

22 April 2010

This supplement has been prepared to present scientific and technical news items that may be of more interest to technical personnel at RDT&E activities and the labs, or the medics rather than the broader readership of the basic CB Daily. Due to the nature of the material, the articles, if available online, are usually only available through subscription services thus making specific links generally unavailable. Thus, usually only the bibliographic citation is available for use by an activity's technical library.

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Chem-Bio News – S&T Edition

1. HISTORICAL DISTRIBUTION AND MOLECULAR DIVERSITY OF BACILLUS

ANTHRACIS, KAZAKHSTAN: *“However, in a recent study B. anthracis DNA from persons affected by the Sverdlovsk accident was assigned to the A.Br.008/009 SNP subgroup (19). Our data and the report that the Sverdlovsk strain was initially isolated in the 1950s in Kirov, Russia (19), underscores the need to genotype additional samples in northern Kazakhstan oblasts and Russia to measure the northern range of this apparently highly successful lineage.....The project research was made possible by support provided by the US Defense Threat Reduction Agency under project KZ-1 and was administered by US Civilian Research and Development Foundation.”*

2. LABORATORY CAPACITY BUILDING IN ASIA FOR INFECTIOUS DISEASE

RESEARCH: EXPERIENCES FROM THE SOUTH EAST ASIA INFECTIOUS DISEASE

CLINICAL RESEARCH NETWORK (SEACRN): *“Each hospital is encouraged to use the MDL[molecular diagnostic laboratory] for purposes other than SEACRN-related research activities, such as HIV, hepatitis, dengue, meningitis, and encephalitis testing by molecular techniques. The philosophy is that it is important and highly beneficial to increase expertise in detecting infectious agents by molecular diagnostics and so stimulate the implementation of other relevant molecular diagnostic tests.”*

3. PHOSPHORUS FRAGMENTS TRAPPED: *“Researchers in the US and Germany have shown how a rare and highly reactive form of phosphorus can be captured and crystallised, making it stable even at room temperature.”*

4. RESEARCHERS IDENTIFY KEY MOLECULAR STEP TO FENDING OFF VIRUSES:

“UT Southwestern Medical Center researchers have determined how a protein that normally latches onto molecules inside cells and marks them for destruction also gives life to the body's immune response against viruses.”

5. PHYSICS STRATEGY TESTED AS SOLUTION FOR ANTIBIOTIC RESISTANCE: *“A Virginia Tech biologist proposes to use a physics strategy called resonant activation to nudge dormant bacteria cells into a stage where they will be sensitive to antibiotics.”*

6. DELIVERING DRUGS IN GELS: *“Scientists have designed and tested biocompatible*

material that forms a gel in vivo and is capable of slowly releasing protein drugs."

7. AUTOMATING CELL CULTURE USING DIGITAL MICROFLUIDICS: "Although applications involving mammalian cells have been extremely popular in the microfluidics community, until now they could only be used for single subcultures -once cells had been seeded and grown, both the device and cells had to be discarded."

8. ALL-WEATHER HYDROGEN PEROXIDE-BASED DECONTAMINATION OF CBRN CONTAMINANTS: "A hydrogen peroxide-based decontaminant, Decon Green, is efficacious for the decontamination of chemical agents VX (S-2-(diisopropylamino)ethyl O-ethyl methylphosphonothioate), GD (Soman, pinacolyl methylphosphonofluoridate), and HD (mustard, bis(2-chloroethyl) sulfide); the biological agent anthrax (*Bacillus anthracis*); and radiological isotopes Cs-137 and Co-60; thus demonstrating the ability of this decontamination approach to ameliorate the aftermath of all three types of weapons of mass destruction (WMD)."

9. SIMULTANEOUS QUANTIFICATION OF FIVE BACTERIAL AND PLANT TOXINS FROM COMPLEX MATRICES USING A MULTIPLEXED FLUORESCENT MAGNETIC SUSPENSION ASSAY: "The antibodies were used along with antibodies against SEB and abrin to establish a highly sensitive magnetic and fluorescent multiplex bead array with excellent sensitivities between 2 ng/L and 546 ng/L from a minimal sample volume of 50 microL."

10. DECONTAMINATION OF CHEMICAL AND BIOLOGICAL WARFARE AGENTS WITH A SINGLE MULTI-FUNCTIONAL MATERIAL: "The HE 4-PAM that was released from the polymer reactivated DFP-inhibited AChE at a similar rate to the oxime antidote 4-PAM."

CB Daily Report

Chem-Bio News

HISTORICAL DISTRIBUTION AND MOLECULAR DIVERSITY OF BACILLUS ANTHRACIS, KAZAKHSTAN

By Alim M. Aikembayev, Larissa Lukhnova, Gulnara Temiraliyeva, Tatyana Meka-Mechenko, Yerlan Pazylov, Sarkis Zakaryan, Georgiy Denissov, W. Ryan Easterday, Matthew N. Van Ert, Paul Keim, Stephen C. Francesconi, Jason K. Blackburn, Martin Hugh-Jones, and Ted Hadfield
Emerging Infectious Diseases
May 2010

"Abstract

To map the distribution of anthrax outbreaks and strain subtypes in Kazakhstan during 1937–2005, we combined geographic information system technology and genetic analysis by using archived cultures and data. Biochemical and genetic tests confirmed the identity of 93 archived cultures in the Kazakhstan National Culture Collection as *Bacillus anthracis*. Multilocus variable number tandem repeat analysis genotyping identified 12 genotypes. Cluster analysis comparing these genotypes with previously published genotypes indicated

that most (n = 78) isolates belonged to the previously described A1.a genetic cluster, 6 isolates belonged to the A3.b cluster, and 2 belonged to the A4 cluster. Two genotypes in the collection appeared to represent novel genetic sublineages; 1 of these isolates was from Krygystan. Our data provide a description of the historical, geographic, and genetic diversity of *B. anthracis* in this Central Asian region.”

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“From a genetic perspective, *B. anthracis* in Kazakhstan was dominated by isolates clustering in the MLVA A1.a group, which is consistent with reports of the A1.a group being widely distributed globally (4,5,6). The widespread occurrence and apparent ecologic establishment of these VNTR genotypes in Kazakhstan supports the hypothesis that the A1.a group represents a very fit strain complex (6). Of the 8 A1.a genotypes in Kazakhstan, 5 were novel (Gkz-2, -3, -4, -5, and -8) and exhibited a previously undescribed pX01 allele (Gkz-5), which is not unexpected considering that this region has been underrepresented in prior MLVA-8 *B. anthracis* studies (4–8).

SNP typing of representative isolates from the A1.a Kazakh MLVA genotypes assigns these isolates to the A.Br.008/009 SNP lineage, which is widely distributed throughout Europe and has been reported in western China (10,18). Notably, the SNP data differentiate the Kazakh genotypes from the related North American genotypes, which are not effectively differentiated by MLVA alone. Since the representative Kazakh isolates in this SNP study were cultured from outbreaks spanning a 50-year period (1952–2002), our data not only expand the understanding of the geographic range of this Eurasian lineage (A.Br.008/009) but also provide insights into its historical incidence and persistence in the country. Because of sampling limitations, the extent to which this dominant lineage is represented in the northern sections of Kazakhstan, and further into Russia, is unknown. However, in a recent study *B. anthracis* DNA from persons affected by the Sverdlovsk accident was assigned to the A.Br.008/009 SNP subgroup (19). Our data and the report that the Sverdlovsk strain was initially isolated in the 1950s in Kirov, Russia (19), underscores the need to genotype additional samples in northern Kazakhstan oblasts and Russia to measure the northern range of this apparently highly successful lineage.

The assignment of Kazakh isolates to the A3.b and A4 MLVA clades and the A.Br.Ames and A.Br.Vollum SNP groups is not surprising considering these MLVA and SNP types are also found in Middle Eastern countries, such as Pakistan and China (10). As first reported by Van Ert et al. (10), and later detailed by Simonson et al. (18), the A.Br.001/002 is common in China, whereas the closely related A.Br.Ames SNP lineage is more restricted geographically. The finding that the Kazakh isolates from the eastern border were assigned to A.Br.Ames SNP group is notable considering that the A.Br.Ames isolates that can be geolocated are found exclusively in Inner Mongolia. These genotypic similarities may reflect historical trade and nomadic routes linking those regions.

The absence of B lineage genotypes in Kazakhstan, as indicated by both MLVA and SNP data, is consistent with the lack of these genotypes in China, including the western province of Xinjiang (10,18), and supports the hypothesis that these lineages are restricted to narrow environmental conditions and, therefore, are more restricted in their global distribution (9). On a more local level, our MLVA data permit strain-level analysis of samples isolated during

outbreaks. In several instances we were able to link strains collected from human anthrax patients to the infection source. For example, we identified the same strain in 10 cultures collected from an outbreak in western Kazakhstan that occurred from July–August 2005. The samples included cultures isolated from livestock, contaminated meat, human victims, and contaminated soil. The MLVA data linked the cultures and provided a mechanism for retrospective epidemiologic trace-back.”

The full article can be found at: <http://www.cdc.gov/eid/content/16/5/789.htm>

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LABORATORY CAPACITY BUILDING IN ASIA FOR INFECTIOUS DISEASE RESEARCH: EXPERIENCES FROM THE SOUTH EAST ASIA INFECTIOUS DISEASE CLINICAL RESEARCH NETWORK (SEAICRN)

By Heiman F. L. Wertheim, Pilaipan Puthavathana, Ngoc My Nghiem, H. Rogier van Doorn, Trung Vu Nguyen, Hung Viet Pham, Decy Subekti, Syahrial Harun, Suhud Malik, Janet Robinson, Motiur Rahman, Walter Taylor, Niklas Lindegardh, Steve Wignall, Jeremy J. Farrar, Menno D. de Jong
PLoS Medicine
April 06, 2010

“Creating Laboratory Capacity

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“Each hospital is encouraged to use the MDL[molecular diagnostic laboratory] for purposes other than SEAICRN-related research activities, such as HIV, hepatitis, dengue, meningitis, and encephalitis testing by molecular techniques. The philosophy is that it is important and highly beneficial to increase expertise in detecting infectious agents by molecular diagnostics and so stimulate the implementation of other relevant molecular diagnostic tests. For example, after implementation of a *Streptococcus suis*–specific molecular test in the MDL of the National Hospital of Tropical Diseases, Vietnam, it was found that *S. suis* is a common cause of bacterial meningitis in adults in Hanoi [6].”

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“Laboratory Quality Enhancement Program

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“Laboratory management staff in each hospital were trained and encouraged to implement a quality management system. This included development and implementation of quality and technical manuals, standard operating procedures (SOPs), and a document control system. To help to improve laboratory quality it was necessary to appoint a senior staff member as a Quality Officer to oversee all aspects of laboratory quality and the quality enhancement program. Furthermore, each laboratory was supported with necessary instruments, and staff were encouraged to establish and monitor an equipment maintenance and calibration program. All staff from each laboratory were trained on standards developed by the technical committee of the International Organisation for Standardisation (ISO15189:2003

and ISO15190:2003, <http://www.iso.org/>) [10].

During the clinical trials it was noticed that occasionally reported biochemistry results were implausible. In response, a continuous improvement system was implemented to ensure that laboratory test results are reviewed by a qualified person to identify performance issues. In addition, advice was given on proper use of internal controls, how to monitor and investigate the possible cause(s) of controls not meeting acceptance criteria, and corrective and preventive action measures when performance issues are identified. Laboratories were encouraged to complement the capacity of each other by establishing a specimen testing referral linkage among them."

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"The ultimate goal of the program was to improve the quality of laboratory services, achieve compliance with GCLP [Good Clinical Laboratory Practice] standards, gain ISO 15189 accreditation, and facilitate better clinical care and collaborative research. Setting such goals makes the process more real and motivates laboratory staff to participate. Several laboratories in Thailand had already started working toward accreditation independently of the SEAICRN, and these efforts helped facilitate the SEAICRN program at those sites. Two laboratories in Thailand received "The Association of Medical Technologists of Thailand" accreditation, and one laboratory in Thailand and two in Vietnam have gained ISO15189 accreditation. In addition, another laboratory in Thailand and one in Indonesia are nearly ready for accreditation inspection."

The full article can be found at: <http://www.plosmedicine.org/article/info%3Adoi%2F10.1371%2Fjournal.pmed.1000231>

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PHOSPHORUS FRAGMENTS TRAPPED

By Simon Hadlington

Chemistry World

April 11, 2010

"Researchers in the US and Germany have shown how a rare and highly reactive form of phosphorus can be captured and crystallised, making it stable even at room temperature.

A team led by Guy Bertrand from the University of California Riverside, with colleagues from Philipps-Universität Marburg, used carbenes - compounds in which a carbon atom has a pair of 'unused' electrons - to trap the rare diphosphorus molecule P₂. The team selectively oxidised the complexes to remove either one electron or two, giving rise to a P₂ cation possessing an unpaired electron - a radical cation - or a P₂²⁺ dication.

Phosphorus atoms are usually not happy in pairs - they prefer to combine with more phosphorus atoms to form species such as P₄. But in 2008, Gregory Robinson's group at the University of Athens in the Georgia, US, showed how two molecules of N-heterocyclic carbene, NHC, could clamp P₂ and stabilise it by donating electrons to it from the electron-

rich carbene centre.

Bertrand's team has now shown that P2 can be similarly stabilised by a pair of different carbene molecules, cyclic(alkyl)(amino) carbene, or CAAC. Furthermore, both the NHC complex and the CAAC complex can be oxidised. 'If you remove one electron from these complexes, they are left with an overall positive charge, and so become cationic,' says team member Gernot Frenking, who carried out the theoretical calculations in the study. 'However, an unpaired electron remains so the complex is at the same time a radical.'

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"Theoretical calculations showed that the electrons were being removed from the P2 centre of the complex but that the subsequent charge deficiency was being compensated for by an increased charge donation from the carbene, which continues to stabilise the P2."

The full article can be found at: <http://www.rsc.org/chemistryworld/News/2010/April/11041001.asp>

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RESEARCHERS IDENTIFY KEY MOLECULAR STEP TO FENDING OFF VIRUSES

Infection Control Today Magazine

April 21, 2010

"UT Southwestern Medical Center researchers have determined how a protein that normally latches onto molecules inside cells and marks them for destruction also gives life to the body's immune response against viruses.

The researchers discovered that a certain form of the "death" protein ubiquitin interacts with another protein, called RIG-I, but does not mark it for destruction. Instead, this form of ubiquitin binds to and activates RIG-I, which is known to trigger the body's immune system when a virus invades a cell.

Dr. Zhijian Chen, professor of molecular biology at UT Southwestern, is senior author of the study, which is available online and in the journal Cell.

Chen and his colleagues reconstituted key elements of the human innate immune system in laboratory test tubes and found ubiquitin forms a unique chain-like structure that associates with RIG-I before RIG-I can get to work fighting viruses. The innate immune system is the body's first generic response against invading pathogens.

"Activation of RIG-I is the first line of our immune defenses against viral infections," said Chen, an investigator for the Howard Hughes Medical Institute at UT Southwestern.

"Understanding how it comes to life is a key step in developing new approaches to antiviral therapies. Having this test-tube system could help us identify substances that enhance the body's antiviral immunity."

Chen said his team's experiments mark the first time innate immunity has been recapitulated in a test tube. The findings provide one of the missing pieces in the complex puzzle of how the body fights off infection, he added.

Chen is now focusing on how activated RIG-I interacts with another protein called MAVS, also essential for immune response."

The full article can be found at: <http://www.infectioncontroltoday.com/hotnews/fending-off-viruses.html>

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PHYSICS STRATEGY TESTED AS SOLUTION FOR ANTIBIOTIC RESISTANCE

Infection Control Today Magazine

April 21, 2010

"A Virginia Tech biologist proposes to use a physics strategy called resonant activation to nudge dormant bacteria cells into a stage where they will be sensitive to antibiotics.

In medicine, resonance means the sound the doctor hears when he or she thumps your chest. In physics, resonance is a periodic force or an oscillation whose frequency is close to that of a natural system's frequency. Sound waves are an example of a natural system that can be altered with resonant activation.

Jianhua Xing, an assistant professor of biological sciences at Virginia Tech who has studied more than a smattering of physics, was considering the problem of antibiotic resistance when he remembered a physics paper on resonant activation that he had read as a student.

One strategy bacterial colonies use to survive antibiotics is to create a few persister cells. Because these cells are dormant or grow very slowly, they can dodge an antibiotic attack that requires active cell wall growth to be effective. Persister cells convert to normally growing cells at a random and slow rate so that there are always a few that remain dormant until the antibiotics are gone. Extending antibiotic treatment can be a dangerous strategy because of severe side effects, such as liver damage.

Persister cells have multiple steady states, with fluctuations in the numbers of proteins as they transition to a normal cell. Xing viewed this fluctuation during synthesis and degradation of proteins as a potential target for resonant activation. Instead of a sound wave or electronic signal, the perturbing signal would be repetitive antibiotic treatment."

The full article can be found at: <http://www.infectioncontroltoday.com/hotnews/physics-solution-for-antibiotic-resistance.html>

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DELIVERING DRUGS IN GELS

By Harriet Brewerton

Highlights in Chemical Science

April 22, 2010

“Scientists have designed and tested biocompatible material that forms a gel in vivo and is capable of slowly releasing protein drugs.

Protein drugs are used to treat a wide range of illnesses but their therapeutic effects are limited by their unstable nature. The drugs are easily denatured in the body and administering them intravenously or via oral pills often causes plasma concentrations that are either too low to have a therapeutic effect or too high and cause toxicity. Delivery of protein drugs over a sustained period of time to a localised area would greatly enhance their therapeutic benefits.

Moon Suk Kim of Ajou University, Suwon, South Korea, and his team, designed and the gel using biocompatible components, sodium carboxymethylcellulose and polyethyleneimine, that electrostatically link to form a gel on exposure to physiological conditions. The gel was found to be porous enough to release the test protein drug, albumen, in a slow and controlled manner for up to 15 days, while preventing biological materials from entering.”

The full article can be found at: http://www.rsc.org/Publishing/ChemScience/Volume/2010/05/gel_drug_delivery.asp

The original article can be found at: <http://www.rsc.org/Publishing/Journals/JM/article.asp?doi=b922614a>

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AUTOMATING CELL CULTURE USING DIGITAL MICROFLUIDICS

By Jennifer Newton

Highlights in Chemical Science

April 21, 2010

“The first lab-on-a-chip platform for complete mammalian cell culture has been developed by scientists in Canada. This could be used to create an automated device to continually grow generations of cells for use in cell biology and tissue engineering.

Although applications involving mammalian cells have been extremely popular in the microfluidics community, until now they could only be used for single subcultures -once cells had been seeded and grown, both the device and cells had to be discarded. Aaron Wheeler at the University of Toronto has used digital microfluidics to develop a device where cells can continuously be seeded, grown and then moved onto fresh culture sites within the same platform for analysis.

Digital microfluidics is a technique where an array of electrodes on a surface is used to exert

electromechanical forces on a droplet, such that you can drag or dance droplets around on a surface as well as dispense them from reservoirs and split or merge them to effect chemical reactions. Wheeler had the innovative idea to translate the technique for use in cell culturing.

In Wheeler's device, traditional flasks used in conventional cell culture are replaced by regions of patterned electrodes, called adhesion pads, where cells are seeded and grown in droplets. New growth media is easily delivered to cells by adding new source droplets that simultaneously sweeps away the old media. Once grown selected cells are moved to new adhesion pads by treating with trypsin, creating generation after generation of cells."

The full article can be found at: http://www.rsc.org/Publishing/ChemScience/Volume/2010/05/automating_cell_culture.asp

The original article can be found at: <http://www.rsc.org/Publishing/Journals/LC/article.asp?doi=c002147d>

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ALL-WEATHER HYDROGEN PEROXIDE-BASED DECONTAMINATION OF CBRN CONTAMINANTS

Journal of Engineering
April 28, 2010

"A hydrogen peroxide-based decontaminant, Decon Green, is efficacious for the decontamination of chemical agents VX (S-2-(diisopropylamino)ethyl O-ethyl methylphosphonothioate), GD (Soman, pinacolyl methylphosphonofluoridate), and HD (mustard, bis(2-chloroethyl) sulfide); the biological agent anthrax (*Bacillus anthracis*); and radiological isotopes Cs-137 and Co-60; thus demonstrating the ability of this decontamination approach to ameliorate the aftermath of all three types of weapons of mass destruction (WMD)."

"Reaction mechanisms afforded for the chemical agents are discussed as are rationales for the enhanced removal efficacy of recalcitrant Co-60 on certain surfaces. Decontaminants of this nature can be deployed, and are effective, at very low temperatures (-32 degrees C), as shown for studies done with VX and HD simulants, without the need for external heat sources."

"Finally, the efficacy of a lower-logistics, dry decontaminant powder concentrate (utilizing the solid active-oxygen compounds peracetyl borate and Peroxydone) which can be reconstituted with water in the field prior to use, is presented."

The full article can be found at: (G.W. Wagner, et. al., "All-Weather Hydrogen Peroxide-Based Decontamination of CBRN Contaminants". *Chemistry Research*, 2010; 49(7): 3099-3105). Link not available.

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SIMULTANEOUS QUANTIFICATION OF FIVE BACTERIAL AND PLANT TOXINS FROM COMPLEX MATRICES USING A MULTIPLEXED FLUORESCENT MAGNETIC SUSPENSION ASSAY

Health & Medicine Week

April 26, 2010

"Proteotoxins such as ricin, abrin, botulinum neurotoxins type A and B (BoNT/A, BoNT/B) and staphylococcal enterotoxin B (SEB) are regarded as potential biological warfare agents which could be used for bioterrorism attacks on the food chain. In this study we used a novel immunisation strategy to generate high-affinity monoclonal and polyclonal antibodies against native ricin, BoNT/A, and BoNT/B."

"The antibodies were used along with antibodies against SEB and abrin to establish a highly sensitive magnetic and fluorescent multiplex bead array with excellent sensitivities between 2 ng/L and 546 ng/L from a minimal sample volume of 50 microL. The assay was validated using 20 different related analytes and the assay precision was determined. Advancing the existing bead array technology, the novel magnetic and fluorescent microbeads proved amenable to enrichment procedures, by further increasing sensitivity to 0.3-85 ng/L, starting from a sample volume of 500 microL. Furthermore, the method was successfully applied for the simultaneous identification of the target toxins spiked into complex food matrices like milk, baby food and yoghurt."

The full article can be found at: (D. Pauly, et. al., "Simultaneous quantification of five bacterial and plant toxins from complex matrices using a multiplexed fluorescent magnetic suspension assay". *Analyst*, 2009; 134(10):2028-39). Link not available.

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DECONTAMINATION OF CHEMICAL AND BIOLOGICAL WARFARE AGENTS WITH A SINGLE MULTI-FUNCTIONAL MATERIAL

Hematology Week

April 26, 2010

"We report the synthesis of new polymers based on a dimethylacrylamide-methacrylate (DMAA-MA) co-polymer backbone that support both chemical and biological agent decontamination. Polyurethanes containing the redox enzymes glucose oxidase and horseradish peroxidase can convert halide ions into active halogens and exert striking bactericidal activity against gram positive and gram negative bacteria."

"New materials combining those biopolymers with a family of N-alkyl 4-pyridinium aldoxime (4-PAM) halide-acrylate co-polymers offer both nucleophilic activity for the detoxification of organophosphorus nerve agents and internal sources of halide ions for generation of biocidal activity. Generation of free bromine and iodine was observed in the combined material resulting in bactericidal activity of the enzymatically formed free halogens that caused complete kill of *E. coli* (>6 log units reduction) within 1 h at 37 degrees C. Detoxification of

diisopropylfluorophosphate (DFP) by the polyDMAA MA-4-PAM iodide component was dose-dependent reaching 85% within 30 min. A subset of 4-PAM-halide co-polymers was designed to serve as a controlled release reservoir for N-hydroxyethyl 4-PAM (HE 4-PAM) molecules that reactivate nerve agent-inhibited acetylcholinesterase (AChE). Release rates for HE 4-PAM were consistent with hydrolysis of the HE 4-PAM from the polymer backbone."

"The HE 4-PAM that was released from the polymer reactivated DFP-inhibited AChE at a similar rate to the oxime antidote 4-PAM."

The full article can be found at: (G. Amitai, et. al., "Decontamination of chemical and biological warfare agents with a single multi-functional material". *Biomaterials*, 2010; 31 (15):4417-25). Link not available.

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