need a confident, unified approach toward a common goal, political in nature, in order to reverse, even if only gradually, the present government policies that are choking Brazilian science. If these policies are not changed, they will lead the country dangerously backwards.

Unity of purpose is also essential at all levels of chemistry teaching, as well as within the industrial sector and in any activity linked directly or indirectly with chemistry. This unanimity must be understood as no mere political strategy but as a true ideology in action, expressing the belief I think all chemists share, that the cultivation and advancement of chemistry are essential for the progress and well being of the country. This undertaking is indeed difficult in the present climate, in which the nation's ruling circles tend to consider advanced quality education and scientific research as luxuries that can be postponed. The preference now is to import everything, rather than to encourage domestic development. We thus face an inversion in the direction of history, by which the nation might be led to a situation reminiscent of that which prevailed before the last half-century.

In addition to immense academic and political effort, there is more to be done. We need to encourage, as individuals and institutions, together with the scientific societies, the popularization of science and the education of the lay population. Without widespread public backing, this inversion process might take much longer. We would do well to remember that, until a few decades ago, awareness that cultivation of science is a necessity was far from unanimous, even in university circles. Moreover, to bring science to the public in general is a relevant task that we cannot shun, as members of a minority who had access to science education at all levels. The goal of this effort is not to find future scientists, but rather to disseminate science among common citizens and to make them scientifically literate. The discovery of scientific talents will be a possible consequence of this action, not its motivation. Insofar as chemistry is the central science, chemists are also at the center of this responsibility. If we criticize those who deny us support for our research, we cannot be omissive in this regard.

Reaching out to the general public, if well conducted, can result in great benefit for the scientific community, too. If we can persuade the general population to accept the idea that science is not only good but indispensable, that it is a manifestation of national pride to have a great number of scientists and institutions doing research, the social status of our activity, as well as our own status, will tend to rise, and we will have more influence on political decisions. In sum, the change in mentality that took place in the universities 30 years ago must now be extended to the rest of the nation.

Acknowledgment

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News from IUPAC

Biodegradation of Chemical Warfare Agents

This article by Dr. Walter Mulbry (wmulbry@asrr.arsusda.gov), a microbiologist at the Soil Microbial Systems Laboratory, USDA/ARS, Beltsville, MD, USA, and Evgenia Rainina, a microbiologist at the Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX, USA, constitutes the report of a Working Party of the IUPAC Ad Hoc Committee on Chemical Weapons Destruction Technologies (chaired by Professor Joseph F. Bunnett, bunnett@chemistry.ucsc.edu). It was originally published in *ASM News*, Vol. 64, No. 6, 1998, pp. 325–331, and it is reprinted here with the kind permission of the American Society for Microbiology. We thank the authors and Patrick Lacey (patlacey@asmusa.org), Production Editor at *ASM News*, for making it possible for us to publish this report.

The Chemical Weapons Convention (CWC), which the U.S. government ratified in 1997, sets an explicit timetable for signatory countries to destroy their chemical weapon stockpiles and related facilities. Although destroying such agents and remediating contaminated sites by conventional means promise to be enormously costly processes, recent research indicates that some of these compounds can be biologically degraded, suggesting an environmentally and economically feasible alternative strategy for addressing these challenges.

The magnitude of the U.S. chemical weapon (CW) stockpile—over 30 000 tons of various blister and nerve agents—presents a formidable challenge to those charged with disposing of it. About 60% of the agents are stored in steel 1-ton bulk containers; the remaining 40% are loaded in several million explosively configured rockets, land mines, mortars, bombs, artillery projectiles, and spray tanks (Figure 1). The "youngest" CW munitions and storage tanks are 30 years old, and the oldest are 53. In addition, U.S. Army officials have identified 215 sites in 33 states that are likely to contain

Figure 1. Artillery shells containing the nerve gas sarin (GB) inside a storage bunker at the Umatilla Chemical Depot in Oregon. (Photo courtesy of Donna Fuzi, Department of the Army)

buried chemical weapons or to be contaminated with chemical agents.

The financial resources needed for these tasks are equally formidable. Since 1985, the U.S. Army has spent USD 3.2 billion on its programs for destroying the U.S. CW stockpile and on planning for the treatment of material at nonstockpile sites (about 4% of these funds were used for research and development). The current cost estimate for the stockpile disposal program, USD 12.4 billion, has increased sevenfold since 1985 and is likely to increase further. In addition, U.S. Army officials estimate that at least another USD 16.6 billion will be needed over the next 40 years to treat buried material at nonstockpile sites.

Although few of the other CWC signatory nations have major CW stockpiles to deal with, several of the independent republics of the former Soviet Union, whose financial resources are already strapped, face cleanup tasks comparable to that of the United States. Other known CW concentrations include Japanese CW munitions abandoned in China in 1945 and an estimated 100 000 tons of German CW munitions that were dumped into the Baltic Sea at the end of World War II.

Newer CW Biodegradation Research Efforts Show Progress

For more than 50 years, the U.S. military and its counterparts in other countries have disposed of obsolete and surplus chemical weapons by a variety of means that are no longer acceptable. For instance, prior to 1969, the U.S. Army disposed of chemical weapons by open-pit burning, evaporative "atmospheric dilution," burial, and placement of munitions in concrete coffins for ocean dumping. In the 1970s, the U.S. Army began using alkaline hydrolysis to deactivate mustard agents known as H and HD (Figure 2).

Because of problems with GB alkaline hydrolysis, U.S. Army officials in 1982 adopted incineration for destroying all classes of chemical weapons. Subsequently, a full-scale CW incinerator facility was built in the Marshall Islands on Johnson Atoll and, more recently, a similar incinerator was built and brought into operation in Utah. Strong and effective opposition to incineration has, however, stalled U.S. Army plans to operate incinerators at seven other U.S. CW stockpiles. Similarly, strong opposition has stalled CW incineration projects in the independent republics of the former Soviet Union.

Historically, chemists and engineers who were involved in developing CW agents also were the specialists who studied how to dispose of these agents. However, in the late 1980s, the U.S. Army began a small intramural program on biodegradation at their Edgewood Research, Development and Engineering Center (ERDEC) in Aberdeen, MD. From 1991–1996, this program was broadened to support extramural research at several universities, including Texas A&M University, Rutgers University, and the University of Washington. In Russia, civilian research groups at Moscow State University, the Pushchino Biological Research Center, and several other government institutes have also been working on disposal strategies for agents in their stockpile.

These biodegradative research efforts are showing progress, particularly with efforts to dispose safely of both nerve and blister agents. Indeed, faced with strong political pressure to avoid incinerating such agents, U.S. Army officials recently changed CW destruction plans at one stockpile site, formally assigning biodegradation a role in treating HD stored in bulk containers.

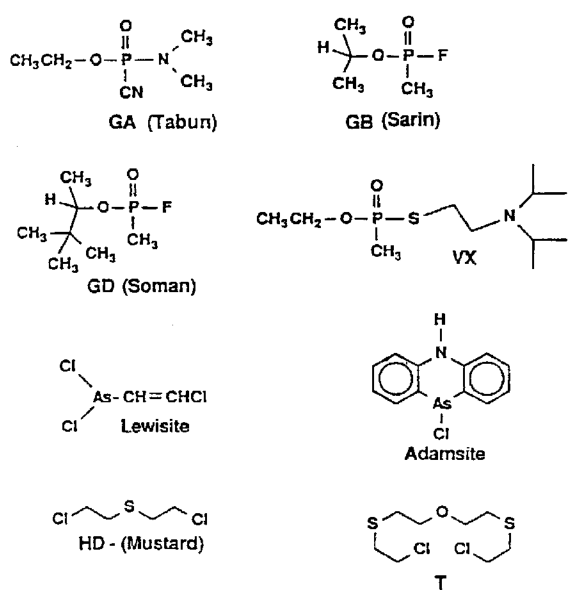

Figure 2. Chemical structures of the primary chemical warfare agents present in the U.S. and Russian stockpiles.

Table 1. Bacterial strains with hydrolytic activities against CW nerve agents

		Hydrolytic activity [kcat (sec^{-1})] against:				
Source Organism	**Enzyme**	**GA**	**GB**	**GD**	**DFP**[a]	**VX**
Pseudomonas diminuta	OPH	ND[b]	56	5	465	0.3
Alteromonas sp. JD6.5	OPAA-2	94	611	3 145	1 650	0
Alteromonas haloplanktis	ND[b]	111	257	1 389	575	0
Alteromonas undina	ND[c]	300	376	2 496	1 239	0

[a] DFP, diisopropyl fluorophosphate. A less toxic analog of the organophosphonate agents.
[b] ND, not determined.
[c] The responsible hydrolytic enzyme in this strain has not been purified.

Several Approaches to Biodegrading Nerve Agents

The organophosphorus (OP) "nerve gases" include VX, GA (tabun), GB (sarin), and GD (soman), all of which are liquid with varied volatility at room temperature (Figure 2). The blistering agents are oily liquids at room temperature and include the "mustard" agents H, HD (agent H that has been purified by distillation), and HT (a 60/40 mixture of HD and agent T), as well as the organo-arsenical agent lewisite (L). Adamsite (DA), a second organo-arsenical agent, is a severe respiratory irritant. Mixtures of the mustard and arsenical agents were also produced, such as agent MLM (mustard–lewisite mixture, a 37/63 mixture of agents HD and L).

In the 1980s, investigators began to search for microorganisms that could metabolize CW agents or structurally related compounds. In the search for microorganisms to act on nerve agents, researchers focused on microbial isolates capable of hydrolyzing OP insecticides. Among a range of microbially derived OP insecticide hydrolyzing enzymes, only the enzyme OPH from *Pseudomonas diminuta* is at all active in degrading nerve agents (Table 1).

Recently, James Wild and his colleagues at Texas A&M University modified the cloned gene for OPH, changing the enzyme's catalytic specificity and increasing its ability to degrade two organophosphonate nerve agents. Thus, following site-directed mutagenesis, cells displayed a 4-fold increase in activity against VX and a 40-fold increase in activity against soman.

Collaborating researchers in other laboratories at Texas A&M University, the U.S. Army ERDEC, the University of Wisconsin, and the University of Pittsburgh are helping to define the catalytic and structural capabilities of the native and modified forms of OPH. For example, in 1994, Hazel Holden and her colleagues at the University of Wisconsin determined a crystal structure for this protein, which can be downloaded from the Brookhaven Protein Data Base on the World Wide Web at http://pdb.pdb.bnl.gov/ (PDB code 1PTA).

Other Microorganisms with CW Hydrolytic Enzymes Identified

In 1989, reasoning that microbial enzymes used in decontamination may need to be resistant to high salt concentrations, Joe DeFrank at the U.S. Army's ERDEC screened isolates from a hypersaline spring in Utah for CW agent hydrolytic activities. Among those isolates, he and his colleagues found several related *Alteromonas* isolates that efficiently hydrolyze nerve agents such as GB, GD, and GF (Table 1).

Subsequently, DeFrank and his colleagues Steve Harvey and Tu-chen Cheng at ERDEC characterized two related enzymes from these *Alteromonas* strains and isolated the gene (*opaA*) that encodes one of these enzymes (termed OPAA-2). The DNA sequence of *opaA* shares a 28% amino acid homology to *Escherichia coli* aminopeptidase P and human prolidase. Moreover, OPAA-2 is highly active in hydrolyzing two dipeptide substrates of prolidase, namely Leu-Pro and Ala-Pro, but is not active against tripeptide substrates of aminopeptidase P or the dipeptides Pro-Leu or Pro-Gly. Such evidence suggests that OPAA-2 is a prolidase having a role in peptide metabolism.

Although no further screening efforts have been undertaken, a more comprehensive screening of microbial dipeptidase activities from different sources may yield entirely new enzymes with higher activities or activities toward other nerve agents such as GA and VX.

Strategies for Degrading Bulk Agents

The Chemical Weapons Convention specifies not only that CW agents be destroyed irreversibly but also that particular by-products be destroyed to ensure that they cannot be reused for military purposes. The by-products also need to be rendered safe for discharge into open environments. Hence, the members of several research groups are studying whether biodegradation will serve to destroy the by-products remaining following either chemical or enzymatic hydrolysis of CW agents. This research has yielded promising results for treating both nerve and mustard agents.

For example, Robin Autenrieth and her collaborators from Texas A&M University and the U.S. Army's ERDEC are studying the steps required to generate an environmentally acceptable waste stream following hydrolysis of sarin. She proposes hydrolyzing sarin with an excess of sodium hydroxide, a process that yields a solution containing sodium isopropyl methylphosphonate

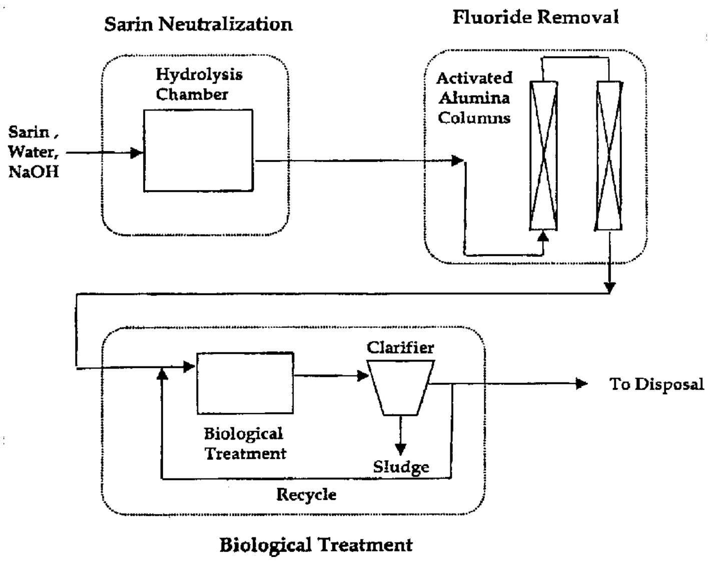

Figure 3. Schematic of a proposed strategy for the treatment of sarin from bulk containers. In this process, sarin is chemically hydrolyzed using excess sodium hydroxide, fluoride is removed by absorption to alumina columns, and the resulting effluent is treated in bioreactors. (Figure courtesy of Robin Autenrieth, Texas A&M University)

(IMPA) and sodium fluoride, both of which must be processed before discharge (Figure 3).

Although attempts to use IMPA as a carbon source to support microbial growth were not successful, IMPA is metabolized as a sole source of phosphorus in bioreactors. In batch experiments where sarin hydrolysate is supplied to microorganisms growing in bioreactors, IMPA levels decrease from 85 mg/liter to their detection limit of 1 mg/liter within 60–75 hours, depending on the microbial consortia in use. However, the consortia require a six-week acclimation period, are inhibited by high levels of the sarin hydrolysate or other sources of phosphate, and require significant amounts of carbon and nitrogen to achieve the required C:N:P ratio of 100:12:1.

The structurally similar nerve agent soman can be treated by comparable hydrolytic and biodegradation steps. However, to reduce the acclimation periods and to raise growth rates when suspended cultures are supplied with soman hydrolysate, Evgenia Rainina at Texas A&M University tested whether immobilized microbial cells metabolize propylmethylphosphonic acid (PMPA) (the primary product of soman hydrolysis) as a sole phosphorus source.

For such experiments, a PMPA-degrading strain from the ERDEC collection is first grown to a high density in rich medium, immobilized within a poly(vinyl) alcohol cryogel, and incubated in phosphate-limited media for 24 hours to reduce endogenous phosphorus pools. In successive batch experiments using immobilized cells, PMPA levels decrease from 164 mg/liter to the detection limit of 1 mg/liter within 60 hours. These results demonstrate the potential utility of the cell immobilization technique for maintaining high enzymatic activities.

Applied separately, water or hydrogen peroxide can effectively hydrolyze another organophosphonate agent, VX, providing a promising first step along the path to degrading this compound. Whether water or hydrogen peroxide is used, ethylmethylphosphonic acid (EMPA) is a by-product that needs to be further degraded. It, too, can serve as a sole phosphorus source for natural microbial consortia in bioreactors.

However, the use of VX, soman, or sarin hydrolysates as phosphorus sources requires the addition of excess nitrogen and carbon, and is rate-limiting. Introducing cells containing constitutive C-P lyase activities into these consortia might accelerate the overall degradative process and thereby reduce the need for adding large amounts of nitrogen and carbon.

Biodegrading the Blistering Agents HD and HT

The poorly soluble blistering agent HD reacts with microbial proteins and is therefore highly toxic to microbial cells, making it a poor candidate for direct biodegradation. Alkaline hydrolysis of HD yields significant amounts of polymerized by-products that also are difficult to biodegrade, according to Yu-Chu Yang and colleagues at the U.S. Army's ERDEC. However, Steve Harvey and his colleagues at ERDEC recently determined that water at 90 °C effectively hydrolyzes HD, yielding 90–95% thiodiglycol (TDG), which is nontoxic and miscible in water.

Because the CWC mandates destruction of TDG, Harvey has been testing an aerobic sequencing batch reactor for its ability to biodegrade hydrolyzed HD products. Using activated municipal sludge as an inoculum and HD hydrolysate as a sole carbon source, this batch process yields a nontoxic effluent. Meanwhile, a cryoimmobilized culture of *Alcaligenes xylosoxidans* from ERDEC metabolizes 150 mM TDG within 24 hours and retains 100% of its initial activity after 4 months of continuous use, according to Evgenia Rainina.

Although the two-step strategy of hydrolysis followed by biodegradation readily applies to bulk containers of HD (purified mustard) and HT, its applicability to stockpiled U.S. munitions containing HD or agent H (unpurified mustard) is less certain. The stockpiles of other nations include the less expensive agent H rather than agent HD. Depending on how those materials were manufactured, agent H typically contains about 30% impurities and may also contain an array of thickeners and other additives. In many cases, during the decades of storage these materials have reacted with one another and with metal casing materials, forming solid or semisolid polymers that are recalcitrant to degradation.

Strategies for Degrading Organo-arsenical Blistering Agents

Although substantial progress has been made toward biodegrading other CW agents, treating the arsenic-containing agents such as adamsite, lewisite, and mustard–lewisite mixtures is problematic because arsenic is toxic. To overcome this problem, Alexander Boronin and his colleagues at the Institute of Biochemistry and Physiology of Microorganisms in Pushchino, Russia developed a three-stage, laboratory-scale process for destroying arsenic-containing CW agents.

For example, their process for treating lewisite entails initial hydrolysis to form 2-chlorovinyl arsine oxide (CAO). Because the remaining high arsenic inhibits further biodegradation, they treat the mixture by electrolysis and electrocoagulation (EC), yielding formate, acetate, and arsenous and arsenic acids; subsequently, during the EC step, arsenic precipitates from solution, reducing its concentration by four orders of magnitude. The remaining organic acids are mineralized in a fluidized bed reactor using a natural consortium of microorganisms immobilized on activated carbon. A similar approach is also effective in destroying mustard–lewisite mixture (MLM) and adamsite, according to Boronin.

Destroying the arsenic-containing CW agents in the Russian stockpile will generate thousands of tons of arsenic. Although this material might prove useful in the microelectronics, optics, and solar power industries, safe storage facilities are needed. Victor Petrov and coworkers at the Russian Institute of Applied Mechanics suggest that converting free arsenic into arsenic sulfide provides a means for safely storing this bulk material.

Unanswered CW Degradation Questions Require Further Research

Despite the progress in developing procedures for destroying CW agents, significant gaps in our knowledge of these compounds limit development of alternative technologies and slow progress on destroying them, according to Joseph Bunnett, an organic chemist at the University of California at Santa Cruz, who has served on a variety of international scientific panels examining CW agent destruction. For example, in 1982, officials in the U.S. Army identified incineration as the best technology to use for this purpose, he points out. Yet, 16 years later, complete reliance on this incineration-based "chemical demilitarization" program has resulted in little destruction of agents and remains stymied because of strong political opposition to incineration.

Citizen opposition to incineration stems in part from a widely held belief that small amounts of intact chemical warfare (CW) agents will be released to accumulate as a "toxic load" in the environment. However, although traces of CW agents released into the atmosphere are likely to be rapidly destroyed by photolysis, hydrolysis, and oxidation, little if any research has been done to document the atmospheric half-lives of most CW agents, according to Bunnett.

Several major CW munitions stockpiles need to be destroyed: the Russian stockpile, most of the U.S. stockpile, the Japanese CW munitions abandoned in China in 1945, and the German munitions that were dumped into the Baltic Sea. Although biodegradation could play a role in destroying these chemical agents, both fundamental and practical questions need to be addressed before even successful laboratory-scale degradative processes can be taken into field-scale use. Yet, unless the pace of research accelerates to meet the deadlines specified by the Chemical Weapons Convention, current gaps in knowledge will sharply limit any use of this promising technology.

Two critical questions need to be addressed soon. One is how well laboratory-scale procedures will perform under field conditions, particularly in settings where partly degraded materials contain a mix of chemical contaminants, as well as an ill-defined range of indigenous microorganisms. Researchers, who typically have tested their processes only on highly purified starting materials, will need access to CW agents from munition stockpiles to see if biodegradative processes will work on complex mixtures.

A second, more fundamental question to address is whether new microbial consortia can be selected or developed that are better suited for carrying out biodegradation of CW agents. One research approach will be to conduct a comprehensive screening of organisms from anaerobic sites or from highly acidic, hypersaline, or metal-contaminated aerobic environments.

In conducting this research, it is crucial that international collaborative efforts be continued and expanded. Although individual nations naturally have a domestic focus when deciding on national research priorities, chemical weapons are an international legacy, and their inappropriate disposal by any nation may have long-lasting consequences. By sharing ideas and resources, the international community stands the best chance of developing and implementing appropriate technology worldwide to prevent further contamination by these dangerous compounds.

Acknowledgments

The IUPAC Ad Hoc Committee on Chemical Weapons Destruction Technologies sponsored this review. We gratefully acknowledge NATO for funding Russian–American linkage grants and for funding the Advanced Research Workshop on Chemical and Biological Technologies for the Detection, Destruction, and Decontamination of Chemical Warfare Agents (12–15 May 1996, Russia). We thank Dr. Steve Harvey for his careful reading of the manuscript and Dr. Joe DeFrank for his suggestions and for calculating the values shown in Table 1.

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News and Notices from Other Societies and Unions

AOAC International Hires New Executive Director

AOAC's Board of Directors has selected E. James Bradford as AOAC International's new Executive Director. He assumed his responsibilities on 6 July 1999.

Dr. Bradford brings to AOAC International a combination of association management, academic, and chemistry experience. Dr. Bradford is former Director of Science, Mathematics, and Technology Education Programs at the American Association for the Advancement of Science (AAAS) in Washington, DC, USA. Prior to that, he was employed for nearly 15 years at the American Chemical Society (ACS) in Washington, DC, USA.

At AAAS, Dr. Bradford directed six science, mathematics, and technology education programs; an NSF-sponsored core competencies project; science books and films; and senior scientists and engineers.

At the ACS, he served as the administrator for Grants and Awards of the Membership Division in 1998 and as assistant director of the Membership Division from 1995 to 1997. Over the years, he was also the ACS special assistant to the executive director, program manager of National Chemistry Week, and manager of the Office of College Chemistry. He established National Chemistry Week as an annual event involving more than 1 000 volunteers reaching out to the public, and he has even appeared on Nickelodeon with Linda Ellerbee in an antismoking program for kids.

As the ACS administrator for Grants and Awards of the Membership Division, he directed a staff of 20 and a budget of USD 20 million to administer 62 national awards in the chemical sciences and a USD 16.3 mil-