The Worldwide Effort to BAN CHEMICAL WEAPONS

Livermore's Forensic Science Center develops novel analytical methods and trains personnel in at-risk nations to respond to incidents involving toxic chemicals.

FOR thousands of years, chemical weapons have been used in warfare. In 600 B.C., the Athenian military poisoned the water supply of the besieged city of Kirrha. During World War I, an estimated 124,000 tons of chlorine, phosgene, mustard, and other chemical warfare agents (CWAs) were released by German forces, killing more than 90,000 soldiers and injuring nearly a million people. As part of the Cold War, both the United States and the Soviet Union maintained stockpiles of CWAs. More recently, events in the Mideast have shown that the threat of chemical weapons continues.

Through an international effort to permanently eliminate existing chemical weapons and prevent their re-emergence, the Chemical Weapons Convention (CWC) treaty entered into force in 1997 and prohibits the development, production, acquisition, stockpiling, or transfer of CWAs. The treaty is implemented through the Organisation for the Prohibition of Chemical Weapons (OPCW), headquartered in The Hague, Netherlands. To support its mission, OPCW relies on a global network of accredited laboratories to analyze samples from suspected chemical weapons production facilities. OPCW laboratories are located in Finland, Sweden, Spain, Singapore, the United Kingdom, the United States, Germany, India, Russia, the People's Republic of China, South Korea, and France, among others.

OPCW requires that samples taken by its inspectors be analyzed by two OPCW-designated laboratories, and U.S. legislation requires that all samples collected in the United States be analyzed within the country. Only two laboratories in the United States are certified for the analysis of environmental samples collected by OPCW: the Laboratory's Forensic Science Center (FSC) and the U.S. Army Combat Capabilities Development Command Chemical Biological Center (formerly known as the U.S. Army Edgewood Chemical Biological Center). The FSC has also earned its OPCW certification for biomedical materials and can thus analyze samples containing any mixture of both sample types.

In addition to supporting OPCW, the FSC performs assessments for homeland security, law enforcement, and intelligence agencies. For example, the FSC partners with the Federal Bureau of Investigation for chemical, radiological, nuclear, and explosive materials analysis.

"The FSC has two intertwined missions," says the center's Director Audrey Williams. "The first is performing cutting-edge research, the second is responding to the urgent needs of our sponsors."

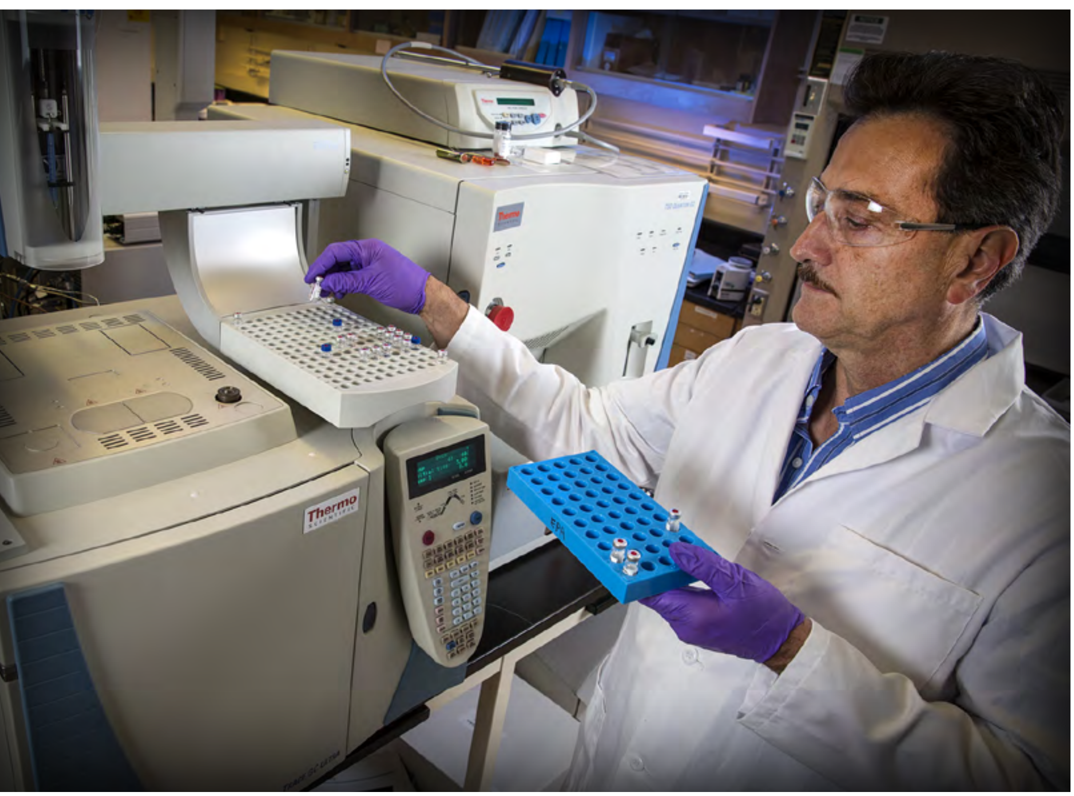
A Dynamic Environment

CWC's controls on toxic chemicals and their precursors are listed on three schedules according to their toxicity, military and commercial utility, and risk. Many chemicals of concern have legitimate civilian uses, so industrial facilities as well as government sites are subject to OPCW inspections.

When OPCW's laboratory accreditation program began in 2001, the U.S. State Department requested that the FSC seek certification because of the Laboratory's strong physical security and environmental controls, as well as the center's recognized technical experience with CWA and expertise in analyzing trace levels of unknown substances, especially nuclear materials. Indeed, the FSC pioneered nuclear forensic analysis and remains the principal U.S. laboratory for analyzing possible nuclear and radiological contraband. (See *S&TR*, July/August 2018, pp. 4–12.)

FSC staff are experts in organic, inorganic, analytical, and nuclear chemistry, environmental and biological sample analysis, and medical countermeasures. Williams acknowledges the challenge in finding people with the needed mix of skills. She says, "Working at the FSC requires not only strong technical expertise, but also an ability to think on one's feet under severe time constraints and in a team environment." As part of their duties, the staff of approximately 25 works to improve detection methods. FSC analysts, for example, sometimes discover that standard sample preparation and analysis methods are lacking, and so must develop new identification protocols. These efforts have included novel techniques to analyze blood and urine and complex extraction protocols for difficult soiled materials.

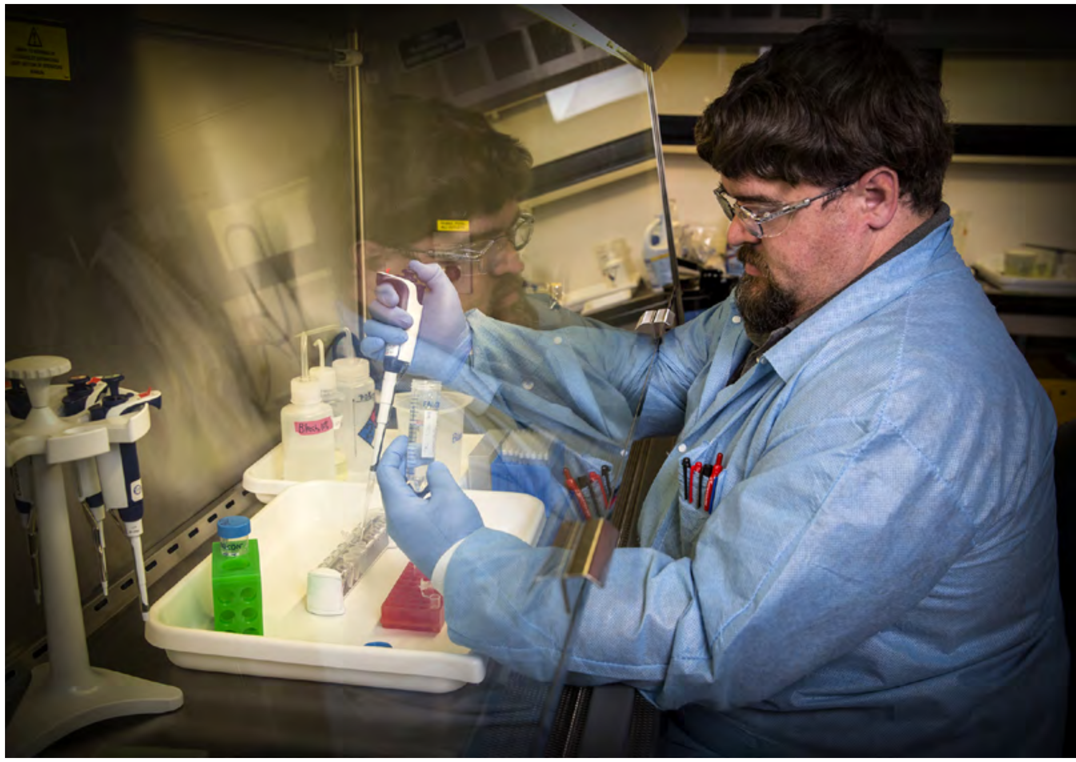
To maintain OPCW accreditation, laboratories are required to participate in extremely challenging annual proficiency tests. The environmental tests typically contain dilute amounts of CWAs, precursor chemicals, degradation materials, and compounds that can hinder successful analysis. For each test, one OPCW-designated laboratory volunteers to formulate the samples and another grades the findings of participating laboratories. FSC staff spend long hours during 15 consecutive days each year isolating possible reportable compounds. "Testing is an immensely stressful time, but it is also intellectually satisfying and rewarding," says Williams. Each laboratory must maintain a three-year rolling average of at least two "A" grades and one "B." Recently, the FSC earned its 10th consecutive "A" grade.



Armando Alcaraz, a chemist in Lawrence Livermore's Forensic Science Center (FSC) prepares samples for analysis using gas chromatography–mass spectrometry. (Photo by George Kitrinos.)

Extending FSC Expertise

Biologist Todd Corzett notes that several years ago, in light of growing evidence of CWAs being used in Mideast conflicts, OPCW recognized the importance of finding biomedical signs of exposure to a CWA. OPCW assembled a worldwide laboratory network modeled after the one for environmental samples but focused this time on analyzing blood and urine. In addition to the FSC, the U.S. Army Combat Capabilities Development Command Chemical Biological Center, Centers for Disease Control and Prevention, and U.S. Army Medical Research Institute of Chemical Defense are OPCW biomedical-designated laboratories. All certified OPCW laboratories equipped for



FSC biologist Todd Corzett prepares a solution for detecting any of a dozen nerve agents in blood plasma. (Photo by George Kitrinos.)

biomedical sample analysis must pass an annual proficiency test, similar to that for environmental accreditation.

Corzett explains that exposure to CWAs leaves behind unique biomarkers that are quickly excreted in urine but can remain in blood plasma for up to 90 days. Concentrations of biomarkers are often lower than CWA concentrations found in environmental samples—5 to 10 parts per billion instead of several parts per million, respectively.

FSC experts work with biomedical samples in a Biosafety Level-2 laboratory, which features strong safety and environmental engineering systems as well as personnel protection. When analyzing blood plasma, the FSC team uses liquid chromatography–mass spectrometry to search for biomarkers called adducts, molecular complexes that form when a CWA binds to a particular protein or enzyme. For example, butyrylcholinesterase (BChE) is a protein found in blood plasma that when exposed to one of a dozen nerve agents produces a persistent adduct. Corzett uses magnetic beads coated in antibodies to extract BChE from blood plasma for biomarker analysis.

Other proteins form adducts upon exposure to different CWAs, and FSC scientists look for those, as well. Corzett notes that knowing the clinical symptoms (for instance, twitching or skin blistering) of the individuals from whom the blood or urine samples were taken can help guide detection protocols, but adds that this information is not always available. “We have to be ready for anything,” he says. “Unlike proficiency tests, real-world samples are often collected and transported in less than ideal conditions, making subsequent analysis more difficult.”

Advanced Equipment Is Critical

FSC staff take advantage of the latest advances in analytical instrumentation that can isolate and identify increasingly more minute quantities of CWAs and other compounds. According to FSC Deputy for Operations Carolyn Koester, an ever-widening family of instrumentation includes gas, liquid, and ion chromatographs coupled with a variety of mass spectrometers; nuclear magnetic resonance (NMR); and infrared and Raman spectrometers.

A recently acquired best-in-class liquid chromatographic tandem mass spectrometer has proven essential for analyzing biomedical samples. The instrument achieves unprecedented sensitivity and fast identification of adducts and other biomolecules. “We’re lucky we work with sponsors who understand the benefits of state-of-the-art instrumentation,” says FSC analytical chemist Brian Mayer. “They recognize that the best equipment helps us get the answers they need. Five years ago, I never imagined we could see down beyond the picogram (10^{-12} grams) level, but it’s now routine.”

On the other hand, instruments’ heightened sensitivity can pose a serious challenge. “We can now detect tens of thousands of different compounds in a single sample, which can be overwhelming,” says Mayer. Williams adds that detection limits are now so low “we can detect nefarious activity that took place years ago, as a trace amount of the target chemical is still there.”

Clues for Chemical Attribution

The FSC has made significant advances not only in detecting CWAs, but also in chemical attribution signatures—trace amounts of synthesis precursors and byproducts, impurities, degradation products, and metabolites, which can provide clues to the likely source of reagents, type of synthesis equipment, or production method used. Williams says, "Even if we don't see a listed chemical warfare agent in a sample, the compounds we identify could be linked to its preparation."

Some CWAs can be made on a small scale with methods that would be difficult to scale up. Likewise, simpler methods typically require chemicals that are strictly controlled, so a terrorist group might choose a more complex method that uses widely available compounds. FSC chemists can often determine which synthesis pathway was implemented, including starting reagents.

In a project sponsored by the U.S. Department of Homeland Security, FSC chemists, in collaboration with the Swedish Research Defence Agency, developed a model to attribute samples containing sulfur mustard to 1 of 11 possible synthesis methods. Samples made with all 11 routes were analyzed to extract chemical attribution signatures as the basis for the model.

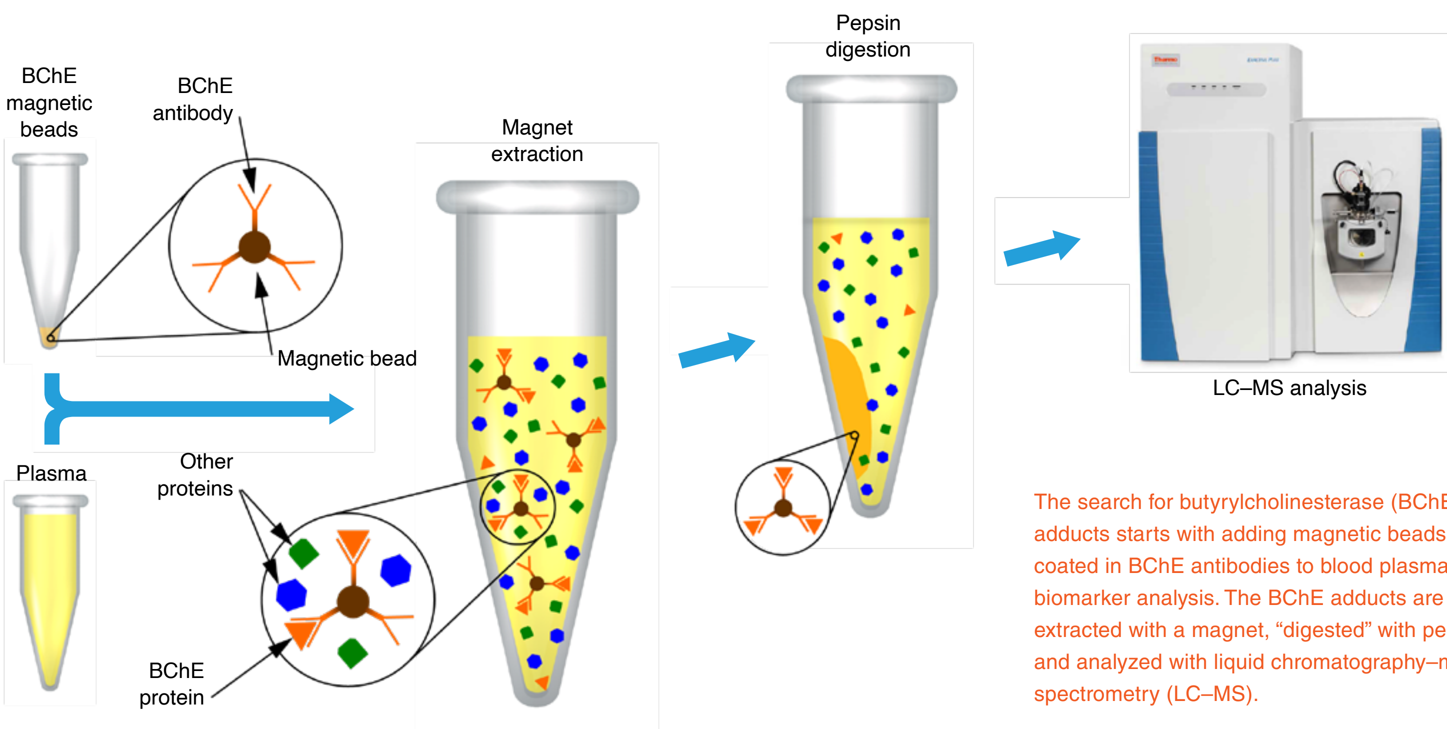
"We developed a machine-learning tool that could give analysts significant insight into how a sample was made. Computers can identify important features in the data that an analyst would simply never notice," says Mayer.

Williams notes that a sample may contain a toxic chemical that is not on a threat list. As an example, in 2018 Sergei Skripal, a former Russian military officer and double agent for the United Kingdom's intelligence service, and his daughter were poisoned in Salisbury, England, with a Novichok nerve agent, which was not on OPCW's list of CWAs. Both recovered, as did a police officer hospitalized after exposure to the remnants of the toxic agent at Skripal's residence. The U.S. State Department concluded that Russia was behind the poisoning. Four months later, two British citizens were hospitalized with Novichok poisoning and one died. British police hypothesized that both individuals had come across the original chemical that had been disposed of haphazardly.

Developing Countermeasures

An important FSC research thrust is developing countermeasures to stop the often-lethal effects of CWAs. Much of the research is funded by the U.S. Department of Defense, whose soldiers may be thrust into theaters of war where they could encounter CWAs. One concern is that an enemy could potentially vaporize an easily manufactured opioid and thereby incapacitate soldiers. Mayer points to a 2002 incident when Chechnyan terrorists took over a Moscow theater and held 850 hostages. Russian special forces vaporized what was believed to be an opioid to subdue the insurgents. All 40 of the insurgents were killed, as were more than 200 hostages.

With funding from Livermore's Laboratory Directed Research and Development Program, FSC researchers in 2016 launched an integrated experimental and computational approach for developing antidotes to nerve agents and incapacitating agents such as fentanyl, a



The search for butyrylcholinesterase (BChE) adducts starts with adding magnetic beads coated in BChE antibodies to blood plasma for biomarker analysis. The BChE adducts are then extracted with a magnet, "digested" with pepsin, and analyzed with liquid chromatography–mass spectrometry (LC–MS).

First-responder trainees participate in a staged exercise wherein a smoke bomb mimics a vaporized nerve agent.

synthetic opioid pain medication that is highly addictive, as well as toxic at low doses. The challenge for FSC scientists is to develop medical countermeasures that can be injected intravenously or intramuscularly, are more effective than current countermeasures with fewer side effects, and have potential as a prophylaxis for soldiers entering combat.

A team led by Mayer and chemist Carlos Valdez used a variety of techniques including NMR to screen candidate compounds identified and then down-select options based on computational chemistry results. They arrived at a promising molecule called subetadex. The U.S. Department of Defense is currently funding the FSC to develop an advanced version of subetadex that would possess enhanced affinities for fentanyl and other synthetic opioids.

In a related effort, a team of scientists from the FSC developed LLNL-02, the first molecule that effectively crosses the protective blood–brain barrier to prevent long-lasting effects on the brain from nerve agents (see *S&TR,* June 2019, pp. 12–15). A significant challenge for researchers developing nerve-agent antidotes is creating a drug that simultaneously protects both the body's central nervous system and peripheral nervous system. The effort, headed by Valdez, was aided by a computer model that simulates the efficacy of potential antidotes. The speedy penetration of LLNL-02 into the brain has been demonstrated in guinea pigs.

No Letup in Effort

In 2019, 98 percent of the world's population was considered under CWC's protection. As of August 2020, 98 percent of all chemical weapon stockpiles declared by possessor nations have been destroyed. However, the threat posed from the acquisition and use of chemical weapons by nations that are non-CWC signatories or terrorists remains. Clandestine production of CWAs has occurred in repurposed facilities (for example, pesticide manufacturing plants); specially built laboratories; and small, makeshift laboratories. Information on how to manufacture and weaponize CWAs has also become more accessible.

With their exceptional analytical expertise, FSC scientists have played a significant role in helping to enforce the tough tenets of CWC through OPCW's aggressive inspection regimes and its worldwide laboratory network. FSC researchers are always mindful of the dangers of chemical weapons—witness Saddam Hussein's killing of 5,000 Iraqis with chemical weapons in 1988, and a Japanese cult's release of a nerve agent in a Tokyo subway that killed 13 people and injured thousands in 1995. Together with the recent incidents in the Mideast, these events have become an urgent reminder that the knowledge to make and weaponize CWAs cannot be taken back.

Furthermore, OPCW's confirmation of a Novichok nerve agent being used

Two Decades of Enhancing Response to Chemical Weapons Use

In 2016, Islamic State fighters launched two chemical attacks involving chlorine and sulfur mustard near the city of Kirkuk in northern Iraq, killing a three-year-old girl and wounding nearly 600 people. The extremist group was thought to have set up a special chemical weapons unit composed of Iraqi scientists, who had worked for Saddam Hussein, and foreign experts. Afterwards, first responders from Iraq's Ministry of the Interior were able to collect valuable evidence related to the attack—thanks in part to training from scientists at Lawrence Livermore's Forensic Science Center (FSC).

As part of an effort to train personnel in at-risk nations to effectively respond to incidents involving toxic chemicals, FSC scientists travel to other countries, especially those in high-priority areas, to instruct first responders and medical personnel on essential protocol. The work is supported by the U.S. Department of State's Chemical Security Program and Biosecurity Engagement Program. Through the Department of State, FSC staff collaborate with foreign government agencies to identify needs and deliver training, including "train-the-trainer" programs that enable local experts to pass on their knowledge.

Since 2013, FSC chemist Armando Alcaraz and colleagues have conducted first-responder training in Iraq, Lebanon, Kenya, Turkey, Malaysia, Jordan, and Yemen. The FSC has conducted eight training sessions with Iraqi civil defense experts, the latest in 2019. In the 2016 sulfur mustard attacks, Iraqi first responders had earlier participated in a mock field-training scenario where a sulfur mustard munition contaminated an area. The same team of first responders responded to the real attack in Kirkuk. The combination of classroom training and realistic field exercises provided the Iraqi responders with the skills they needed to effectively address the situation. The FSC received recognition from the Iraqi Ministry of the Interior for their efforts. "We potentially saved lives and kept responders safe from exposure," says Alcaraz. "They were very appreciative of the training."

In 2019, first responders from Latin American and the Caribbean attended the first regional training course for chemical warfare agent (CWA) sampling and analysis in contaminated areas. The five-day class, run by the Organisation for the Prohibition of Chemical Weapons (OPCW), was held in Bogota, Columbia, for participants from Argentina, Barbados, Belize, Brazil, Chile, Colombia, Costa Rica, the Dominican Republic, Guatemala, Ecuador, Mexico, Panama, Peru, and Saint Lucia. FSC scientists supported the course through the Instructor Development and Exchange Program administered by the OPCW Assistance and Protection Branch.

Iraqi first responders conduct a training exercise during a simulated chemical warfare agent attack. The same first responders, trained by FSC staff, later performed admirably when terrorists launched an actual attack in the city of Kirkuk in 2016.

Alcaraz says, "We teach the basics: explaining what chemical warfare agents are and their effects, what methods are available for detection and decontamination, how to collect evidence, and how to use and remove chemical protective clothing." He notes that the training, which sometimes includes the establishment of a forensic laboratory, helps to ensure governments remain committed to the nonproliferation of CWAs.

A key part of training involves realistic field exercises. During these activities, participants practice with detection equipment; collect samples for forensic laboratories; don protective equipment such as respirators, face shields, and chemical-resistant clothing; and follow procedures to prevent contamination. The most realistic training occurs at specialized facilities in the Netherlands, Czech Republic, and Belgium. Training at these sites features staged incidents, in which participants must identify the agent involved and respond appropriately.

Back at home, FSC staff have been providing incident response support for more than a decade. Since 2008, the FSC has been the Chemical Agent Reference Laboratory for the Environmental Protection Agency (EPA) for developing reliable, accurate, and extremely sensitive analytical methods and providing standards (milliliter quantities of 10 parts per million CWAs). EPA is the federal agency responsible for environmental cleanup following acts of terrorism involving CWAs as well as toxic industrial compounds.

Livermore analytical chemist and FSC Deputy for Operations Carolyn Koester is the principal investigator for the partnership with EPA. As part of their duties, FSC scientists work to improve detection methods in partnership with EPA. "We want to help EPA laboratories ensure that all public areas are safe after an incident involving chemical weapons," she says. Following an attack on U.S. soil, EPA's Environmental Response Laboratory Network (ERLN), which includes the FSC, would quickly become involved. Response teams, using Livermore-developed techniques, would determine the nature and extent of the contamination and then help monitor decontamination and restoration activities. As part of ERLN, the FSC could also provide overflow chemical analysis capacity, if requested.

The EPA work is part of a larger Livermore effort to help federal and state agencies plan for efficient recovery from a chemical weapons release and reduce the remediation effort by days or weeks. (*S&TR*, March 2010, pp. 4–10.) Toward that goal, Livermore scientists have also strengthened scientific understanding of how CWAs interact with different building and office materials.

In this training exercise, a first responder wearing protective clothing examines a human mannequin covered with skin blisters at a makeshift laboratory. The blisters are indicative of exposure to sulfur mustard.

in the 2018 Salisbury attack resulted in the organization recently adding four more compound families, including the Novichoks, to the list of tightly restricted chemicals under the CWC. This revision marked the first time any class of chemical had been added to the CWC schedules since the treaty came into force more than two decades ago. As a result, the FSC is actively investigating the characteristics of the four additional compound families and their precursors, environmental degradation, and decontamination products. Says Williams, "One or more chemicals from the newly added chemical families could be spiked in a future OPCW environmental proficiency test sample."

Although FSC researchers continue to acquire state-of-the-art instrumentation and develop more effective protocols for detecting CWAs, FSC staff, including chemist Armando Alcaraz, conduct further work with national ministries to provide training for detecting and responding to chemical threats (see the box on p. 10). He notes OPCW is hoping to launch training programs in countries such as Morocco, Algeria, Nigeria, and several Latin American nations. OPCW is also desirous of establishing accredited laboratories in Africa, Latin America, and the Mideast. Clearly, the fight to eliminate chemical weapons must be a worldwide endeavor.

—Arnie Heller

Key Words: Biosafety Level-2, butyrylcholinesterase (BChE), Biosecurity Engagement Program, Chemical Security Program, chemical warfare agent (CWA), Chemical Weapons Convention (CWC), Environmental Protection Agency (EPA), Forensic Science Center (FSC), Organisation for the Prohibition of Chemical Weapons (OPCW), subetadex, sulfur mustard, Swedish Research Defence Agency, U.S. Army Combat Capabilities Development Command Chemical Biological Center.

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