious Diseases (NIAID), the branch of NIH that sponsors most of its vaccine-related research, a new generation of novel adjuvant formulations are being developed. These formulations are intended to enhance and optimize immune responses to vaccines; enable easier delivery of antigens, and reduce the amount of antigen and the number of immunizations required for protective immunization. Squalene is a common component of these new formulations. As with all drugs and biological products, the absolute safety of adjuvant formulations can never

be guaranteed.<sup>3</sup> Safety concerns have been cited<sup>4</sup> regarding the use of novel adjuvant formulations in vaccines, including squalene, and the associated adverse reactions.<sup>5</sup> It has also been suggested that the safety of vaccines containing these formulations must be evaluated in conservative ways.<sup>6</sup>

## DOD and NIH Performed and Sponsored Research With Squalene

DOD and NIAID officials reported that, to help develop more effective vaccines, they conducted research using adjuvant formulations with squalene. In all, they performed or sponsored 28 clinical trials on vaccines using adjuvant formulations with squalene, and 1,749 human subjects participated in these trials. Prior to the Gulf War, both organizations were devising ways to induce a rapid response to several vaccines using adjuvant formulations with squalene. DOD officials stated that they considered, but decided against using vaccines with adjuvant formulations—including those with squalene—to protect Gulf War troops.

## DOD Research

Between 1988 and 1998, DOD sponsored 101 clinical trials on vaccines as part of a process required by FDA for licensing investigational new drugs (IND). At least 21 of these trials involved vaccines with adjuvant formulations, and 5 of these 21 involved adjuvant formulations containing squalene. These formulations were available from U.S. firms.<sup>7</sup> (See app. I for specific information on these firms and the development of adjuvant formulations with squalene.) In the five trials involving squalene, 572 human subjects volunteered and participated. Of the five trials, two began before the Gulf War. DOD officials could not confirm whether any of the

<sup>3</sup>J. L. Bussiere et al., "Preclinical Safety Assessment Considerations in Vaccine Development" In Powell, M.F. and Newman, M.J. (Eds.) (1995). *Vaccine Design: The Subunit and Adjuvant Approach* (New York: Plenum Press), pp. 61-75.

<sup>4</sup>Goldenthal, K. L. et al., "Safety Evaluation of Vaccine Adjuvants: National Cooperative Vaccine Development Meeting Working Group," *AIDS Research and Human Retroviruses*, vol. 9 (1993), pp. S47-S51. Lorentzen, J.C. "Identification of Arthritogenic Adjuvants of Self and Foreign Origin." *Scandinavian Journal of Immunology*, vol. 49 (1999), pp. 45-50.

<sup>5</sup>Adverse reactions are local or systemic. Local reactions include pain and swelling at the injection site. Systemic reactions include fevers and toxicity of organs and systems.

<sup>6</sup>M.F. Powell and M.J. Newman, *Vaccine Design: The Subunit and Adjuvant Approach* (New York: Plenum Press, 1995)

<sup>7</sup>This information was derived from DOD data submitted to FDA and may not include cooperative research efforts with others.

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volunteers in studies that DOD sponsored had deployed to the Gulf War. The five trials are described as follows:

- In April 1988, DOD's first clinical trial of an experimental malaria vaccine with an adjuvant containing squalene was approved,<sup>8</sup> but according to DOD, doses were actually administered from June 1989 to January 1990. Five volunteers were given the vaccine.
- In August 1990, another trial of the malaria vaccine was approved, using the same adjuvant with squalene on 12 volunteers.<sup>9</sup>
- In 1994, DOD began another study on a malaria vaccine containing an adjuvant with squalene.<sup>10</sup> Both 110 experimental subjects and 11 control subjects were given the adjuvant. An additional arm of the study, using human subjects from Gambia, was withdrawn before any vaccines were given because of concerns about the stability of the product.
- In 1995, through a cooperative research and development agreement, the Chiron Biocine Company and the Walter Reed Army Institute of Research began a clinical trial of a vaccine for Human Immunodeficiency Virus (HIV) that contained an adjuvant with squalene.<sup>11</sup> The vaccine containing squalene was given to 41 healthy volunteers in Thailand, and the adjuvant with squalene without the rest of the vaccine was given as a placebo to 13 people in a control group.
- In 1997, the Walter Reed Army Institute of Research began to cosponsor another study in Thailand on an HIV vaccine with an adjuvant formulation containing squalene, which is ongoing.<sup>12</sup> This study will give both the experimental and control subjects the adjuvant formulation with squalene. Three hundred and eighty subjects have been recruited for this study; 3 are Americans and the remaining are Thai citizens.

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<sup>8</sup>IND 2699. "Safety and Immunogenicity of a Plasmodium falciparum Malaria Sporozoite Vaccine, R32NS1<sub>81</sub> With DETOX™ As An Adjuvant."

<sup>9</sup>IND 3714. "The Protective Efficacy of a Plasmodium falciparum Vaccine, R32NS1<sub>81</sub> and MPL/CSW as an Adjuvant."

<sup>10</sup>IND 6043. "Plasmodium falciparum Circumsporozoite Antigen Vaccine (Recombinant, Yeast) with Alum, QS21, MPL and SB62 Adjuvant Combinations."

<sup>11</sup>IND 4096. "A Phase I Trial of Biocine HIV SF2 gp 120/MF59 Vaccine in Seronegative Thai Volunteers."

<sup>12</sup>IND 7172. "A Phase I/II Double-blind, Placebo-controlled study of the Chiron HIV Thai E gp 120/MF59 Vaccine Administered alone or Combined with the Chiron HIV SF2 gp120 Antigen in Healthy HIV-Seronegative Thai Adults."

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Appendix II provides further details on these studies, and appendix III provides a list of DOD research publications on those trials involving human subjects.

In addition, DOD has conducted several experiments on animals, using vaccines with adjuvant formulations containing squalene, for a wide range of diseases, including anthrax, toxic shock, and malaria. The anthrax vaccine experiments with adjuvant formulations containing squalene began in 1987, and some of the results have been presented at conferences and published in several medical journals. (See app. IV for a list of some of DOD's animal research on adjuvant formulations with squalene). DOD's animal studies are of interest for two reasons. First, because tests on animals are generally performed before human trials, they represent the first step of vaccine research and provide a more complete picture about the state of research on adjuvant formulations with squalene before the Gulf War. Second, since vaccines against biological warfare cannot be tested for efficacy in humans, animal research is considered essential by researchers.

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### NIH's Research on Vaccines With Adjuvant Formulations Containing Squalene

NIAID officials stated they have sponsored vaccine trials on various adjuvant formulations, including several with squalene. NIAID's research on vaccines and adjuvant formulations has increased substantially over the last 10 years. The total number of active vaccine projects more than doubled, from 212 in 1987 to 539 in 1997. Research involving adjuvant formulations expanded at an even faster pace, from 13 studies in 1987 to 59 active projects in 1997. NIAID's clinical research on novel adjuvant formulations involving human subjects began in 1988.

NIAID-sponsored basic/preclinical studies on adjuvant formulations with squalene began in 1987, and clinical trials began at the same time as Operation Desert Storm, in January 1991. Since then, NIAID has sponsored at least 23 trials of vaccines involving adjuvant formulations with squalene, with 1,177 human volunteers.<sup>13</sup> Nineteen of the 23 trials involved an HIV vaccine tested on a total of 935 volunteers; the 4 remaining trials involved a vaccine for herpes with 242 subjects. (See app. V for a list of the 23 studies.

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<sup>13</sup>Establishing the exact number of studies is difficult because NIAID's databases often do not specify the adjuvants used in both preclinical and clinical studies. Also, 2 years after the studies are completed, the records are routinely destroyed and only an index is maintained.

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## DOD Officials Report They Considered, but Decided Against, Using Vaccines With Novel Adjuvant Formulations, Including Squalene

In August 1990, DOD established various committees to address its concerns about the threat of Iraqi biological warfare agents and the insufficient supply of vaccines to immunize all troops against these agents. These committees identified several problems. They determined that DOD had neither a sufficient quantity of vaccine nor the manufacturing capacity to protect the force. It also did not have sufficient time to administer the required six anthrax shots over 18 months and faced formidable logistical problems in giving multiple shots to troops in various locations in the Persian Gulf region.

According to DOD officials, the use of novel adjuvant formulations for the anthrax vaccine was rejected because any alteration in the licensed vaccine would require relicensure, and DOD would not receive FDA approval in time. Other alternatives were pursued. DOD requested help from commercial U.S. and foreign vaccine manufacturers; NIH, through its National Cancer Institute facility at Fort Detrick, Maryland; and additional military production facilities at Fort Detrick and Porton Down, United Kingdom. According to the commercial manufacturers, they turned DOD down because developing a safe and effective vaccine takes sustained investment and planning and DOD had not previously been willing to invest the money and time. DOD began immunizing troops in January 1991. However, it should be noted that even if the manufacturing capacity had been increased, DOD never had the 18-month time span needed to fully immunize the troops in the Gulf War because of the war's short duration.

Although DOD awarded contracts to the National Cancer Institute to produce additional anthrax vaccine and began planning production of additional botulinum toxoid vaccine at the U. S. Army Medical Research Institute of Infectious Diseases, also located at Fort Detrick, the two institutes were unable to begin production before the war. DOD officials said that botulinum toxoid vaccine was acquired from Porton Down, United Kingdom, but was not used. Consequently, according to DOD, the only vaccines against biological warfare agents—anthrax and botulinum toxoid—given during the Gulf War were produced by the Michigan Department of Public Health. It subsequently became an independent agency, the Michigan Biologic Products Institute, and was recently privatized as BioPort. Officials at BioPort said that they have never used adjuvant formulations containing squalene.

We cannot say definitively whether or not Gulf War-era veterans were given vaccines with adjuvant formulations containing squalene for a number of reasons. Although DOD officials told us they did not administer such



vaccines, they stated they did not have documentation on the process and results of decision-making related to the administration of vaccines at the time of the Gulf War. Also, some officials involved in the decisions were no longer employed with DOD at the time of our review, and we were either unable to locate them or they declined to be interviewed.

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## Independent Researchers State They Have Detected Squalene Antibodies in Gulf War-Era Veterans

In examining the pathology of illnesses afflicting Gulf War-era veterans, independent researchers examined whether antibodies to squalene were present in patients who had and had not been deployed to the Gulf War. Using an assay that they developed the researchers stated that they detected squalene antibodies in the blood of sick Gulf War-era veterans. The immunologist who headed this study and laboratory researchers at a major university medical center that were involved in the study shared their methodology and findings with us. The results of the research have been submitted to a medical journal to be peer reviewed and published. As of February 1999, there was no set date for publication. According to the researchers, the antisqualene antibody assay that they developed in their study is a variant of the common Western Blot assay<sup>14</sup> and is similar in format to a test cited in a published report on silicone antibodies.<sup>15</sup>

Using the antisqualene antibody assay, the independent researchers stated they found most veterans with Gulf War illnesses in their research had the antibodies to squalene, regardless of whether they were deployed or not. Non veterans in the research that were known to have received adjuvant formulations with squalene as volunteers in clinical trials of experimental vaccines also had the antibodies to squalene and had an array of symptoms similar to symptoms of the Gulf War patients. On the other hand, those participants (in the control groups) that were healthy with no autoimmune symptoms, those non-Gulf War veterans with autoimmune diseases of unknown origin, and those who had received other adjuvant formulations were found not to have antibodies to squalene. The independent researchers concluded that, while the reason for the presence of the

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<sup>14</sup>The Western Blot assay applies a protein or polymer such as squalene to test strips, which are then incubated with patient serum. If the antibody of interest is present, test strips turn bluish black. A darker color indicates a higher concentration of antibodies.

<sup>15</sup>S. A. Tenenbaum et al., "Use of anti-polymer antibody assay in recipients of silicone breast implants," *The Lancet*, vol. 349 (1997), pp. 449-454. For correspondence concerning this study see "Antipolymer antibodies, silicone breast implants, and fibromyalgia," *The Lancet*, vol. 349 (1997), pp. 1170-1173.

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squalene antibodies remains unclear, the presence of these antibodies could potentially be a contributing factor to Gulf War illnesses.

DOD officials stated they could develop an assay, or test, for detecting antibodies to squalene. According to these officials, it would not be expensive to develop the assay and test it on a sample of Gulf War-era veterans that are sick. However, they believed that since DOD did not use adjuvants with squalene, DOD does not need to develop such an assay or to screen the veterans for the antibodies. Second, squalene is a substance that occurs naturally in the human body, and they doubted that an assay could be developed to differentiate antibodies to natural and manufactured squalene. Third, they noted that squalene is also found in numerous topical creams that some soldiers could have used. Finally, DOD officials do not believe that funding squalene antibodies in veterans would prove that the antibodies caused Gulf War illnesses. Consequently, DOD intends to wait until the independent researchers publish their research in a peer-reviewed journal before deciding whether to conduct testing.

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## Conclusions and Recommendation

Time is critical for many Gulf War-era veterans who continue to suffer from illnesses and have been waiting for the past 7 years for an explanation about the nature of their illnesses. It is therefore important that DOD takes advantage of any opportunity to obtain and evaluate additional information on the veterans' symptoms and potential contributing factors. Independent researchers, using an assay that they state is similar to standard research assays, have concluded that squalene antibodies are present in sick Gulf War-era veterans that participated in their research and are a potential contributing factor to these veterans' illnesses. DOD officials stated that it is feasible to develop and apply an assay to test for squalene antibodies. Yet for various reasons, including its assertion that it did not use adjuvant formulations with squalene, DOD plans to wait until the researchers' research is published before considering whether to conduct its own testing. However, publication is usually a lengthy process and may take more than a year. Given that Gulf War-era veterans have already waited a significant amount of time for information on their illnesses, we believe that DOD should act now to expand on the research already conducted. Although the origin of the antibodies may be important to assess, the first step is to determine the extent to which they are present in a larger group of sick Gulf War-era veterans. We therefore recommend that the Secretary of Defense review the independent research that researchers report has revealed the presence of squalene antibodies in the blood of ill Gulf War-era

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veterans and conduct its own research designed to replicate or dispute these results.

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## Agency Comments

In written comments on a draft of our report, DOD disagreed with our recommendation to test for antibodies for squalene in the blood of ill Gulf War-era veterans. DOD stated there is no basis for believing veterans were exposed to vaccines containing squalene. DOD further believes that the proposed testing for the presence of squalene antibodies will not appropriately address or assist in resolving the issue of whether such antibodies may be a contributing cause to the illnesses of Gulf War-era veterans.

Specifically, DOD stated no experimental vaccines with squalene had been used in U.S. troops during the Gulf War and that the manufacturer of vaccines verified it had never used adjuvant formulations containing squalene. DOD noted that we concluded there was no evidence that Gulf War-era veterans were given adjuvant formulations containing squalene, and it therefore believes our proposal to test veterans seems scientifically and fiscally irresponsible. DOD suggested that our report be titled “Gulf War Illnesses: Gulf War Veterans Did Not Receive Vaccine Adjuvant Formulations Containing Squalene.”

DOD further stated the assay developed by independent researchers has not been validated through peer review or publication in scientific literature and that it is correctly adhering to widely accepted standards by awaiting such validation before considering the use of the assay in Gulf War illness studies. It also believed our recommendation to test for squalene antibodies showed a lack of understanding of scientific methods. In particular, DOD stated the presence of antibodies would not establish an association with adverse health outcomes and establishing an association would require a statistically meaningful study of randomly selected Gulf War veterans and non deployed veterans. DOD noted that any experimental design to test for this association must be evaluated for scientific merit through independent peer review.

DOD misstated our finding on whether Gulf War-era veterans may have received vaccine adjuvant formulations containing squalene. We did not conclude that Gulf War era veterans were not given adjuvant formulations containing squalene. Rather, we cannot say definitively whether or not Gulf War-era veterans were given these formulations. We have modified the report text to make this point clear. Furthermore, it was not our

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intention to focus on how squalene antibodies may have been introduced into the blood of the veterans. Rather, the focus should be on the opportunity to resolve whether such antibodies are present in the blood of ill Gulf War-era veterans, and if so, whether or not they play a role in their illnesses. In this respect, the results of the independent research suggesting that antibodies to squalene are present in ill Gulf War-era veterans participating in their research cannot be ignored.

We continue to believe that DOD should take this opportunity to begin addressing and potentially resolving the question of whether or not squalene antibodies may be contributing to the illnesses of Gulf War-era veterans. Specifically, DOD should conduct research designed to replicate or dispute the independent research results that revealed the presence of squalene antibodies in the blood of ill Gulf War-era veterans. We modified our recommendation to clarify this position. If DOD's research affirms the presence of these antibodies, additional research should be conducted that is designed to assess the significance of that finding. This would simply be a first step in the research process and would not finally resolve the issue of whether or not squalene antibodies are a marker for, contribute to, or cause the illnesses. Follow-on research would be required to address those issues.

DOD also provided technical comments, which we incorporated as appropriate. DOD's comments are printed in their entirety in appendix VI.

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## Scope and Methodology

To develop the information in this report, we conducted multiple literature searches of public and agency databases and reviewed both published and unpublished literature on the use of adjuvant formulations in vaccine, including DOD research protocols and agency documentation. In addition, we interviewed officials at DOD, NIH, FDA, and the Veterans Administration. We interviewed vaccine experts in academia, pharmaceutical firms, and the American Medical Association and confirmed the validity of using assays as a means of determining the presence of antibodies. We also interviewed the immunologist who headed the independent research and laboratory researchers from Tulane University in New Orleans who developed the anti-squalene assay, and they shared their methodology and findings with us. Finally, we interviewed responsible officials at BioPort.

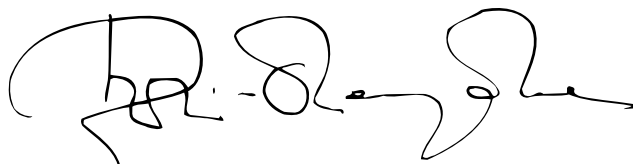
Our work was completed between August 1997 and August 1998 in accordance with generally accepted government auditing standards.

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We are sending copies of this report to other interested congressional committees. We are also sending copies to the Honorable William Cohen, Secretary of Defense; the Honorable Togo D. West, Jr., Secretary of Veterans Affairs; and the Honorable Donna E. Shalala, Secretary of Health and Human Services. Copies will also be made available to others upon request.

If you have any questions or would like additional information, please contact me at (202) 512-3092. Major contributors to this report were Sushil K. Sharma and Dan Rodriguez.

Sincerely yours,

A handwritten signature in black ink, appearing to read 'Kwai-Cheung Chan'. The signature is fluid and cursive, with a large initial 'K' and a long horizontal stroke at the end.

Kwai-Cheung Chan  
Director, Special Studies  
and Evaluations

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**Abbreviations**

DOD	Department of Defense
FDA	Food and Drug Administration
HIV	Human Immunodeficiency Virus
IND	Investigational new drug
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health

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# Development of Adjuvant Formulations With Squalene

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Biotechnology research and development of adjuvant formulations with squalene began in the 1970s and the first clinical study began in 1984. At the time of the Gulf War, at least three firms—Ribi ImmunoChem Research Inc. of Hamilton, Montana; Chiron of Alameda, California; and Syntex of Palo Alto, California—had developed adjuvant formulations with squalene and were distributing them for vaccine research and development. Research on adjuvant formulations with squalene has continued. At least seven biotechnology and pharmaceutical firms have developed nine different adjuvant formulations that may contain squalene. In five of the adjuvant formulations, squalene or squalane is always a component, and in the other four, it is used optionally (see table I.1). According to Chiron, its adjuvant formulation with squalene has been tested on over 9,000 human subjects. Ribi ImmunoChem reports that its adjuvant formulations with squalene have been tested on over 1,000 human subjects.

**Appendix I  
Development of Adjuvant Formulations With  
Squalene**

**Table I.1: Pharmaceutical Firms' Adjuvant Formulations That May Contain Squalene or Squalane**

<b>Name of adjuvant formulation</b>	<b>Name of pharmaceutical firm</b>	<b>Compound used</b>	<b>Always contains squalane or squalene</b>	<b>Squalene or squalane is used optionally</b>
Antigen Formulation	IDEC Pharmaceuticals Corporation	Squalane	Yes	No
CRL 1005 (Block Copolymer P1205)	Vaxcel Corporation	Squalane	No	Yes
Detox	Ribi ImmunoChem Research, Inc.	Squalane	Yes	No
Gerbu Adjuvant	CC Biotech Corporation	Squalane	No	Yes
GMDP	Peptech, Ltd., UK	Squalane	No	Yes
MF59	Chiron	Squalane	Yes	No
MPL®	Ribi ImmunoChem Research, Inc.	Squalane	No	Yes
Ribi adjuvant system	Ribi ImmunoChem Research, Inc.	Squalane	Yes	No
Syntex adjuvant formulation (SAF)	Syntex Research	Squalane	Yes	No

Note: Much of this information in this table is from F.R. Vogel and M.V. Powell, Chapter 7, "A compendium of Vaccine Adjuvants and Excipients," *Vaccine Design: The Subunit and Adjuvant Approach*, M.F. Powell and M.J. Newman, (New York: Plenum Press, 1995). Additional and updated information was gathered from F.R. Vogel and other sources.

# DOD's Clinical Trials on Novel Vaccines With Adjuvant Formulations Containing Squalene

The following table identifies vaccine trials with adjuvant formulations that contained squalene and squalane conducted by DOD under the Food and Drug Administration's (FDA) process for approving investigational new drugs (IND). New drugs and vaccines under development generally have to be tested in humans for safety and efficacy before they are approved for general human use. Therefore, FDA grants IND waivers allowing human subject experiments after reviewing information on the product, its manufacture and testing, and the proposed clinical study.

Date IND approved for human subject research <sup>a</sup>	IND number	Number of human subjects	Country of subjects	Vaccine	Adjuvant containing squalene or squalane
4/27/88	2699	5	United States	Malaria	Detox
8/8/90	3714	12	United States	Malaria	Detox
12/7/94	6043	121 <sup>b</sup>	United States	Malaria	MPL
2/8/95	4096	41 vaccine, 13 placebo <sup>c</sup>	Thailand	HIV	MF59
9/18/97	7172	300 vaccine, 80 placebo <sup>c</sup>	377-Thailand 3-United States	HIV	MF59
Total	5	572		Malaria HIV	Detox MPL MF59
INDs using U.S. citizens	3	138		Malaria HIV	Detox MPL MF59
INDs using foreign citizens	2	434		HIV	MF59

<sup>a</sup>Date IND approved by FDA's Human Subject Research Review Board.

<sup>b</sup>As of December, 1997.

<sup>c</sup>The control group received a placebo consisting of the adjuvant MF59 alone without the rest of the vaccine.

# DOD's Published Research on Vaccines With Adjuvant Formulations Containing Squalene That Involved Human Subject Volunteers

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Rickman, L. et al. "Use of adjuvant containing mycobacterial cell-wall skeleton monophosphoryl lipid A, and squalene in malaria circumsporozite protein vaccine." Lancet. Vol. 337, 1991, pp. 998-1001.

Hoffman, S. L. et al. "Safety, immunogenicity, and efficacy of a malaria sporozite vaccine administered with monophosphoryl lipid A, cell-wall skeleton of mycobacteria, and squalene as adjuvant." American Journal of Tropical Medical Hygiene. Vol. 51/5, 1994, pp. 603-612.

Stoute, J. A. et al. "A preliminary evaluation of recombinant circumsporozoite protein vaccine against plasmodium falciparum malaria." New England Journal of Medicine. Vol. 336, 1997, pp. 86-91.

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# DOD's Animal Research on Adjuvant Formulations With Squalene

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## Anthrax

Iacono-Connors, L. et al. "Protection against Anthrax with Recombinant Virus-Expressed Protective Antigen in Experimental Animals," Infection and Immunity. Vol. 59, 1991, pp. 1961-1965.

Ivins, B. et al. "Experimental anthrax vaccines: efficacy of adjuvants combined with protective antigen against an aerosol *Bacillus anthracis* spore challenge in guinea pigs." Vaccine. Vol. 13, 1995, pp. 1779-1784.

Ivins, B. et al. "Experimental Anthrax Vaccines: Efficacy Studies in Guinea Pigs." Abstracts of the 93rd General Meeting of the American Society for Microbiology. 1993, p. 160.

Ivins, B. et al. "Comparative efficacy of experimental anthrax vaccine candidates against inhalation anthrax in rhesus macaques." Vaccine. Vol. 16, 1998, pp. 1141-1148.

Ivins, B. et al. "Cloned Protective Activity and Progress in Development of Improved Anthrax Vaccines." Salisbury Medical Bulletin Special Supplement. 1990, pp. 86-88.

Ivins, B. et. al. "Immunization against Anthrax with *Bacillus anthracis* Protective Antigen Combined with Adjuvants." Infection and Immunity. Vol. 60, 1992, pp.662-668.

Ivins, B. et. al. "Adjuvant Efficacy in Experimental Anthrax Vaccines: Protection Studies in Guinea Pigs." Abstracts of the 91st General Meeting of the American Society for Microbiology. 1991, p. 121.

Ivins, B. et. al. "Vaccine Efficacy of *Bacillus Anthracis* Protective Antigen Produced in Prokaryotic and Eukaryotic Cells." Abstracts of the 94th General Meeting of the American Society of Microbiology. 1994, p. 150.

Little S. F. et. al. "Protection against experimental anthrax infection using fragments of Protective antigen." Proceedings of the International Workshop on Anthrax. Vol. 87, 1996, p. 129.

Little S. F. et al. "Passive Protection by Polyclonal Antibodies against *Bacillus anthracis* Infection in Guinea Pigs." Infection and Immunity. Vol. 65, 1997, pp. 5171-5175.

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**Malaria**

Malik A. et al. "Induction of cytotoxic T lymphocytes against the Plasmodium falciparum circumsporozoite protein by immunization with soluble recombinant protein without adjuvant," Infection and Immunity. Vol. 61, 1993, pp. 5062-5066.

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**Toxic Shock Syndrome**

Stiles, B. et al. "Biological Activity of Toxic Shock Syndrome Toxin 1 and a Site-Directed Mutant, H135A, in a lipopolysaccharide-Potentiated Mouse Lethality Model." Infection and Immunity. Vol. 63, 1995, pp. 1229-1234.

# National Institute of Health Studies Using Adjuvants With Squalene

Date Investigational New Drug (IND) study began	Vaccine	Institute	IND number	Adjuvant with squalene	No. of subjects
1/28/91	HIV	NIAID/AVEG <sup>a</sup>	005A	MF 59	16
3/22/91	HIV	NIAID/AVEG	005B	MF 59	46
10/1/91	Herpes	NIAID/DIR <sup>a</sup>	92-I-0267	MF 59	40
10/29/91	HIV	NIAID/AVEG	005C	MF 59	14
12/19/91	HIV	NIAID/AVEG	007A	MF 59	32
2/04/92	HIV	NIAID/AVEG	007B	MF 59	17
11/16/92	HIV	NIAID/AVEG	007C	MF 59	10
07/28/92	HIV	NIAID/AVEG	008	MF 59	14
10/1/92	Herpes	NIAID/DIR	93-I-0141	MF 59	174
12/09/92	HIV	NIAID/AVEG	201	MF 59	149
5/19/93	HIV	NIAID/AVEG	012A	MF 59	15
6/03/93	HIV	NIAID/AVEG	015	MF 59	30
6/03/93	HIV	NIAID/AVEG	015	MPL	30
6/03/93	HIV	NIAID/AVEG	015	SAF/2	30
10/1/93	Herpes	NIAID/DIR	94-I-0086	MF 59	18
10/12/93	HIV	NIAID/AVEG	012B	MF 59	59
5/11/95	HIV	NIAID/AVEG	022	MF 59	59
9/14/95	HIV	NIAID/AVEG	024	MF 59	30
10/1/95	Herpes	NIAID/DIR	96-I-0024	MF 59	10
7/10/96	HIV	NIAID/AVEG	022A	MF 59	129
2/06/96	HIV	NIAID/AVEG	026	MF 59	85
7/31/96	HIV	NIAID/AVEG	029	MF 59	28
5/22/97	HIV	NIAID/AVEG	202	MF 59	142
<b>Total INDs and subjects</b>			<b>23</b>		<b>1177</b>

<sup>a</sup>NIAID is the National Institute of Allergy and Infectious Diseases, AVEG is the AIDS Vaccine Evaluation Group, and DIR is the Division of Intramural Research.

# Comments From the Department of Defense



THE ASSISTANT SECRETARY OF DEFENSE  
WASHINGTON, DC 20301-1200

Mr. Henry L. Hinton, Jr.  
Assistant Comptroller General  
National Security and International Affairs Division  
U.S. General Accounting Office  
Washington, DC 20548

Dear Mr. Hinton:

This is the Department of Defense (DoD) response to the General Accounting Office (GAO) draft report "GULF WAR ILLNESSES: Questions About the Presence of Squalene Antibodies in Veterans Can Be Resolved," dated October 20, 1998 (GAO Code 713014, OSD Case 1711). The DoD strongly disagrees with the GAO recommendation that the Secretary of Defense should test for antibodies to squalene in the blood of ill Gulf War era veterans, and suggests that the title of the report be changed to reflect the facts of the GAO investigation. The proposed testing for the presence of squalene antibodies will neither appropriately address nor assist in resolving the issue of whether squalene antibodies may be a contributing cause to the illnesses of Gulf War era veterans. In view of the GAO's conclusion that Gulf War era veterans did not receive vaccine adjuvant formulations containing squalene, the GAO proposal to test Gulf War veterans for the presence of squalene antibodies seems scientifically and fiscally irresponsible. The DoD suggests that the report be titled "GULF WAR ILLNESSES: Gulf War Veterans Did Not Receive Vaccine Adjuvant Formulations Containing Squalene."

The DoD is committed to competition and independent peer review to secure the very best research performers, hypotheses, and experimental designs, from all possible sources, including the Federal, civilian, national and international communities. This commitment follows an appreciation at all levels within the Department of our responsibility to achieve an optimal investment of dollars, to assist our Gulf War veterans secure diagnoses and treatments for their disabilities and illnesses, and to prevent such disabilities and illnesses as a consequence of future deployments.

Detailed comments to the GAO recommendation are enclosed. In addition, a copy of the draft report with separate comments and annotations listing our recommendations for changes to improve the clarity, consistency, and accuracy of the GAO report was provided to the GAO staff. The DoD appreciates the opportunity to comment on the GAO draft report.

Sincerely,

  
Dr. Sue Bailey

Enclosure:  
As stated



GAO DRAFT REPORT DATED OCTOBER 20, 1998  
(GAO CODE 713014) OSD CASE 1711

“GULF WAR ILLNESSES: QUESTIONS ABOUT THE PRESENCE OF SQUALENE  
ANTIBODIES IN VETERANS CAN BE RESOLVED”

DEPARTMENT OF DEFENSE COMMENTS TO  
THE GAO RECOMMENDATION

GAO RECOMMENDATION: The GAO concluded that DoD, for a small investment, has a timely opportunity to address and potentially resolve the issue of whether or not squalene antibodies may be a contributing cause to the illnesses of the Gulf War era veterans. The GAO recognized the DoD views that squalene is a naturally occurring substance and is also used in commercial products, but while acknowledging that the origin of the antibodies may be important to determine, stated that the first step is to determine the extent to which squalene antibodies are present in a larger group of sick Gulf War era veterans. The GAO, therefore, recommended that the Secretary of Defense test for antibodies to squalene in the blood of Gulf War era veterans. (pp.17-18/GAO Draft Report)

DoD RESPONSE: The DoD strongly disagrees with the GAO recommendation that the Secretary of Defense should test for antibodies to squalene in the blood of sick Gulf War era veterans.

- There is no basis for believing that Gulf War-era veterans were exposed to squalene-containing vaccines. The DoD has indicated that no experimental vaccines with squalene-containing adjuvants had been used in U.S. troops during the Gulf War. The Michigan Biologic Products Institute, producer of vaccines against the biological warfare agents, anthrax and botulinum toxoid, verified that they have never used adjuvant formulations containing squalene in their vaccines. The GAO itself concludes that “...we found no evidence to conclude that Gulf War-era veterans, either military or civilian personnel, were given adjuvants containing squalene.” In view of their own conclusion, the GAO proposal to test veterans for anti-squalene antibodies seems scientifically and fiscally irresponsible.
- The assay for anti-squalene antibodies developed by the independent researchers has not been validated through peer review or publication in the scientific literature. The DoD is correctly adhering to widely accepted scientific and medical standards by awaiting such validation before considering the use of this assay in studies of Gulf War veterans and their illnesses. The fact that “...they (the independent researchers) believe [the assay] is valid...” does not add scientific credibility. Further, the concern that “...time is critical for many Gulf War-era veterans...” does not justify using unproved technology, especially in human studies where the use of such assays is unethical. Data obtained from a methodology that has not been validated have significant potential to harm or mislead Gulf War veterans through the medical misinformation the data may support.

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**Appendix VI**  
**Comments From the Department of Defense**

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- The presence of anti-squalene antibodies in the blood of Gulf War-era veterans would not establish an association of squalene or squalene antibodies with illnesses among Gulf War veterans. The approach recommended by the GAO to test for antibodies to squalene in the blood of sick Gulf War-era veterans demonstrates a lack of understanding of scientific methods. Scientifically assessing the causal association of the presence of squalene antibodies and adverse health outcomes would require a statistically meaningful, population-based study of randomly selected Gulf War veterans and non-deployed veterans. Fundamental principles of epidemiological investigation underscore that relationships between potentially toxic substances and ill health require accurate and unbiased assessments of the health status, type of exposures, and the intensity of exposures of individuals representative of the entire group at risk. Scientifically defensible inferences about relationships between exposures and outcome are possible only in studies where both exposure and outcomes are measured objectively on population-based samples with high participation rates. The GAO might consult with scientists at the National Academy of Sciences Institute of Medicine regarding the value of GAO's proposed research and the nature, complexity, and cost of an appropriate study design.
- The hypothesis that squalene antibodies may be a contributing cause to the illnesses of the Gulf War era veterans and any experimental design to test that hypothesis must be evaluated for scientific merit through independent peer review. The GAO recommendation to proceed with efforts to test for antibodies to squalene in the blood of ill Gulf War era veterans disregards the federal agencies' commitment to fund the highest quality research programs based on the key guiding principle of independent merit review. The funding of research efforts outside the competitive peer review process has been severely criticized by the Presidential Advisory Committee (PAC) on Gulf War Veterans' Illnesses and other members of the scientific community. The Institute of Medicine (IOM) in 1995 and the PAC in its 1996 report emphasized the importance of external competition in order to ensure the scientific merit, level of priority, and relevance of research proposals. The PAC noted in its 1997 Special Report that these issues are especially crucial when spending involves public money during times of shrinking budgets. The PAC emphasized that the interests of veterans are not well served by research that is not meritorious. The Senate Investigation Unit most recently endorsed the PAC and IOM recommendations and stated that "...federal research programs should be guided by sound scientific principals, which is best assured when all research funding is subject to a rigorous and independent peer review process."

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