CHANCE FAVORS THE PREPARED MIND:

A History of the DoD Cholinesterase Monitoring Program and the DoD Cholinesterase Reference Laboratory

PUCHENG KE, RALPH A. STIDHAM, ALEXUS H. RAMIREZ-WIGGINS, MARISOL S. CASTANETO, ROBERT B. CROCHET, ADRIENNE M. FORBES, & MATTHEW D. WEGNER

"Chance favors the prepared mind." (Forte adiuvat animus paratus)

-Louis Pasteur

Introduction

The well-known time-honored quote above from the French chemist, Louis Pasteur, whose contributions were recognized with saving many lives by developing vaccines against anthrax and rabies, can mean several things as it relates to chemical weapon countermeasures. First, it could purport that the better prepared and more knowledgeable a person is, the more that person may be able to benefit from of any chance opportunities or observations. Persons having vision, curiosity, and ability related to the situation may position themselves to exploit any unseen "pearls" initially concealed at the beginning of their inquiry.

Due to the distinctive and extremely volatile nature of chemical warfare nerve agents, the U.S. government established the Department of Defense (DoD) Cholinesterase Monitoring Program, a massive clinical testing and occupational health monitoring program to periodically assess employee's potential exposure to chemical warfare nerve agents. For decades, with the ingenuity of laboratory and specialized personnel using sophisticated laboratory techniques, the program has monitored tens of thousands government military and civilian employees working in chemical weapon storage and destruction depots, as well as in the Chemical Defense industries and emergency preparedness programs. The potential deployment of chemical weapons by Russia and other adversaries have become an undeniable reminder that it is still crucial for the military to maintain and improve a robust cholinesterase activity testing program to better protect Americans and our allies worldwide.

In comparison to other types of chemical weapons, chemical warfare nerve agents have always played a particularly important role in part due to their superior lethality, long lasting effects, excellent survivability in the field environment, and ease in production. The long history of the U.S. government's research on chemical weapon development and countermeasures can be traced back to the WWI era.[1]

After WWII, the government started focusing on the safer-to-handle and easier-to-conceal binary chemical weapons. As a result, a large quantity of traditional chemical weapons and their related munitions were consolidated and delivered to multiple military depots for long term storage or demilitarization (destruction). The process was initiated by the Congress in the 1950s and accelerated during the U.S.-Soviet détente (1969-1979).

Similar to many organophosphate and carbamate pesticides, chemical warfare nerve agents target human cholinesterase by electrophilically attacking the cholinesterase serine residue to render the enzyme inactive.[2, 3] Without cholinesterase-induced breakdown, the neurotransmitter, acetylcholine can quickly accumulate and disrupt the normal acetylcholine synaptic concentration levels. The resulting rapid over-stimulation in the human neuromuscular system can cause acute poisoning and lead to a serious cholinergic crisis. The symptoms of cholinergic crisis are often summarized by the mnemonic 'SLUDGE' (Salivation, Lacrimation, Urination, Defecation, Gastrointestinal distress, and Emesis), in addition to miosis and muscle spasm.[4] 'SLUDGE' is the consequence of excessive exposure to cholinergic neurotransmitters and the resulting over excitation of central nervous system neurons. With high degree cholinesterase activity depression, a person can suffer from flaccid paralysis and respiratory failure, which may eventually result in death.

Unlike commercially available organophosphorus and carbamate pesticides, chemical nerve agents can undergo a fast secondary *in vivo* dealkylating transformation (commonly known as 'aging') to form a significantly more stable agent-cholinesterase complex.[5] The formation of the 'aged' agent-cholinesterase complex cannot be reversed by any conventional medical treatment. Due to these unique and extremely dangerous features, the U.S. government reached to a conclusion that it was necessary to establish a massive clinical testing and occupational health monitoring program to periodically assess employee's potential exposure to chemical warfare nerve agents. This later became the DoD Cholinesterase Monitoring Program.

Scope of the DoD Cholinesterase Monitoring Program Services

The DoD Cholinesterase Monitoring Program started testing government employees in the 1950s. The program significantly expanded in the 1970s through the 1990s. During this period, several DoD cholinesterase testing laboratories were established at or near the nerve agent storage and demilitarization sites. The program experienced peak activity in the mid-1990s through the early 2000s, especially after both the U.S. and Russia signed the treaty under the Chemical Weapons Convention and accepted its obligations in 1993. At the time, there were more than 25 satellite cholinesterase testing laboratories and one Cholinesterase Reference Laboratory under the DoD Cholinesterase Monitoring Program.[6] The program provided direct and crucial support to the Army National Guard Weapons of Mass Destruction Civil Support Teams (WMD-CSTs) in all 50 states, all OCONUS and CONUS DoD medical facilities, the Defense Threat Reduction Agency, the DoD Chemical Surety Program, and numerous auxiliary personnel engaged in missions supporting the nation's destruction of chemical warfare nerve agent munitions. The program has also collected a large amount of testing data and enabled the DoD to establish a normal range of human red blood cell acetylcholinesterase activity.[7]

Analytical Methods for Cholinesterase Activity Testing *in vitro*

Because chemical warfare nerve agents are Cholinesterase Inhibiting Substances, an important biomarker for potential exposure to nerve agents is to evaluate and measure depressed human cholinesterase activity. With the necessity to save lives and innovate, scientists who took Dr. Pasteur's longstanding maxim to heart have developed two widely utilized analytical methods, the electrometric Michel delta-pH method and the colorimetric Ellman method.[8, 9] The Michel method, published by the Army in 1949, measures cholinesterase activity by tracking cholinesterase catalyzed acetylcholine hydrolysis reaction rate in a patient's blood sample *in vitro*.[10] The dissociation of acetylcholine generates choline and acetic acid. The increased acidity of the blood sample is reflected by the decreased pH value. The change of pH can be accurately captured by a calibrated pH meter. Acetylcholine hydrolysis rate over a given time period can thus be calculated as 'delta-pH per unit time period' with a simple mathematical formula. As an alternative, Ellman in 1961 reported a unique method using the sulfur-substituted acetylcholine analog, acetylthiocholine as the substrate.[11] After the cholinesterase catalyzed hydrolysis, the resulting thiocholine reacts with 5, 5-dithiobis-2-nitrobenzoate to produce the highly conjugated chromophores bearing 5-thio-2-nitrobenzoic acid anion in yellow. Therefore, the rate of acetylthiocholine hydrolysis directly links to the color change rate of the reaction mixture and can be accurately measured at 412 nm wavelength (wavelength of the substituted benzene ring absorption light) by a calibrated photometer.

Although the Ellman method has proved to be more sensitive to exposures to extremely low dose nerve agents, the Michel method has significant advantages over the Ellman method for military use. Chief among these is that it requires fewer special reagents with less complex testing procedures. Additionally, although both methods can be applied to a variety of human biological matrices, e.g., red blood cells, serum, and plasma, the Ellman method is prone to be affected by the background

colors. Therefore, the DoD decided to adopt the Michel method for the Cholinesterase Monitoring Program. The original Michel method takes about 60 minutes for the hydrolysis to complete. It was later improved by the scientists at the Army Edgewood Arsenal Biomedical Laboratory by markedly shortening reaction time to 17 minutes in 1973.[12] After extensive comparison studies and discussions, the DoD officially designated the 17-minute modified Michel method as the standard methodology for the Cholinesterase Monitoring Program in 1978.[13] The modified method greatly improved testing efficiency by enabling the laboratory to test up to 51 samples every 17 minutes and to shorten testing turnaround time to less than 72 hours.

Quality Management Issues of the DoD Cholinesterase Monitoring Program in Early Days

Rapid expansion also created challenges in quality control and quality assurance. In the early 1970s, the DoD started receiving alarming concerns over a lack of standardized operating procedures across the DoD Cholinesterase Monitoring Program.[14] Although all the testing laboratories used the Michel method, individual laboratories independently purchased their preferred equipment and sometimes even modified testing steps or reaction conditions without proper method validation or verification.[15] In addition, there were no standardized requirements or policies on blood sample collection, submission, and accession. Moreover, none of the cholinesterase testing laboratories under the DoD Cholinesterase Monitoring Program were accredited.[16] The variations in laboratory practices among the laboratories led to inconsistencies, which made it difficult for healthcare providers to interpret results. The inter-laboratory testing result variations at the time were so significant that it was almost impossible to conduct individual cholinesterase activity baseline establishment or long term intra-individual cholinesterase activity monitoring unless all the samples from the individual were tested at the same laboratory.[17] Moreover, even the testing results from the same laboratory were often inconsistent or irreproducible.[18] Another challenge was the absence of proficiency testing program and mandatory requirements to include internal Quality Control samples for each testing batch.[19] At the time, there was no commercial or government reference laboratory to prepare and supply reliable Quality Control materials.[20]

To combat the 'chaotic' situation, the DoD tasked the Army to establish a practical and effective program to establish and enforce standardized testing procedures, minimize inter-laboratory testing variations, and improve the overall testing result reliability.[21] In response, the Army Health Services Command started a pilot Quality Assurance program consisted of the cholinesterase testing laboratories at Fitzsimons Army Medical Center (FAMC), Dugway Proving Ground, and Tooele Army Depot in 1974 - 1975.[22] The program included: (a) daily quality assurance testing; (b) periodic proficiency testing; (c) blind batch quality control testing where feasible; and (d) retesting of duplicate samples shipped to FAMC.[23] To minimize systemic bias, the testing equipment across the three testing sites was identical. FAMC was responsible for providing standardized and mandatory training for all testing technicians at the three sites.[24] All reagents and equipment were verified and certified at FAMC prior to delivery to the other two sites.[25] The feedback and results were encouraging, and the Army quickly incorporated these quality management measures in the first official DoD cholinesterase testing regulation, Technical Bulletin (TB) MED 292 in May 1975.[26] The pilot Quality Assurance program later expanded to Rocky Mountain Arsenal in 1976.[27] In the same year, the Army further changed the requirement of testing blind quality control samples in each testing batch from optional to mandatory.[28] Followed by this policy change, the Army developed a practical way to monitor long-term and short-term quality control sample testing result trends. After extensive data review, internal and external inspections, and comparison, the Army selected FAMC as the DoD Cholinesterase Monitoring Program reference testing center in February 1976, and fiscal and manpower resources were gradually implemented through the rest of the year.[29] In 1977, the DoD Cholinesterase Reference Laboratory was officially established at FAMC and became fully functional.[30] The DoD Cholinesterase Reference Laboratory was tasked to: (a) prepare and send out freeze-dried blind quality control samples at least at two (low and high) concentration levels; (b) conduct limited primary cholinesterase activity testing; (c) re-test selected (no less than 20% of) primary testing samples previously tested at and submitted by other testing sites for verification; (d) prepare and provide quarterly proficiency testing samples; (e) provide standardized and centralized training and certification for all testing personnel under the DoD Cholinesterase Monitoring Program. This included initial and annual refresh training; (f) purchase, verify, certify, maintain, and repair major testing equipment used at all testing sites in order to ensure all major equipment was identical and interchangeable among different testing sites; and (g) provide technical support and troubleshooting to other testing laboratories. These efforts greatly improved the DoD Cholinesterase Monitoring Program testing reliability and significantly reduced inter-laboratory testing variations.[31] Consequently, the DoD was able to utilize the large amount of data collected all over the country to establish a general human

cholinesterase activity baseline for government employees.[32] Ideally, the individual cholinesterase activity baseline is recommended to be used for every patient due to natural inter- and intra-individual cholinesterase activity variations. However, in reality, cholinesterase activity testing has not been readily available for general population or even all military members. Thus, individual baselines of most people are unknown and it is not economically feasible to include everyone in the long-term monitoring program. As a result, a reliable population-based cholinesterase activity normal range becomes crucial for quick, massive, and early screening for chemical nerve agent-induced acute poisoning for force protection.

Current Operations of the DoD Cholinesterase Reference Laboratory and Cholinesterase Monitoring Program

Under the Base Realignment and Closure Commission's recommendations, the DoD Cholinesterase Reference Laboratory was reassigned to the Directorate of Laboratory Sciences and relocated to the U.S. Army Center for Health Promotion and Preventative Medicine (USACHPPM) at Aberdeen Proving Ground in 1996.[33] In 2008, the DoD Cholinesterase Reference Laboratory moved down to the USACHPPM-South at Fort Sam Houston due to the reorganization of the DoD preventive medicine assets. The USACHPPM-South later became Public Health Command Region-South, which eventually was redesignated as Public Health Command, West (PHC, W) as of October 2023.

Currently, the DoD Cholinesterase Reference Laboratory is under the DoD Food Analysis and Diagnostic Laboratory, PHC, W located at Joint Base San Antonio-Fort Sam Houston. The DoD Cholinesterase Monitoring Program routinely conducts clinical testing and occupational health monitoring for not only federal, state, and local government employees in chemical weapon storage and demilitarization under the Chemical Surety Program, but also government contractors, national research laboratories, certain private sector employees, and local general population in agricultural industries with potential danger of exposure to organophosphate and carbamate pesticides.

In order to maintain high testing reliability, all testing laboratories under the DoD Cholinesterase Monitoring Program have been operated in accordance with updated regulations, e.g., TB MED 590 and DA PAM 40-8.[34, 35] The DoD Cholinesterase Reference Laboratory has been under extensive audits and accredited under the DoD Clinical Laboratory Improvement Program (CLIP) since the early 1990s. Recently, although COVID-19 pandemic caused operational difficulties, the DoD Cholinesterase Reference Laboratory underwent its first ISO 15189:2012 and CLIP combined assessment through the American Association for Laboratory Accreditation (A2LA) and successfully earned dual accreditation in April 2021. Dual accreditation of the laboratory was later re-verified in Summer 2023 and is currently in transition to ISO 15189:2022/CLIP.

Since the U.S. military accelerated chemical weapon demilitarization in the 2000s, many nerve agent storage and destruction sites have been consolidated or closed. This has resulted in a rapid decrease in the number of cholinesterase activity testing sites and human blood specimens processed over the past two decades. In July 2023, the DoD announced the destruction of all U.S. chemical weapons stockpile was completed ahead of the Chemical Weapons Convention elimination deadline.[36] Currently, there are only 7 federal government-owned cholinesterase activity testing sites, including the DoD Cholinesterase Reference Laboratory, for periodically monitoring a population of slightly more than 3,000 people. In comparison, there were still 12 DoD cholinesterase activity testing sites inside the CONUS alone in support of over 25,000 personnel in 2009. Although the reduction in testing sites and samples was logical for economic purposes, it has nevertheless created a potential capability gap in the military protection and sustainment war fighting functions against chemical weapons.

The DoD Cholinesterase Monitoring Program in Future

While the DoD Cholinesterase Monitoring Program and the DoD Cholinesterase Reference Laboratory have downsized with demilitarization of the U.S. chemical weapon stockpiles coming to an end, recent use of chemical nerve agents in terrorist attack in Japan, the Syrian civil war in the Mideast, political assassinations in the U.K. and Malaysia,[37, 38] and current rapidly changing geopolitical situations that may result in peer or near-peer large scale combat operations (LSCO) involving potential enemy use of lethal chemical nerve agents have made us seriously rethink the value and future of the DoD Cholinesterase Monitoring Program in force and general population protection.

As the world's biggest chemical weapon developer and maintainer, Russia (including former USSR) has kept synthesizing, testing, weaponizing, and stockpiling highly toxic nerve agents after it signed the Chemical Weapons Convention in 1993. It has been reported that Russia even secretly used

the Western financial aid for chemical weapon destruction in the development of the 4th generation nerve agents, the A-series agents, or 'Novichoks' (literal meaning 'newcomers') between 1971 and at least up to mid-1990s.[39] Novichok agents were designed to significantly enhance lethality in the field environment in comparison to the traditional G- and V-series agents. As a group, Novichoks possess highly modified substituent patterns while sharing some structural similarities to legitimate organophosphorus pesticides in order to cheat the United Nations and Chemical Weapons Convention inspections that follow molecular fragments. Novichoks have also posed remarkable challenges to chemical analysis and clinical testing communities. Even until today, exact structures and toxicological properties of many Novichok agents remain unknown. The lack of detailed structural elucidation information has created formidable obstacles in developing practical testing panels with modern highly specific technologies, e.g., mass spectrometry for Novichoks induced poisoning detection, identification, and surveillance.[40] Due to the nature of mass spectrometry-based analytical methods, if the target is not on the testing panel, the instrument will most likely miss it. In addition, detecting human metabolites of chemical warfare nerve agents in blood or urine often cannot, in theory provide necessary confirmatory evidence to identify specific nerve agents, since some of them share identical primary or secondary metabolites. This limitation has created noteworthy challenges in forensic, clinical, and toxicology communities. Moreover, mass spectrometry-based methods usually require sophisticatedly designed extraction procedures to minimize matrix effects or interferences.[41] Subsequently, extraction often leads to low instrument responses caused by poor recoveries in addition to difficulty in ionization of certain nerve agent metabolites. In contrast to mass spectrometry methods, the method that the DoD Cholinesterase Monitoring Program currently uses is a broad-spectrum, easy to operate, fast turnaround, and highly cost-effective technology that can readily be applied in the field environment. Instead of chasing specific molecular fragment 'fingerprints', the method sensitively and quantitatively measures the affected biological effects caused by cholinesterase inhibiting substances. As a result, knowing chemical structures of the analytes is no longer a prerequisite for testing capability. Due to the unique advantages, the DoD Cholinesterase Monitoring Program has served as a powerful and indispensable tool for screening human potential exposure to cholinesterase inhibiting substances for decades and proved its critical and long-lasting value in future civilian and military applications. ■

Maj. Pucheng Ke

is an Army Biochemist and currently the Chief of Quality Assurance at the DoD Food Analysis and Diagnostic Laboratory, U.S. Army Public Health Command, West, Joint Base San Antonio-Fort Sam Houston, TX. He has a B.S. in Chemistry from Lanzhou University and a Ph.D. in Organic Chemistry from Indiana University, Bloomington. He was previously assigned as the Chief of the DoD Cholinesterase Reference Laboratory at the DoD Food Analysis and Diagnostic Laboratory, U.S. Army Public Health Command, West, Joint Base San Antonio-Fort Sam Houston, TX. His email address is pucheng.ke.mil@health.mil.

Dr. Ralph A. Stidham

is the Regional Epidemiologist in the Epidemiology and Disease Surveillance Division at the U.S. Army Public Health Command, West, Joint Base San Antonio-Fort Sam Houston, TX. He has a B.A. in Sociology from San Diego State University, A Certificate of Public Health from the University of Washington, a M.P.H. from Emory University, and a Doctorate in Health Sciences (D.H.Sc) from Nova Southeastern University. His email address is ralph.a.stidham.civ@health.mil.

Ms. Alexus H. Ramirez-Wiggins

is currently a graduate research intern (from Oklahoma State University) at the DoD Cholinesterase Reference Laboratory, the DoD Food Analysis and Diagnostic Laboratory, U.S. Army Public Health Command, West, Joint Base San Antonio-Fort Sam Houston, TX. She has a B.S. in Forensic Chemistry from Sam Houston State University. Her email address is alexus.ramirez-wiggins@okstate.edu.

Lt. Col. Marisol S. Castaneto

is an Army Biochemist and currently the Program Manager of the Army Forensic Toxicology Program, U.S. Army Medical Command, Joint Base San Antonio-Fort Sam Houston, TX. She has a B.S. and an M.S. in Biochemistry from Kansas State University, and a Ph.D. in Toxicology from University of Maryland School of Medicine . She was previously assigned as the Chief of Quality Assurance at the DoD Food Analysis and Diagnostic Laboratory, U.S. Army Public Health Command, West, Joint Base San Antonio-Fort Sam Houston, TX. She contributed to the manuscript while she was assigned at the DoD Food Analysis and Diagnostic Laboratory. Her email is marisol.s.castaneto.mil@health.mil.

Capt. Robert B. Crochet

is an Army Biochemist and currently the Chief of the DoD Cholinesterase Reference Laboratory at the DoD Food Analysis and Diagnostic Laboratory, U.S. Army Public Health Command, West, Joint Base San Antonio-Fort Sam Houston, TX. He has a B.S. in Biochemistry and Ph.D. in Structural Biology from Louisiana State University. He was previously assigned as the Chief of Immunodiagnostics at the at the DoD Food Analysis and Diagnostic Laboratory, U.S. Army Public Health Command, West, Joint Base San Antonio-Fort Sam Houston, TX. His email address is robert.b.crochet.mil@health.mil.

Ms. Adrienne M. Forbes

is currently the DoD Cholinesterase Monitoring Program Manager at the DoD Cholinesterase Reference Laboratory, the DoD Food Analysis and Diagnostic Laboratory, U.S. Army Public Health Command, West, Joint Base San Antonio-Fort Sam Houston, TX. She has a B.S. in Biology from Columbia Union University, a Clinical Laboratory Scientist ASCP Certification, and a Clinical Research Associate Certification. She was previously assigned as a Clinical Laboratory Scientist at the Department of Veterans Affairs with concentrations in Clinical Chemistry and Microbiology.

Col. Matthew D. Wegner

is an Army Veterinary Pathologist and currently the Director of the DoD Food Analysis and Diagnostic Laboratory at U.S. Army Public Health Command, West, Joint Base San Antonio-Fort Sam Houston, TX. He has a B.S. in Biology, a M.S. in Veterinary Microbiology and Preventive Medicine, and a D.V.M., all from Iowa State University. He was previously assigned as the Chief of Comparative Pathology and Veterinary Medical Research at the U.S. Armed Forces Medical Directorate in Bangkok, Thailand, and as a Chemical Defense Research Pathologist and Chief of Laboratory Services at the U.S. Army Medical Research Institute of Chemical Defense at Aberdeen Proving Ground, MD. His email address is matthew.d.wegner2.mil@health.mil.

Notes

1. Al Mauroni, “The U.S. Army Chemical Corps: past, present, and future,” The Army Historical Foundation, accessed March 12, 2024, https://armyhistory.org/the-u-s-army-chemical-corps-past-present-and-future/

2. Robin Black, “An Overview of Biological Markers of Exposure to Chemical Warfare Agents,” *J Anal Toxicol* 32, no. 1 (January – February 2008): 2-9, https://doi.org/10.1093/jat/32.1.2.

3. Vassiliki Aroniadou-Anderjaska et al., “Acetylcholinesterase Inhibitors (nerve agents) as Weapons of Mass Destruction: History, Mechanisms of Action, and Medical Countermeasures,” *Neuropharmacology* 181, (December 2020): 108298, https://doi.org/10.1016/j.neuropharm.2020.108298

4. Hiroyuki Ohbe et al., “Cholinergic Crisis Caused by Cholinesterase Inhibitors: a Retrospective Nationwide Database Study,” *J Med Toxicol 14*, no. 3 (September 2018): 237-241, https://doi.org/10.1007/s13181-018-0669-1

5. Daniel Quinn et al., “Why is Aged Acetylcholinesterase So Difficult to Reactivate,” *Molecules* 22, no. 9 (September 2017): 1464-1469, https://doi.org/10.3390/molecules22091464.

6. Unpublished Memoranda, 1950s - 1990s, U.S. Army Public Health Command, West, Archives, Joint Base San Antonio-Fort San Houston, TX.

7. Unpublished Memoranda, 1950s - 1990s.

8. Harry Michel, “An Electrometric Method for the Determination of Red Blood Cell and Plasma Cholinesterase Activity,” *J Lab Clin Med* 34 (1949): 1564-1568.

9. George Ellman et al., “A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity,” *Biochemical Pharmacology* 7, no. 2 (July 1961): 88-95, https://doi.org/10.1016/0006-2952(61)90145-9.

10. Michel, “An Electrometric Method for the Determination.”

11. Ellman et al., “A New and Rapid Colorimetric Determination."

12. Robert Ellin, Barry Burkhardt, Russell Hart, “A Time-modified Method for Measuring Red Blood Cell Cholinesterase Activity,” *Arch Environ Health* 27, no. 1 (July 1973): 48-49, https://doi.org/10.1080/00039896.1973.10666307.

13. Unpublished Memoranda, 1950s - 1990s.

14. Unpublished Memoranda, 1950s - 1990s.

15. Unpublished Memoranda, 1950s - 1990s.

16. Unpublished Memoranda, 1950s - 1990s.

17. Unpublished Memoranda, 1950s - 1990s.

18. Unpublished Memoranda, 1950s - 1990s.

19. Unpublished Memoranda, 1950s - 1990s.

20. Unpublished Memoranda, 1950s - 1990s.

21. Unpublished Memoranda, 1950s - 1990s.

22. Unpublished Memoranda, 1950s - 1990s.

23. Unpublished Memoranda, 1950s - 1990s.

24. Unpublished Memoranda, 1950s - 1990s.

25. Unpublished Memoranda, 1950s - 1990s.

26. Unpublished Memoranda, 1950s - 1990s.

27. Unpublished Memoranda, 1950s - 1990s.

28. Unpublished Memoranda, 1950s - 1990s.

29. Unpublished Memoranda, 1950s - 1990s.

30. Unpublished Memoranda, 1950s - 1990s.

31. Unpublished Memoranda, 1950s - 1990s.

32. Department of the Army, *Technical Bulletin (TB) MED 590: Red Blood Cell Cholinesterase Testing and Quality Assurance* (Washington, D.C.: Department of the Army, 2001).

33. Unpublished Memoranda, 1950s - 1990s.

34. Department of the Army, *Technical Bulletin (TB) MED 590*.

35. Department of the Army, *Department of the Army Pamphlet (DA PAM) 40-8: Occupational health guidelines for the evaluation and control of occupational exposure to nerve agents GA, GB, GD, and VX* (Washington, D.C.: Department of the Army, 1990).

36. “US Completes Chemical Weapons Stockpile Destruction Operations,” U.S. Department of Defense, accessed March 12, 2024, https://www.defense.gov/News/Releases/Release/Article/3451920/us-completes-chemical-weapons-stockpile-destruction-operations/.

37. Georgios Pampalakis, Stavroula Kostoudi, “Chemical, Physical, and Toxicological Properties of V-Agents,” *Int J Mol Sci* 24, no. 10 (May 2023): 8600, https://doi.org/10.3390/ijms24108600

38. Peter Chai et al., “Novichok agents: a historical, current, and toxicological perspective,” *Toxicol Commun* 2, no. 1 (June 2018): 45-48,https://doi.org/10.1080/24734306.2018.1475151.

39. Chai et al., “Novichok agents."

40. Harald John, Horst Thiermann, “Poisoning by Organophosphorus Nerve Agents and Pesticides: An Overview of the Principal Strategies and Current Progress of Mass Spectrometry-Based Procedures for Verification,” *Journal of Mass Spectrometry and Advances in the Clinical Lab* 19 (January 2021): 20-31,https://doi.org/10.1016/j.jmsacl.2021.01.002.

41. John, Thiermann, “Poisoning by Organophosphorus."