

**10th International Symposium on
Protection against**



**Chemical and
Biological
Warfare Agents**

Stockholm, Sweden

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ABSTRACTS

ERRATA

POSTER PRESENTATIONS

New posters:

Advances in Handheld, Field-Based Chemical Identification - Duane Sword

New Methods for Small Scale Synthesis of CWA - Richard Wärme

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Advances in Handheld, Field-Based Chemical Identification

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Short description:

- How are innovations in chemical identification changing field response tactics?
- Countering evolving threats from commonly available explosive materials (HMEs and TICs)
- Integrating robots and analytical instruments = faster, safer response
- Complementary technologies with Raman & FTIR

Long description:

Advances in portable instrumentation are significantly altering the tactics and procedures used for chemical identification in the field. Rather than removing a sample from the hazard zone, responders now rely on handheld instruments for rapid identification directly on-scene, saving time and increasing responder safety. Proven technologies like Raman and FTIR are gaining inroads as instruments meeting the ruggedness, analysis speed and true portability requirements are being deployed in theater across the world.

Standoff chemical identification has also long been a critical need for responders. As miniaturized, rugged instrumentation gets smarter, and robot technology continues to evolve, integrating sensors and robots now enables this long requested capability for responders in the field.

Raman spectroscopy is well-suited for field identification of liquids and powders encountered in weapons caches or clandestine laboratories. Raman enables responders to quickly identify potentially dangerous unknown substances through sealed containers, including commercial and homemade explosives and their precursors, CWAs, and toxic industrial chemicals.

FTIR spectroscopy, another proven analytical technique, has become more viable in the field due to greater ease of use, faster response time and truly handheld instruments. New technology minimizes sample preparation while advanced chemometrics eliminate user interpretation for consistent, accurate ID results.

This session will discuss the use of handheld instruments for field-based identification of chemical threats. Analytical performance will be presented along with case studies and user scenarios.

Dear Symposium Delegate,

We celebrate the 10th anniversary of our symposium, 1983-2010, where we meet every third year to discuss a variety of aspects on protection against chemical and biological warfare agents. The symposium, initially focused only on chemical warfare agents, has successively grown, and in 1995 biological issues were included. During these 27 years, remarkable changes have occurred in the world, both regarding the political situation and in science, and the term Weapons of mass destruction has been expanded to CBRN – chemical, biological, radioactive and nuclear agents.

The character of the BCW defence research has changed considerably from the first symposium in 1983, when most activities were performed within national defence programmes. The trend of the last decade has been a successively increased international cooperation to counteract the threat from biological and chemical warfare agents and CBRN terrorism, between individual states as well as within organisations such as the European Union and NATO. Much emphasis has been directed to improve the chemical and biological safety and security.

The main focus of protection measures has shifted from scenarios with military use in state-to-state conflicts to attacks by non-state actors. According to the current scenarios, a wider range of possible agents is of concern and new concepts of protection and crisis management are needed. As a result of the world-wide fear of possible terrorist attacks with chemical or biological agents, the funding for research and development for improved protection against such agents has been raised worldwide.

In the parallel sessions of the symposium various topics will be discussed, such as Emerging threats, CBRN crisis management, concepts for early warning, detection and identification technology, development of improved means of medical countermeasures, physical protection and decontamination, and also issues relating to non-proliferation and demilitarization.

We hope that you during these four days of the Tenth International Symposium on Protection against Chemical and Biological Warfare Agents will find much inspiration for your important work for counteractions to the threat imposed by biological and chemical agents.

Umeå in May 2010

Lena Norlander

Head of the Steering Committee of the Symposium

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TERRORISTS AND WEAPONS OF MASS DESTRUCTION

Albert Jongman, Dutch Ministry of Defense, Irislaan 253, Oegstgeest, The Netherlands

In response to the recent failed Times Square bombing American terrorism expert Bruce Hoffman argued that operations like this are meant as distraction in order to consume the attention of law enforcement and intelligence agencies in the hope that these distractions will allow more serious operations to slip by unnoticed. This begs the question whether we are to expect more serious operations and if so will they include CBRN-attacks. Will terrorist organisations make the leap to more technologically advanced operations? Several experts have indicated that there is no logical progression from truck bombs to nuclear bombs. So how does the risk equation look like? In the presentation some remarks will be made concerning the intentions and the capabilities of terrorist organisations, in particular jihadi organisations, when it comes to CBRN-attacks. Some recent qualitative and quantitative research findings will be presented on the basis of recent research in the US. After discussing some recent threat assessments several remarks will be made with respect to the scenarios formulated in 2008 by the Center for the Study of Weapons of Mass Destruction of the US National Defense University. Several actual developments will be presented that are making the outcome of one of the scenario's more likely. The presentation will be concluded with some remarks on policy measures and the Dutch approach concerning CBRN-E. Over the past years attempts have been made to install a layered defense which could reduce the risks to the extent that terrorist organisations may conclude that it would be a waste of effort to continue with their plans.

THE SECOND GENERATION BIOLOGICAL INTEGRATED RECONNAISSANCE DEFENCE (BIRD) SYSTEM – ENHANCING EUROPEAN CBRN DEFENCE CAPABILITY

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Biological warfare agents (BWA) bear unique features that differentiate them markedly from chemical and radiological toxic substances. European military forces must be equipped and trained to operate in the unique environment created by BWA and associated hazards within the framework of European Common Security and Defence Policy (CSDP) operations. To reach this goal, the capability to detect, identify and monitor (DIM) the presence of toxic or pathogenic biological agents in the environment is essential.

There is therefore a need for an Integrated Biological Defence System (BIRD) able to detect within seconds a biological hazardous concentration and subsequently as required identify the involved agents so as to ensure that appropriate physical protection and post-incident response measures can be taken in a timely manner, in order to mitigate their overall effects.

Over the last 4-5 years, European Defence Agency (EDA) partner Member States (pMS) have developed a series of capability orientated measures in order to address the military need in Biological DIM capability. Notably these were an EU CBRN DIM concept for CSDP Operations that was noted by the EU Military Committee, and a Common Staff Target (CST) for a Biological Integrated Reconnaissance Defence System (BIRD). Further to this EDA pMS launched a study into the design of an Integrated Biological Defence System Architecture (IBDSA), the result of which was the launch of an Ad Hoc Project Cat B Biological Equipment Development and Enhancement Programme – Preparation Phase (BIO EDEP PREP) by EDA Defence Ministers at their Steering Board Meeting on the 15 May 2009. Currently the Project is supported by 9 EDA Member States and Norway.

The specific aim of the preparation phase is to develop Common Staff Requirements (CSR) for Individual Soldier Protection, Non-Specialist all-arms CBRN Defence Unit Protection, and CBRN Defence Specialists Equipment Capability leading to options for the realisation of a wide range of Biological DIM Equipment Capability. More specifically, it will result by the end of 2010 in the development of 8 CSR documents detailing the requirements specific for each of the 8 sub-systems of the BIRD: a biological aerosol collector and its reader for individual biological hazard surveillance and monitoring providing a detect-to-treat capability, second and future generation rapid deployable tactical area biological surveillance and monitoring networks providing a detect-to-warn capability, a rapid deployable and a second generation armoured biological field reconnaissance platforms, a second generation deployable tactical field analysis sub-system, a biological residue detection sub-system for decontamination control, and an information management sub-system that will provide interface between the sub-systems and warrant BIRD's coherence and seamless integration within pMS and EU Communication Information Systems (CIS). The programme expects to enhance currently fielded equipment and develop where required new equipment, in order to allow pMS military forces to operate in a biological environment and enable operational and tactical commander's freedom of action and movement.

BODY PROTECTION AGAINST CHEMICAL AND BIOLOGICAL AGENTS: ACHIEVING A BALANCE OF CAPABILITY FOR THE CURRENT AND FUTURE SECURITY THREAT ENVIRONMENT

Scott Duncan, PhD

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Individual protective equipment (IPE) offers an obvious capability enhancement, permitting safer operations in a contaminated environment by reducing the likelihood of casualties from exposure to toxic materials. At the same time, other mission activities potentially may be impaired due to an increase in physiological burden and the reduction in functionality that result from wearing IPE. The focus of Canada's effort in next generation body protection against chemical and biological (CB) agents is the integration of CB agent protection into a combat uniform. The intent is to provide the user with a minimum level of protection available all of the time rather than having to choose between no protection, or an over garment that offers too much protection that is not needed most of the time. A balanced approach is sought that includes protection commensurate with the security threat environment and risk based planning in a modern operational context. Our approach specifically addresses the military's capability goals for the asymmetric counter - terrorism and counter - insurgency environments, which demand rapid response, high in - theatre mobility, technical superiority and maintenance of operational tempo. New materials are being designed specifically to bring their protection performance in-line with the asymmetric CB operational window of between 2 and 6 h. This affords new opportunities to optimise air permeability and evaporative and thermal resistance, in an effort to increase the total heat loss (THL) through the material systems. By incorporating features into the design of a uniform that passively or actively aid in the reduction of thermal burden there will be less of a necessity for the evaporative heat loss requirement to be accommodated in its entirety through the protective material. Designing IPE for very specific threat states, and to protect against realistic hazards for only as long as operational requirements demand, should lead to a new generation of IPE for the asymmetric threat environment which will raise the combat capability level and increase the probability of mission success.

**THE HEALTH PROTECTION AGENCY AND
THE MANAGEMENT OF CHEMICAL INCIDENTS**

Professor Virginia Murray

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Society as a whole is extremely dependent upon chemicals. Chemical incidents are not infrequent and may occur as a very rapidly obvious release, such as a chemical spill, fire and explosion, or as less immediately apparent event such as contamination of a product or land contamination. Incidents can occur accidentally or deliberately.

In many chemical incidents it may be very difficult to identify the chemicals released. Indeed adequate risk assessment data may be limited as one of the main difficulties of dealing with a chemical incident is obtaining rapid information on the identity of the chemical or mix of chemicals involved and their health hazards.

The Health Protection Agency is an independent UK organisation that was set up by the government in 2003 to protect the public from threats to their health from infectious diseases and environmental hazards. It does this by providing advice and information to the general public, to health professionals such as doctors and nurses, and to national and local government.

This presentation summarises the work of the Health Protection Agency, its wider partnership in preparedness and response, provides examples of incident response and includes some of the areas of research at local, national and international levels which build the evidence base knowledge that is needed for chemical incident response.

**Commercial developing technologies:
the role of large industries, SMEs and service providers**

Brigitte Serreault

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The Chemical and Biological industrial sector has developed on basis of the defence capabilities existing in Europe and globally in close relationship with the academic sector and the “end users”, the MoDs, including through NATO. Performing products were developed mainly at **sensor or individual equipment levels** for among others bio point detection and identification, protection or chemical detection.

With the evolution of terrorism, the new threats to civil population, the involvement of civil first responders or of armed forces for civil operations, new needs have appeared in this comprehensive security context. They cover a wide range of activities from potential illegal chemical factories detection, to protection, crisis and casualties management in large civil areas such as stations or commercial centres, water and food chain security, or luggage chain security. These new needs call for a holistic approach including human and social sciences, human factors for first responders and human behaviour for the public as well as legal, ethical and environmental issues. They call also for a **system approach** which is the new paradigm, **from the basic technologies (“underpinning”) to the system integration**.

The challenges are high and require at least a European scale to succeed. Industry already started to prepare itself especially with the European Commission, in the PASR (with BODE on bio stand off detection, BIO3R etc) and then in FP7 security as well as in other themes such as transport, health or environment. These projects already created a suppliers “community” in industry, SMEs and RTOs working together in EDA or national projects too

This is also needed to create a multi-national market with standardized or at least interoperable equipment and modules as initiated by the MIC mechanism and the DG JLS CBRN action plan. Then industry will be able invest with a long term perspective.

Industry is willing to contribute to setting this **long term R&D roadmaps** in cooperation with the academic sector and the users to propose the right products for the market. Projects like FP7 CBRNemap aim at setting this approach. An interesting initiative is the **Integrated Mission Groups on Security**, which is an open forum inviting on a free and voluntary basis industry, academia and if possible users to prepare together a Strategic Research Roadmap on security research, following the ESRAB and ESRIF reports. A theme is dedicated to CBRNE, the TA6. You are all invited to join by contacting the IMGS secretary.

Considerations for Forensic Analysis of Chemical and Biological Evidence

Robert Bull Ph.D. FBI Laboratory, Quantico, VA, USA

In recent years there has been an increase in the focus on the development of tools for chemical and biological forensics. Several cases over the past decade have driven the development of these fields of forensics. While the materials that are examined present special challenges in the case of chemical threat agent and infectious materials, the goal of determining the link between a questioned and a known remain the focus of the forensic laboratory. Although, public health priorities drive the testing for chemical and biological agents, special considerations must be made to ensure that the traditional forensic methods, such as latent print trace evidence, tool marks, and questioned document exams can proceed. The Amerithrax case in the United States has accelerated the development of capabilities to link samples through genetic or chemical attributes of evidence from crime to potential material source. The methods that are used to achieve the goals of a forensic exam of biological or chemical agents are not unique to forensics. The applications of the scientific methods do require special considerations to ensure that scientific evidence is accepted into court. In the United States we need to demonstrate that a method meets the challenges set out in the Daubert standard. This requires that for scientific data to be excepted into court that it meet four criteria: 1. Analytical methods with testable hypothesis, 2. Peer reviewed products, 3. Understood error rate, incorporate standards and control, 4. Generally accepted by the relevant scientific community. By applying rigorous scientific standards to evidence regardless of the hazard, the forensic community can achieve the goals of a complete, accurate, and timely analysis in support of the investigative process.

**ADDRESSING CONTEMPORARY AND FUTURE CBW PROLIFERATION
CHALLENGES:
ARMS CONTROL IN A CHANGING WORLD**

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We live in a changing world. For example, since the conclusion of the negotiation of the Chemical Weapons Convention (CWC) in 1992 and even more so since the negotiation of the Biological Weapons Convention (BWC) in the early 1970s, we have witnessed many interesting and in some cases quite unpredictable changes, including: changes in international relations; changes in the international security environment; the revolution in information and communication technologies; discoveries and developments in the life sciences; and the increased globalisation of chemical and biological industries.

This presentation commences by providing an overview of the contemporary and possible future CBW proliferation challenges, based on some of the lessons of history in a changing world. It then considers the roles of the CWC and BWC, which were negotiated during the Cold War, in addressing security concerns in the 'post Cold War - post 9/11' security environment, including the challenges posed by CB terrorism. This will include the potential roles of the two treaties in enhancing 'pathogen security' and 'chemical security' as a means to raise the barriers to CB-terrorism, with reference to recent activities, including within the Organisation for the Prohibition of Chemical Weapons (OPCW), to support such activities.

The relationships between the two treaties and other CBW non-proliferation agreements and mechanisms are also explored, including CBW export controls, the UN Security Council Resolution 1540, and the Proliferation Security Initiative, and their roles in addressing these CBW proliferation challenges.

Finally, measures that may be necessary to ensure the continuing relevance of the BWC and CWC in a changing world will be considered, including the possibility for amendments to be made to these treaties, and the difficulties caused by the BWC Institutional Deficit', and how that problem might be resolved.

THE AUM SHINRIKYO'S WMD TERRORISM

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The Aum Shinrikyo, an obscure cult religious group, attacked the Tokyo subways employing sarin gas in March 1995, which was viewed as a mark of a new era in terrorism. The Aum Shinrikyo remains the one empirical example of a religiously motivated cult with an affluent amount of financial and human resources and motivations to use unconventional weapons. The Aum Shinrikyo's leaders included the scientific elite of a young generation as well as former *Yakuza* members who had ties with criminal organization. Aum succeeded in establishing an extensive network to procure weapons, material, and drug, involving many countries, even including North Korea.

Despite the fact that the law enforcement authority had already obtained various pieces of information that reasonably indicated that Aum was producing sarin by late 1994, the law enforcement authority decided to wait in order to gather preponderance of evidence until it was too late. Japan's experience with the Aum Shinrikyo's terrorism provides valuable insights for the international efforts to prevent the future WMD terrorism.

This presentation explains the Aum Shinrikyo's WMD terrorism, focusing on the following elements: 1) Aum's intention and capability; 2) details of the Aum's WMD programs, both biological weapons (anthrax, botulinum toxin, etc.) and chemical weapons (sarin, soman, VX, yperite, phosgene, hydrocyanic acid gas, etc.); 3) weapon systems and mode of attacks, including their target selections; 4) the lessons learned for the prevention and crisis/consequence management for CBW terrorism, focusing on perspectives of firefighters, police, military, and private medical institutions.

This presentation explains the Aum's WMD programs visually by using the pictures of the Aum's WMD, incorporating newly available information from the court trials that continued since the late 1990s.

CASE STUDIES: THE UTILISATION OF LARGE-SCALE TEST FACILITY

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Abstract

The National Institute for NBC Protection, as a technical-service body of the State Office for Nuclear Safety and an organisation providing expertise for the Integrated Rescue System of the Czech Republic in compliance with the National Action Plan to Combat Terrorism, performed lots of studies and conducted real tests evaluating readiness and resistance of important elements of critical infrastructure of the Czech Republic in emergency situations caused by the intentional release/spread of dangerous chemical or biological agents. Three basic types of critical infrastructure were selected for the purpose of such studies: a congress hall, the buildings of the central bodies of the state administration, the public transport network. The data detected from individual studies were used in the process aimed at the improvement of security of the aforementioned premises against terrorist attacks.

The SUJCHBO, within the context of such public-security requirements for the performance of more comprehensive experiments, has built a large scale facility with testing hall of 600 m² and a volume of 4200 m³ making it possible to perform a comprehensive study of the processes of spreading and impact of dangerous CBRN agents on the elements of critical infrastructure and to monitor the effectiveness of the measures leading to the reduction of negative impact of such agents. The used construction materials of the test hall, technological background and the level of their protection enable to conduct the experiments with TICs, selected CWAs and routinely used simulants for the individual types of biological agents.

Key Words: critical infrastructure, large scale facility, CWA, B agents

THE THREAT OF TERRORIST IMPROVISED CHEMICAL DEVICES (ICDS)

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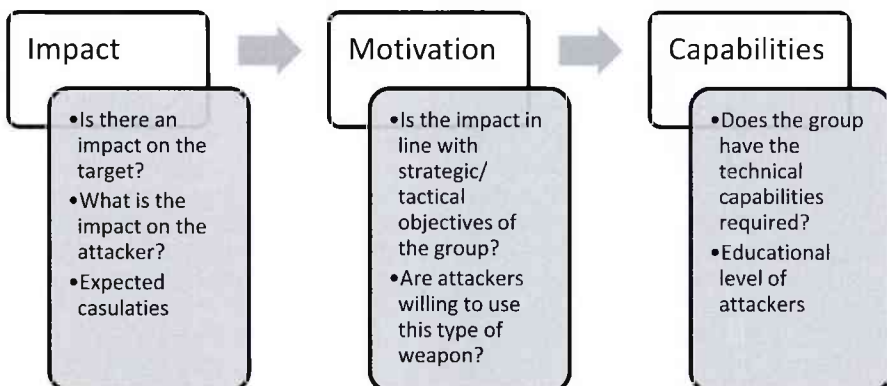
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The use of Improvised Explosive Devices (IEDs) in both areas of war, such as Afghanistan and Iraq, but also in Western societies, such as the attacks in London and Madrid, has started a new chapter of deadly force. However, the number of attacks with improvised CBRNe devices, including Improvised Chemical Devices (ICDs) is rather limited. The Chechnyan threat of use of radiological dispersion devices (dirty bombs placed in Moscow and Chechnya), the Tokyo attacks and the Iraqi Chlorine attacks have been some of the few examples of terrorist use of CBRN weapons. The internet however, is full of recipes and manuals to make and use these devices. The question is how big is the threat?

Estimating the threat of improvised CBRNe devices is a new and difficult task, for which the authors propose a new methodology. The analytical framework used for analysis of the different recipes, when available in combination with instructions for use, is simple and straight forward. For each recipe or manual, we use three standardized 'target cases' to make an assessment.

The assessment is made using three stages. First, the impact of the attack is assessed. The authors propose to assess the direct physical impact, the lasting impact and the societal impact. Second, when the attack has an impact, the impact is compared with different motivational variables for different groups to assess whether this type of attack would fit within their objectives and whether the group members would be willing to carry out such a type of attack. Third and finally, the technical capabilities needed to carry out the attack are assessed. This method is graphically shown below.



The authors have applied this methodology on a number of manuals from both the internet and from field research to assess the threat.

THE WEIGHTED-BIT ASSESSMENT TABLE OF HAZARDOUS CHEMICALS

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The Fraunhofer-Institute for Technological Trend Analysis has developed an assessment tool for dangers associated with the liberation of hazardous chemicals (chemical warfare agents, toxic industrial chemicals, and bio-toxins). In this system substances are evaluated as to their *availability*, *deployability*, *toxicology*, and the possible *socioeconomic consequences* of their release. Each of the 24 sub-categories is qualified as either “true” (value 1) or “not true” (value 0), to minimise ambiguity. An exception to this rule is made in some sub-categories of the toxicology section where the value 0.5 is allowed for practical reasons.

As the Weighted Bit Assessment Table derives sorted lists from the information in the matrix by simple multiplication and addition procedures, the use of this method, e.g. to simulate specific scenarios, can be learned and understood within 30 minutes. Plotting 2D-charts is very simple, too, as basic functionalities of spreadsheet programmes are used as a technical background for the matrix.

The advantages of this system are: i) low ambiguity because of the “one bit” character, ii) a wide range of substances and hazards can be assessed and compared in just one table, iii) a specific “pattern” that can be further analysed is generated for each substance, and iv) the weighting factors can be adapted to assess different scenarios.

These features render our system capable of enabling easy, yet sound, communication between persons with different backgrounds and thus make it a valuable planning tool.

SCENARIO-ORIENTED ASSESSMENT OF HAZARDOUS BIOLOGICAL AGENTS

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Based on the concept of a “Weighted-Bit Assessment Table of Hazardous Chemicals”, that was developed by Fraunhofer INT, we have now developed and will further refine an analogous tool for biological hazard assessment.

The aim is to elaborate a system that will enable easy, yet sound, communication between persons with different backgrounds on the topic of dangers associated with the liberation of biological agents. The tool is designed to assist planning procedures, e. g. for identifying, discussing and assessing possible gaps in security concepts and associated research needs. At this stage of our project, we focus on human pathogens; animal and plant pathogens are not yet included, toxins are currently treated as chemicals.

As expected, the categories used to describe chemicals (availability, deployability, toxicology, possible socioeconomic consequences) could be used only as an initial set, with epidemiology instead of toxicology. Regarding biological agents it turned out that the set of categories and subcategories needs to be broadened and deepened. One important step in the process is to verify that biological agents can be adequately described by a limited set of agent-specific attributes. Once such a set of attributes is identified, it can be applied to characterise biological agents and explore their hazardous potential in different scenarios.

Effect of chemical war weapon on visual pathway using visual evoked potential

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Abstract

Chemical weapons have variety of adverse effects on different organs of human body. Lung & respiratory system are among the primary sites to be affected during early exposure & therefore there exists large number of references available for the effect of chemical weapon on the mentioned organ. One of the organ which might be affected, is a part of visual system i.e. visual pathway for which fewer references are available in this respect. The possible reason for this drawback is the late effect of chemical weapons on the visual system. In the present study the influence of chemical weapons on the visual system is of interest.

Visual evoked potential (VEP), which is a potential obtained by light stimulation of visual system was used as a tool to screen the visual pathway of chemical war victims. Latency & amplitude of VEP, P_{100} were recorded in fifty patients. SPSS a statistical program was used to analyze the results obtained. Significant delay was obtained in VEP, P_{100} Peak of the victims in comparision to healthy group.

Demyelination might be considered for the cause of this delay. therefore demyelination may be considered as a long-term effect of chemical weapons. which will be discussed in detail in full paper.

Key words: Visual pathway. Chemical war weapon & Visual evoked potentials.

DHS S&T CHEMICAL AND BIOLOGICAL DETECTION SYSTEMS

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The Science and Technology Directorate (S&T) of the U.S. Department of Homeland Security (DHS) invests in cutting edge research and development to increase the Nation's preparedness against chemical and biological threats through improved threat awareness, advanced surveillance and detection, and responsive countermeasures. To support this mission, the Chem/Bio Division is developing sensors to rapidly detect releases of chemical or biological agents in the environment in order to enable an immediate response by the local community to mitigate the effects of such a release. Timely detection and effective response to a chemical or biological incident has the potential to save lives and reduce the impact of the attack and the extent of contamination in existing infrastructure.

This presentation will cover several of the development projects and testing efforts for rapid chemical and biological detection systems within DHS S&T. In addition, some requirements for future detection systems will also be discussed.

**BIO-EDEP - SECOND GENERATION BIOLOGICAL RECONNAISSANCE
PLATFORMS - RESULTS OF THE EDA STUDY 09-CAP-031 "PLATFORM
SURVEY STUDY FOR BIOLOGICAL FIELD RECONNAISSANCE"**

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Over the last three years, the European Defence Agency (EDA) has launched a serie of initiatives in order to address the shortfall in the biological detection identification and monitoring (BIO DIM) capability of its members: elaboration of a CBRN DIM concept that was adopted by the EU Military Committee (EUMC), and the Common Staff Target (CST) document for a Biological Integrated Reconnaissance Defence System (BIRD), followed by the design of an Integrated Biological Defence System Architecture (IBDSA). Based on these studies, EDA has launched the preparation phase of the future Biological Equipement Development and Enhancement Programme (Bio-EDEP). The products of this preparation phase are the Common Staff Requirements (CSR) for height sub-systems covering individual soldier protection, non-specialist all arms unit protection and CBRN defence specialists equipments and leading to options for the realisation of Bio-EDEP.

Two of these sub-systems are aimed at field bio-reconnaissance, one concerns a rapid deployable platform, the other one concerns a second generation of armoured platform. The rapid deployable platform will enable dismounted CBRN specialists to carry out bio-reconnaissance tasks on sensitive site and sampling according to SIBA procedures. The armoured platform will enable multi-mission detection and provisional identification while ensuring best protection of operators though armour and remote sampling.

In order to support the redaction of the CSR on these two sub-projects, EDA, in November 2009, contracted the 09-CAP-031 study "Platform survey study for biological field reconnaissance" to Rheinmetal Landsysteme GmbH and its sub-contractors Thales Security Solutions & Services and TNO Defence Security and Safety. More specifically, the aim of this study is to develop a set of general applicable technical specifications for biological sensor packages especially concerning the interfaces between these sensors packages and platforms considered relevant for biological reconnaissance.

The main task of this study is to conduct a survey on available and future reconnaissance platforms and sensor packages taking into account the conceptual basics defined within the CBRN DIM concept and the CST BIRD. This task is divided into five workpackages (WP). The first WP, describes the operational surroundings, the missions and thus the core capabilities required. WP-2 and WP-3 concern sensors and platform (including UAV and UGV) and assess the suitability and feasibility of those packages for the fourth WP which aims (integration, interfaces, sample and waste management, data processing and management, power supply etc...). The last WP brings together the results of the other workpackages, deduces a set of relevant technical specifications and recommendations and describes a potential way ahead for both subsystems.

Chemical-Biological Detection Overview

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State-of-the-art and emerging techniques are under research for chemical and biological (CB) contaminant detection. Spectroscopic approaches include mass spectrometry for bio-identification, Raman spectroscopy for CB identification, and differential scattering (DISC) for standoff bio-discrimination. Basic research efforts that hold much promise for the future include rapid proteomics mass spectrometry combined with bioinformatics tools for the identification of biological samples. This method has also been used to identify viruses responsible for the outbreak of novel influenza strains such as H1N1 and causative mechanisms for the honey bee Colony Collapse Disorder. Variations of Raman spectroscopy that include imaging show great promise to improve CB surface sensing performance with non-contact, high-specificity advantages. Algorithm optimization continues to improve biological standoff detection with DISC lidar approaches. Field trials in tunnel, open air, high humidity, and littoral environments with over 300 releases of 21 different simulants and 14 interferents demonstrated dramatic improvements in sensitivity and successfully demonstrated excellent discrimination during both day and night. The results of these efforts are providing a research foundation for evolving CB sensing needs, in the near-term and well into the future.

BUILDING A GLOBAL BIOLOGICAL DETECTION SYSTEM : THE FRENCH APPROACH

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French biodetection programs have gone through several major gates in the recent months.

Most significant are :

- entry in service in January 2010, of a major operational capability, SABL (*Système d'Alerte Biologique de Théâtre*), a global surveillance system associating a supervision launching of the DETECBIO v1 program, materialized by the award of important contracts respectively covering development and production of the system, and development of necessary biological reagents. Procurement of three DETECBIO v1 systems is now scheduled, with expected deliveries in 2012 and 2013
- near completion of the development of two new equipments for CBRN reconnaissance platoons, namely "Célia" lightweight, high-efficiency, cyclonic biocollector and "Kim" portable field identification device (relying on magnetically enhanced immunoagglutination), whose delivery is scheduled in 2010 and 2011
- award of several R&T contracts in the field of biological early warning and identification, one of which aiming at the development of an improved biological alarm monitor "MAB" from Proengin.

These actions, combined to other projects conducted either at a state level (development of critical reagents) or in cooperation under the aegis of the European Defence Agency (essentially through BioEDEP project, which is covered by a separate presentation by J. Pourtau), illustrate the incremental building process of a global detection system, combining surveillance and reconnaissance functions. This process converges on the DETECBIO v2 program, which should be fielded by 2017/2018.

Our presentation will provide detailed information on these systems, with a special focus on the architecture of SABL and DETECBIO v1. Examples of actions heading to an enhanced satisfaction of operational needs will also be presented, such as the performance / ergonomics / maintainability balance. We will conclude with a summary of expected capabilities of DETECBIO v2, and of technological actions engaged to overcome current gaps. Integration of the biological detection capability in global, integrated CBRN defense system (to be presented separately by P. Tuffigo & S. Meunier) will also be discussed.

BODE : A STANDOFF DETECTION STUDY FOR EARLY WARNING OF BIO-AEROSOLS

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In the frame of the FP7 sponsored by the European Commission, BODE (Biological Optical Detection Experiment) was a project coordinating the efforts within six countries: FRA, GBR, GER, ITA, SWE, EST. The study was carried from January 2007 to March 2009, to prepare European capabilities to face biological threat with a stand-off detector using active detection (LIDAR). The system was designed for short range detection (100-300 m).

The main subsystems of the BODE LIDAR system are described including:

- Laser emission with a specific OPO (Optical Parametric Oscillator) setup built in order to produce the most effective UV wavelengths for fluorescence generation. In order to improve the classification capability, the laser source has been designed through a dual UV excitation scheme, the laser alternatively fires two specific UV wavelengths
- Optoelectronic reception with a telescope as the concentrating optics, the fluorescence emission from the bio particles at some distance being focused on dichroic filters a multichannel photomultiplier array.
- Man Machine Interface (MMI) and data processing was developed, containing the main information: camera display for steering monitoring, cloud position and level of alarm (no alarm, bio alarm, danger alarm).

The demonstrator has been tested and evaluated at FOI facility in Umeå (Sweden). Trials did go through chamber tests, for first calibration phase and data bank realisation. Then tests were performed on artificial outdoor cloud dissemination.

After the completion of the tests, the raw data were processed in delayed time to define and adapt the numerical processes (for instance identify the most suitable numerical filtration) and furthermore to analyse the results. The ROC analysis (Receiver Operational Characteristics), a useful tool to analyse performances, has been used to determine sensitivity.

PCA analysis (Principal Component Analysis) was also used for selectivity measurement and demonstrated good classification capabilities.

Keywords: Standoff, LIDAR, Laser Induced Fluorescence, ROC analysis, PCA analysis

NORMALISED BIOAEROSOL "BACKGROUND" FOR THE STANDARDISATION OF
THE EVALUATION OF BIO-DETECTION TECHNOLOGIESV. Ramisse, L. Berchebru, D. Descroix, G. Hersen, A. Plecis and Y. MorelDGA CBRN Defense, Le Bouchet - B.P. n°3 - 91710 Vert-le-Petit - France
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The intentional release of pathogen aerosol is the main biological threat faced by both military and civilian populations. Its timely detection is a critical component of a global CBRN defence system. However, biodetectors have to overcome the "false positive" signals stemming from the natural occurrence of a bioaerosol "background", comprised of viable micro-organisms and various biomaterial debris. Indeed, airborne bacteria and viruses are found anywhere, anytime and some of them are close in size, structure and chemical content to biothreat agents. This is a special challenge for biodetectors compared to R, C or even E detectors for which naturally occurring interfering substances are clearly less of a problem.

Therefore it is important for biodetector R&T and even more for the test and evaluation (T&E) process to address the bioaerosol background issue quantitatively and qualitatively. Both aspects fluctuate at random as a function of time from very brief scale (seconds) to seasonal trends. The types of environments (indoor, urban or countryside), geographic locations and climatic conditions are also critical parameters. Modelling and predictions seem unlikely to be possible.

Regarding T&E, these fluctuations raise two currently unsolved and difficult problems: (i) relying on natural biobackground would require long and costly statistic studies to have a proper view and even then (ii) such testing would not be reproducible (and could not be compared across different test centres). In view of a harmonisation or standardisation of the T&E process for biodetection equipments, we developed a "normalised biobackground" made up of selected predominant bacterial strains isolated from outdoor air at our facility. This mixture can be generated at will and used under lab conditions to test identification technologies and aerosol alarm monitors. The main drawback is evidently that such a mixture is not sufficiently representative of the real natural most complex background. However, the great advantage is to offer a possible standard reference allowing comparative testing in different laboratories and facilities as well as a stable reference in time. The latter point is critical for the T&E or contractual validation of R&D studies outputs and industrial contracts.

In order to implement the use of this "normalized biobackground", basic data are required, mainly the bacterial composition of the natural background (necessary to select the different bacterial phyla proportions) and the quantitative aspect. Therefore, in addition to a bibliographic review, we performed the long term studies necessary to assess the bacterial composition of the bioaerosol background as well as the monitoring of the (culturable) concentrations. Cumulating more than 2000 biocollection air sample analyses over more than one year, we could assess what natural bioaerosol peak concentrations biodetectors are likely to be faced with. Finally we investigated the practical storage and reproducibility of the normalised biobackground. It is currently used for the R&D studies in French biodefense programs.

**CATSI EDM – A MILITARIZED SENSOR FOR PASSIVE STANDOFF DETECTION
OF CWAS**

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ABSTRACT:

Defence Research and Development Canada (DRDC) – Valcartier has developed a ruggedized passive standoff sensor for the detection of chemical warfare agents (CWAs) based on differential Fourier-transform infrared (FTIR) radiometry. This system is referred to as the Compact Atmospheric Sounding Interferometer (CATSI) Engineering Development Model (EDM). CATSI EDM is a passive infrared double-beam Fourier spectrometer system designed for real-time and autonomous standoff detection and identification of chemical vapours at distances of up to 5 km. It is based on the successful passive differential detection technology developed and demonstrated by DRDC Valcartier's from 1996 to 2003. The sensor takes advantage of a well balanced dual-beam FTIR interferometer with two adjacent field of view to optically suppress the spectral background and the instrument self emission. This sensor produces target gas spectra with reduced background contribution, thus making possible detection of weak IR emission in strong background emission. In this paper, the CATSI EDM components will be described. In particular, the optical head mounted on a pan and tilts tripod, the control and processing computer with an integrated local console and the remote console will be reviewed. Results obtained from a number of laboratory and open-air trials will be presented. The analysis of these results clearly demonstrates the attribute of the passive differential radiometric approach for the standoff detection and identification of chemical vapours of up to few kilometres.

KEYWORDS:

CATSI, EDM, FTS, hyperspectral, Spectrometer, FTIR, standoff detection, CWA

IN SITU AND REMOTE DETECTION OF CHEMICAL SURFACE CONTAMINATION

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Laser Induced Breakdown Spectroscopy (LIBS) offers a unique in-situ capability to detect a chemical contamination on any surface by analysing the spectral response of the material to a laser interrogation. Detection is performed in real time, without sample preparation, and even remote detection is possible. The French atomic energy commission (CEA) has first studied LIBS devoted to nuclear applications, and transportable systems have been developed in collaboration with the French company IVEA which industrializes and commercializes LIBS systems.

In the frame of the French interministerial program of CBRN, the CEA, in collaboration with IVEA, has also developed systems devoted to security applications. Several systems have been developed, including a hand-held probe, connected to the central unit with optical fibers, and a remote system allowing detection up to several meters. The portable probe is eyeware-safe, since a security prevents the laser from lasing when there is no contact with a surface. The remote system is constituted of a telescope and a central unit; it is fully transportable and ready for use within a few minutes.

A chemical warfare agent is an organic molecule containing a few non-metallic specific atoms, typically phosphorous, sulfur, chlorine, fluorine and arsenic. Detecting these elements and their elemental ratios, and moreover the whole spectrum, gives a fingerprint of a chemical warfare agent. Dedicated data acquisition and treatment software are developed to perform spectral signature recognition relative to a predetermined spectral library of contaminants and surfaces.

Different results obtained both with the handheld probe and the remote system are reported, including detection of a contamination with a harmless sarin simulant, as DMMP, and interferents, all spread on real surfaces obtained from public locations. Further performances are discussed.

THE ANALYSIS OF SIGNAL-TO-NOISE (SNR) RANGE-RESOLVED
CHARACTERISTICS FOR LIDAR STANDOFF DETECTION OF B-AGENTS

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The detailed analysis of lidar technique performance adopted for standoff detection of bio-aerosols is presented. The aim of this approach was to evaluate the possible ranges of detection in case of selected bacteria and spores and in different environmental conditions. Additionally, the obtained mathematical formulas allow to perform the optimization procedures of construction parameters of any lidar system to meet the specified requirements, concerning its working range. The knowledge of fluorescence excitation-emission spectral characteristics ("fingerprints") of both b-agents and naturally existing interferents is a necessity to reduce the number of false alarms.

The analysis discussed is based on a regular, range-resolved elastic lidar equation, which is transformed into fluorescence-type target equation. The values of laser induced fluorescence cross sections and concentrations are taken into account as variables at this stage. To perform a real-value analysis of achievable working ranges, the signal-to-noise ratio (SNR) characteristics were developed. They include the impact not only of the optical fluorescence echo power level impinging on the detector, but also the detector noise parameters and natural optical background as well. These two factors appear to have tremendous effects concerning the overall method efficiency, mainly due to the following:

- fluorescence emission spectra in concern fall mainly within the visual spectral range which is full of natural daylight,
- fluorescence emission signal is extremely weak, because of low cross section of LIF phenomenon.

The obtained characteristics enable to identify, depending on the desired system specifications, the optimal detection method taking into account direct detection (single-pulse), multi-pulse detection (summation), or PRM (Pseudo Random Modulation) technique.

**SCREENING AND IDENTIFICATION OF ORGANOPHOSPHORUS COMPOUNDS
RELATED TO THE CHEMICAL WEAPONS CONVENTION WITH LC-NMR AND
LC-SPE-NMR TECHNIQUES**

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Nuclear magnetic resonance (NMR) spectroscopy is one of the most important analytical techniques for structural characterization of unknown molecules. Unambiguous NMR identification of the relevant chemicals directly from environmental samples or other complex matrices can be demanding due to the overlapping background resonances. In last decades, hyphenation of NMR with liquid chromatography (LC-NMR) [1,2] and the improved technique that utilizes combined use of LC separation and postcolumn solid-phase extraction (LC-SPE-NMR) [3,4] have emerged to alleviate the analysis of complex samples. Here, we describe the use of LC-NMR with on-flow one-dimensional proton-phosphorus heteronuclear single quantum coherence (1D ¹H-³¹P HSQC) spectrometry in selective detection of characteristic organophosphorus degradation products of nerve agents sarin and soman, namely methylphosphonic acid (MPA), isopropyl methylphosphonic acid (IPMPA), and pinacolyl methylphosphonic acid (PMPA) [5]. These chemicals were monitored during chromatographic separation at level 10 µg with high time resolution (1 spectrum per 2 s) while the eluent peaks and impurities were almost completely suppressed. In this way, the retention times of these non-UV-detectable analytes were determined. Consecutive LC-SPE enrichment yielded off-line NMR samples which were analyzed using a micro coil probe head with high mass sensitivity [6,7].

The usefulness of LC-NMR and LC-SPE-NMR techniques was demonstrated in decontamination solution analysis. MPA, IPMPA, and PMPA, at level 150µg/ml in 10% KOH H₂O/EtOH solution, were separated and enriched with sufficient yield; no significant impurity background was present in the off-line NMR samples, and identification of the extracted analytes was straightforward.

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**COMBINATION OF SOLID PHASE EXTRACTION AND SOLID PHASE
DERIVATIZATION USING
STRONG ANION EXCHANGE POLYMERIC DISK FOR THE
ANALYSIS OF NERVE AGENT'S MARKERS**

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Anion exchange disk-based solid phase extraction (SPE) has been combined with in vial solid phase derivatization (SPD) and GC-MS analysis for the determination of Alkylphosphonic acids (APAs) in aqueous samples. The optimization of critical method parameters, such as the SPD reaction, was achieved using statistical experimental design and multivariate data analysis. The optimized method achieved quantitative recoveries in the range from 83% to 101% ($n = 13$, RSD from 4% to 10%). The method was sensitive, with LODs in SIM mode of 0.14 ppb, and demonstrated excellent linearity. For forensic applications, aqueous samples containing APAs at concentrations exceeding 14 ppb were concentrated and target analytes were successfully identified by spectral library and retention index matching. Method robustness was evaluated using aqueous samples from the official OPCW Proficiency Test (round 19) and all APAs present in the sample were conclusively identified. The SPE disk retained the underivatized APAs in a stable condition for extended periods of time. No significant losses of APAs from the disk were observed over a 36-day period. Overall, the method is well suited to the qualitative and quantitative analysis of degradation markers of OP nerve agents in aqueous matrices with simplicity, a low risk of cross-contamination and trace level sensitivity.

DETERMINATION OF SULPHUR MUSTARD AND RELATED COMPOUNDS IN
ENVIRONMENTAL SAMPLES BY HEADSPACE-TRAP GAS CHROMATOGRAPHY –
MASS SPECTROMETRY

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New methods for determination of sulphur mustard (HD) in water and soil have been developed using headspace-trap in combination with gas chromatography - mass spectrometry (HS-trap GC-MS). As HD is unstable, especially in water, methods for determination of some of the cyclic decomposition products have also been investigated. The HS-trap technique allows focusing and concentration of the analytes prior to chromatographic analysis, thus enhancing the sensitivity considerably compared to applying conventional static HS. Detection limits of HD in water and soil were 1 ng/ml [1] and 3 ng/g [2], respectively. For soil samples, this is two orders of magnitude lower than what has been reported in the literature. The more stable cyclic sulphur compounds 1,4-thioxane, 1,3-dithiolane and 1,4-dithiane could be detected at levels of 0.1 ng/ml in water and 0.2-0.7 ng/g in soil. As the HS-trap extraction and sample introduction system requires almost no sample preparation, the total time for sample handling and analysis was less than one hour.

The HS-trap GC-MS technique was successfully employed for determination of HD related compounds in a sediment sample collected from an old dumping site for chemical munitions in Skagerrak in 2002. Besides of the HD degradation products detected in 2002, an additional cyclic sulphur compound was unambiguously identified. The result showed that the technique worked well for determination of the analytes, also after many years retention in the sediment.

The sensitivity of the HS-trap technique makes it promising for trace determination of other types of semi-volatile chemical warfare agents as well. Moreover, the versatility of the system allows for determination of the analytes in a variety of sample matrices (like vegetation, clothing and building materials) with minimal sample preparation.

[1] B.T. Røen, *et al.*, J. Chromatogr. A 1217 (2010) 761-767.

[2] B.T. Røen, *et al.*, J. Chromatogr. A 1217 (2010) 2171-2178.

COMPARATIVE ANALYSIS OF METHODS OF RETROSPECTIVE DETECTION OF
SULFUR MUSTARD METABOLITES AND ITS PROTEIN ADDUCTS IN RATS

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We report that nonlethal exposure to the chemical warfare agent sulfur mustard can be monitored by both GCMS and MALDI-TOF methods up to 7 day after exposure. Male rats were exposed by 2 mg/kg of sulfur mustard in 0.9 % NaCl percutaneously. Samples of urine and plasma were collected at 6, 24, 48, 72, 144 and 168 hours after exposure. Thiodiglycol (TD) and thiodiglycol sulfoxide (TDS) were detected in urine, plasma and red cell extract samples. TD was detected in urine at the levels of 3 ug/ml in 6 h and 17 ng/ml in 48 h after exposure. Both TD and TDS were detected in urine after 7 days at the level of 7 ng/ml (approx. 5 times more than the control level).

The adduct formation of sulfur mustard with albumin and hemoglobin in rat blood has been studied in the experiment. We detected alkylated adducts with both albumin and hemoglobin using MALDI-TOF mass-spectrometry. Albumin was analysed after trypsin digestion of whole plasma and an adduct with Cys34 of peptide CPYEEHIK was detected 7 days after exposure. Globin from red blood cells of exposed rats was purified, trypsinised and analysed using proteomics techniques. We have identified a new site of mustard alkylation of rat beta-globin. Peptide GTFATLSELHCDK with alkylated Cys93 and MH⁺ 1525.724 was detected after 7 days of exposure. A number of alkylated adducts with Asp and Glu residues of beta-globin were also detected.

In conclusion, both methods have been adapted for monitoring the exposure to sulfur mustard. Thiodiglycol and thiodiglycol sulfoxide in urine are good markers of sulfur mustard exposure only for several days. Protein adducts were detected 7 days after exposure by MALDI-based proteomics method and this approach can be useful for sensitive detection of exposure of an organism to sulfur mustard.

**IDENTIFICATION AND QUANTIFICATION OF METABOLITES OF SULPHUR
MUSTARD IN URINE BY GC/MS/MS**

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The use of chemical warfare agents is considered to be a real threat in the context of terrorist's attacks against civil and military population. The identification and the quantification of excretion products present in biological samples like urine allow to prove the use of those chemical weapons.

We developed a method to extract, detect and quantify the urinary specific metabolites of sulphur mustard derived from the β -lyase pathway. Sample preparation of rat or human urine was based on the reduction of two metabolites, 1,1'-sulfonylbis[2-(methylsulfinyl) ethane] (SBMSE) and 1-methylsulfinyl-2-[2-(methylthio) ethylsulfonyl]ethane (MSMTESE) by acidic titanium chloride (TiCl_3) in order to obtain 1,1'-sulfonylbis[2-(methylthio) ethane] (SBMTE). Sample preparation (SPE cartridge, concentration) was optimized. Satisfying recovery rates ($> 85\%$) and clean extracts were obtained.

The identification and the quantification were performed by GC/CI/MS/MS on triple quadrupole (Varian) in MRM mode (Multiple Reaction Monitoring) in presence of the corresponding deuterated internal standard. The sensitivity of the method was evaluated (Limit of quantification = $10 \text{ pg}/\mu\text{L}$).

The analytical method was tested on urine samples collected from two individuals after accidental exposure to sulphur mustard. Results obtained by GC/MS/MS and LC/MS/MS analytical techniques were compared.

Complementary studies will be further realized in particular to determine the urinary excretion profiles in function of routes of exposure.

New fluorinated derivatization reagents for screening and identification of nerve agent markers in aqueous samples by GC-MS

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A novel rapid screening and identification method based on derivatization and gas chromatography mass spectrometry (GC-MS) have been developed for the determination of alkylphosphonic acids (APAs), the degradation products of organophosphorus nerve agents. The method involves a rapid 5 min direct derivatization of 25 µL aqueous sample with new highly fluorinated derivatisation reagents. This resulted in derivatives of APAs, screened as phosphonate anion by sensitive GC-MS negative ion chemical ionization (NICI) and identification by GC-MS EI and positive ion chemical ionization (PICI). The conditions for the derivatization were optimized using statistical experimental design and multivariate data analysis. Method robustness was evaluated using aqueous samples from an official OPCW Proficiency Test and all APAs present in the sample were conclusively identified.

Keywords: Chemical Warfare Agents, Alkylphosphonic Acids, Direct derivatization, Negative ion chemical ionization

Hydrolysis of Mustard Simulant 2-Chloroethyl Ethyl Sulfide in the O/W Microemulsion

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Abstract

Hydrolysis of mustard simulant 2-chloroethyl ethyl sulfide (2-CEES) was investigated in the oil-in-water (O/W) microemulsion which is made up of cetyltrimethylammonium bromide (CTAB), 1-butanol, n-hexane and water. The degradation products were identified by GC-MS, NMR and LC/MS. Intensities of products were real time monitored by ^1H -NMR. The mechanism and process of hydrolysis of 2-CEES in the O/W microemulsion were described by pseudoternary model. It was showed that (1) The degradation products of 2-CEES in the O/W microemulsion are hydrolysates except small amounts of alcoholysate, bromation product and elimination product. The hydrolysis of 2-CEES forms 2-hydroxyethyl ethyl sulfide (2-HEES), open chain sulfonium ions $(\text{CH}_3\text{CH}_2)(\text{CH}_2\text{CH}_2\text{Cl})\text{S}^+(\text{CH}_2\text{CH}_2\text{SCH}_2\text{CH}_3)(\text{ECT})$ and $(\text{CH}_3\text{CH}_2)(\text{CH}_2\text{CH}_2\text{OH})\text{S}^+(\text{CH}_2\text{CH}_2\text{SCH}_2\text{CH}_3)(\text{EHT})$. Sulfonium ions that can't be detected by GC/MS were successfully identified by NMR and LC/MS. Although some signals of target ions overlap with the resonances of background compounds in ^1H -NMR spectra, the data from high resolution LC/ESI-MS provides an important complementarity for structural confirmation. (2) The hydrolysis of 2-CEES in the O/W microemulsion proceeds via a cyclic sulfonium ion intermediate. Cyclic sulfonium ion is produced in the interface phase of the O/W microemulsion. Subsequent reaction of water-soluble cyclic sulfonium ion with water and 2-CEES proceeds in the interface or water phases to form 2-HEES and ECT. Water-soluble 2-HEES and ECT convert to EHT in water phase. The interface phase made up of CTAB and 1-butanol is the predominant zone where degradation reaction proceeds.

Keywords: Hydrolysis, 2-CEES, sulfonium ions, mechanism

EBLN – the European Biodefence Laboratory Network

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The objective of this project is the establishment and management of a strategic European Biodefence Laboratory Network (EBLN) to increase European preparedness for protection against Biological Warfare Agents (BWA). The project will improve the European capability to verify the use of B-agents in the context of Biological and Toxin Weapon Convention (BTWC). In the case of an event, indicating the use of BWA, unmistakable identification of the agent has to be performed. The forensic proof of use of these agents must be such that it cannot be refuted. This is a very demanding task that needs increased multinational collaboration.

Twelve countries, Austria, Belgium, Czech Republic, Finland, France, Germany, Italy, Netherlands, Norway, Poland, Spain and Sweden, participate in this project. The project involves the design and construction of a shared database with high-resolution typing data which is necessary common resource for unmistakable typing and identification of B-agents. Agreement on standardized protocols and methods is needed for inter-laboratory reproducibility and comparison of results within the EBLN. Identification of agents and sources in a forensic context relies on a spectrum of features and therefore the effort to set up such a comprehensive system surpasses the resources of individual nations and thus depends on coordination of multinational cooperation.

Many different technological approaches to BWA typing are employed and compared on common reference strain collections and the resulting high-resolution typing data are included in a common shared database. This project is very successful in bringing together twelve countries to work together on common tasks.

DEVELOPMENT OF RAPID IDENTIFICATION AND TYPING METHOD FOR
BACTERIA USING MALDI-TOF

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In spite of progress in identification of bacterial agents, routine laboratory detection still relies on laborious, time-consuming and costly biochemical techniques. Moreover, these procedures suffer from higher false-positive and -negative rates and mostly, are not able to identify the bacteria deeper than on *species* level. On the other hand, precise genetic approaches demand primers and their design. Furthermore the time needed for analysis also does not meet the desired criteria for modern biological crisis management.

MALDI-TOF typing is now widely clinically employed because it is the low-cost procedure shortening the time for identification from the bacterial culture up to a few minutes. This robust technique, based on mass spectrometry principle, is inherently connected with higher mass accuracy of detected molecules and therefore the differentiation as deep as to *subspecies* is feasible. Therefore, the MALDI-TOF typing is rapid and reliable tool for the identification of highly virulent microorganisms including those classified as potential biological warfare agent.

However, nowadays commercially available systems are not tailored for detection of highly infectious microorganisms. The procedures for preparation of bacterial lysates have to be able to provide sample sterility on one hand, but also sufficient protein content on the other hand. Moreover, since the success of the analysis is tightly connected with databases that may be underrepresented for rarer infectious microorganisms, the differentiation between non-virulent and virulent strains could also fail.

In our study, we compared two methods for bacterial protein extract preparation together with an effect of α -Cyano-4-hydroxycinnamic acid and sinapic acid as matrices for MALDI-TOF bacterial identification on a range of gram-positive and gram-negative bacteria. We investigated the possibilities for the identification and typing using statistical and clustering tools in available software.

DETECTION OF FUNCTIONAL TOXINS: APPLICATION TO VEGETAL AND
BACTERIAL TOXINS OF THE BIOLOGICAL THREAT

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The development of rapid and accurate detection methods for toxins is required. Many detection methods targeting the entire organism producing the toxins are based on the nucleic acid amplification. Other techniques involve immunoassays or mass spectrometry which target either the whole microorganism or a specific toxic component. Although these methods are sensitive, they may lack of specificity and there is a need of confirmation techniques.

Numerous toxins consist of a component that binds to cell surfaces and a catalytic unit that modifies cell homeostasis leading to deleterious effects in cell targets. Owing to the exquisite specificity and high catalytic activity of their enzymatic components, there is considerable interest in using these activities as a mean to monitor infection in biological fluids and in various environmental media. The assays are based on the reaction of the toxin with its specific substrate and the monitoring of the reaction product through mass spectrometry or enzyme immunoassay.

In this context we have applied this strategy to ricin from *Ricinus communis*, botulinum toxins from *Clostridium botulinum* and lethal and edema factors from *Bacillus anthracis*. The lowest limits of detection were in the picomolar range (*Anal Chem.* 79, 659, 2007 and 81, 5935, 2009). For instance for the edema factor, upon reaction with ATP and in the presence of calmodulin, the enzymatic activity was monitored by measuring the production of cAMP using a competitive enzyme immunoassay. This allowed a sensitivity of 1 pg/mL for edema factor in human plasma. The method is also very fast (< 2 hours) and is now presented as a detection kit which may be used in hospitals. In conclusion, these new approaches allow the detection of active toxins and prove to be useful in diagnostics and environmental detection.

DEGRADATION PRODUCTS OF CWAs IN DECONTAMINATION

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Abstract

Decontamination of CWAs on contaminated equipments and terrain requires diverse decontamination solutions and emulsions. Nowadays several different decontamination procedures, such as alkaline and oxidative solutions, are used. However, there is only a little knowledge on the actual CWA degradation chemicals, which are formed in the decontamination solutions. The purpose of this study was to screen and identify the main degradation products of CWAs, namely HD and VX, in three commercially available decontamination solutions, BX-24, GD-5 and GDS-2000, and in Finnish emulsion E-2000. The decontamination was investigated in two reaction times, 30 minutes and 24 hours, at room temperature. Several sample preparation and analytical methods, i.e. gas chromatography (GC), gas chromatography – mass spectrometry (GC-MS), liquid chromatography – mass spectrometry (LC-MS), liquid chromatography – tandem mass spectrometry (LC-MSMS) and nuclear magnetic resonance spectroscopy (NMR), were utilized in order to obtain reliable information of the decontamination products. The results of our study will be discussed.

PREPARATION AND EVALUATION OF A PROTOCOL TO EVALUATE THE PERFORMANCES OF MASS DECONTAMINATION UNIT

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In case of CBRN event, an important action in this process is to decontaminate as soon as possible valid and invalid victims. The objectives are to reduce the hazard and the risk of cross contamination to other victims, to authorize medical treatment and to restore the contaminated space.

One important aspect to evaluate properly a mass decontamination unit efficiency is to measure the level of toxic gas inside the decontamination shelter evaporated or the biological agents dispersed from the cloths after their coming into the mass decon unit.

A protocol has been developed to measure these two parameters when the decon unit is in operation, which is representative of a real situation. The chemical simulant is methyl salicylate (MeS) with a UV absorber, and the biological simulant is *Bacillus atrophaeus*. Victims are contaminated with a solution of MeS and a solution of *Bacillus atrophaeus* at different part of the body.

Bubblers, collecting tubes and a portable chemical detector (LCD Detector) are set at different part of the unit to measure the level of chemical contamination. The portable chemical detector is used to quantify precisely the amount of chemical agent inhaled because it can be set close to the respiratory tracts of the victims all along the unit. Slit Samplers are set on the floor where the victims are undressed to check if bio agents are dispersed into the shelter.

Different mass decon unit have been evaluated. Results shows that the cleaning of the air is sometimes difficult even if the undressed part is physically separated from the shower part which is itself separately separated from the dressed part. In that condition, the concentration of the chemical agent into the unit can increase when the number of the victims treated increased.

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Building Interior Cleanup Studies - Utility of Biological Indicators

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The anthrax-tainted letters disseminated in the fall of 2001 resulted in contamination of infrastructure, posing an unprecedented challenge of decontaminating over 20,000,000 cubic feet (~ 1 million sq. ft.) of combined building interior space. A number of liquids and gaseous products are registered (with the EPA) for use as disinfectants, however, none is currently registered for use against *Bacillus anthracis*. Additionally, their efficacy in the context of large building volumes with complex materials was at best speculative. Some early work provided indications on requirement of a dose of ~9,000 ppmv-hr of chlorine dioxide (CD) gas to achieve effective cleanup. Re-occupation of such buildings was subject to extensive surface sampling and recovery of zero viable *B. anthracis* spores.

An extensive number of biological indicators (BIs) were used to qualitatively assess the desired fumigation dose. Biological Indicators are defined as “*A standardized preparation of bacterial spores on or in a carrier serving to demonstrate whether sterilizing conditions have been met. Spores of different organisms are used for different methods of sterilization*” (Fifth Edition “Disinfection, Sterilization, and Preservation – The Essential, by Seymour S. Block). Specific questions addressed in this study were: a) what dose of CD gas is required for complete sterilization of BIs; b) how does the spore kill profile on BI compare to that on selected building interior surfaces; c) what is the most appropriate surrogate spores for sporicidal action of CD gas; and d) what material type will serve as an appropriate backing for BIs?

The results show that the D values (time for a decimal reduction) for spore (*B. atrophaeus*) inactivation on BIs range between 5 - 7 min (with 1,500 ppmv) and between 7 – 10 min (with 500 ppmv) and require a CT value of <3,000 ppmv-hr. In contrast, the D values for spore (*B. anthracis* NNR1Δ1) inactivation on wood are significantly higher and range between 12 – 17 min (1,500 ppmv) and 22 – 24 min (500 ppmv) and require a CT value of >9,000 ppmv-hr. In order to develop a BI that was more consistent with *B. anthracis* spore kill on relevant building materials, BIs (spores on stainless steel backing) were prepared with spores from four surrogate species: *B. atrophaeus*, *B. cereus*, *B. subtilis*, and *Geobacillus stearothermophilus*. Surrogate spore kill on the new BI was compared with *B. anthracis* spore kill on a wood surface. The results show that of the four surrogates, *G. stearothermophilus* spores appear to be most resistant to kill by CD gas initially (D value of 35 min compared to 8-10 min for the other three surrogate species @ 1500 ppmv). In addition, the CT values required for a complete kill of *G. stearothermophilus* spores ranged between 5,000 – 7,000 ppmv-hr. In conclusion, based on the results summarized here, the BIs used in the building cleanup efforts do not provide confirmatory indication for achieving a dose of 9,000 ppmv-hr. Furthermore, the data indicate that BIs made with spores of *G. stearothermophilus* more closely represent the spore inactivation profile observed for *B. anthracis* on building surfaces (e.g., wood.)

(Sponsored by and in collaboration with the US-EPA National Homeland Security Research Center's Decontamination and Consequence Management Division)

CB^{plus} CHEMICAL-BIOLOGICAL PROTECTIVE COMBAT OPERATIONS UNIFORM: NEXT GENERATION LOW BURDEN PROTECTIVE CONCEPTS

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Canada has just completed the demonstration of daily wear combat uniform concepts that provide just-in-time dermal protection against CB hazards, to be worn in theatres where there is an increased risk of asymmetric use of agents. As first outlined here in 2001, the CB^{plus} combat uniform concept was born from the recognition that the threat to military forces has changed from the Cold War to smaller scale but less predictable battlefield scenarios. This uniform is not intended to replace existing CBRN protective equipment, but to replace the continual use of such high burden equipment at times when the probability of encountering a hazard is relatively low. In more detail, the uniform should protect at a certain level against various CB hazards, while offering comfort and weight closer to that of the current daily-wear combat uniforms (Figure 1). This necessitates an innovative approach to individual protection that addresses new toxic challenges, differing levels of exposure in varied theatres of operation, and a need to reduce the physiological burden on the wearer.

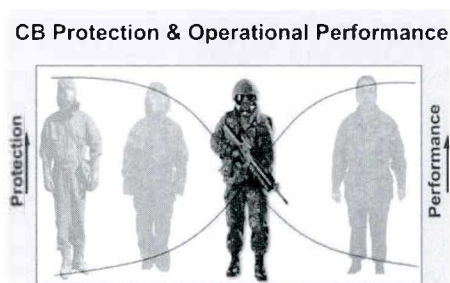


Figure 1: Positioning of CB^{plus} concept within protection/human performance tradeoff

The overall objectives of the project were to:

1. Develop an operational concept for a uniform incorporating just-in-time chemical and biological protection suitable for the asymmetric threat environment;
2. Develop/select appropriate criteria and evaluation methods for uniform performance;
3. Develop appropriate novel lightweight materials, closures and designs;
4. Develop and demonstrate uniform concepts for chemical/biological protection.

Selected concept uniforms produced under the project will be discussed, in the context of the trade-offs between protection, functionality, and physiological burden achieved, as well as concept of use.

Using scoring methods to assess the performance of CBRN personal protective systems in different scenarios

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Elaborate testing programs are available in the area of personal CBRN protection, aimed at guaranteeing that the materials and systems protect according to specifications. These tests provide crucial insight in the design of protective equipment and its effect on the overall protection of and the thermo physiological burden for the wearer. Results regarding these separate aspects need to be combined to enable an overall assessment of the actual operational performance of the protective system.

In an exploratory effort we have built a pragmatic system that should allow the analysis of the performance of different systems in different scenarios by assigning scenario dependent weighting factors to test results. We have built a list of parameters that are relevant for the performance of CBRN protective systems, for protection, burden and use. As the list of parameters is too large to handle in a simple system, we have broken down the list by grouping the parameters in relevant classes and continued the research with a selection of these classes. Then, we have developed different concepts (on paper) of CBRN protective systems that should be applicable to different scenarios and scored these systems in relation to their required performance in these classes. Although this project was largely a paper exercise, several tests have been performed on representative systems and materials. Additionally, five different scenarios were selected and the required values for the classes were also scored. Comparing these scores in a structured way provides information on the relevant factors of the CBRN protective systems. The result of our analysis was that we have gained much knowledge on the practical use and limitations of these scoring methods and insight into the way ahead for the method development. The follow-on steps will be discussed in brief.

**COMPLEX PERFORMANCE EVALUATION OF
PERSONAL PROTECTION ENSEMBLE**

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Current testing of personal protective ensemble (PPE) focuses primarily on the barrier material properties and the individual components. However, once the type of the PPE had been design, it has to be evaluated in very complex manner, in order to perform configuration control of the PPE from material and design point of view, test the integrity of an ensemble with individual components in dynamic conditions, as well as evaluate physical and heats stress properties affecting users. The feedbacks from complexity PPE performance evaluation are needed for the improvement of the PPE during R&D phases, certification for deployment and the control of the PPEs during manufacturing and maintenance, as well as for the improving current safety and health standards. The aim is to keep emergency responders protected against exposure of CBRN hazardous materials, physically fresh and unencumbered while performing response mission in contaminated environment, where actual quality of the PPEs and human factors play an important role in emergency responder's safety and health.

The National Institute for Nuclear, Biological and Chemical Protection v.v.i. in Czech Republic is applying complex process for PPE's research and development, testing and certification continuously for several decades. The establishment utilises CBR laboratories for swatches testing with live CBR agents, configuration and integrity control of the PPE is performing in the Gas Test Chamber with volunteers and robotic mannequin "Golem" under dynamic conditions. Human factors evaluating physical and heats stress properties of the PPE, and also fitness of individual users are performing in the Climatic Test Chamber enable set up variable environmental conditions.

The presentation is providing several examples of complexity approach for technology development and the evaluation of the PPEs, applying the Czech novel testing method Visual-Man-In-Simulant Test (V-MIST) with sensing underwear for integrity evaluation of chemical PPE, the improvement of the PPE protection factor through artificial ventilation and the thermovision survey of human body thermal behavior wearing the PPE.

Key Words

Personal protective ensemble, PPE, CBR agents, gas test chamber, climatic test chamber, mannequin "Golem", Visual-Man-In-Simulant Test, heat stress, thermovision.

PROTECTIVE AND TEMPORAL EFFECTS OF CLOTHING ON THE *IN VITRO* SKIN ABSORPTION OF CHEMICAL WARFARE AGENTS AND SIMULANTS.

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The deliberate release of chemical, biological, radiological and nuclear (CBRN) materials poses a significant threat to civilian populations as exemplified by the 1995 Tokyo subway incident¹. A major step in the process of decontamination is the removal of clothing², the effectiveness of which has not previously been subject to extensive research. Therefore, the objective of this study was to assess the influence of clothing on the dermal absorption of sulphur mustard (HD), VX, soman (GD) and methylsalicylate (MS).

Studies were performed using Franz-type glass diffusion cells³ containing dermatomed pig skin with clothing applied to the skin surfaces, either in single layers or in various combinations. In one study, the various swatches of clothing were left *in situ* for the duration of the (24 hour) study period. In a second study, the clothing was removed at various times post exposure. Unclothed skin was used as controls in both studies. The fabrics investigated included cotton, denim, acrylic and nylon garments. Each experiment was started by the addition of a 10 µl droplet of ¹⁴C-radiolabelled chemical contaminant to the surface of the clothing or skin, with subsequent measurements of the amount of radioactivity penetrating the skin being used to calculate dermal absorption kinetics.

In the absence of disrobing, clothing significantly reduced both the rate and extent of penetration of all contaminants over the 24 hour period with multiple layers providing the most protection. Disrobing further enhanced the level of protection but this effect decreased exponentially with time; the greatest levels of protection were achieved 5 minutes post-exposure. However, there was a measurable reduction in the dermal absorption of all contaminants when disrobing was delayed to 360 minutes post exposure. These data indicate that every effort should be made to remove contaminated clothing as soon as possible following exposure in order to limit subsequent health affects arising from percutaneous exposure to hazardous chemicals.

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TESTING THE PROTECTIVE PERFORMANCE OF CLOTHING MATERIALS AGAINST AEROSOLS

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Abstract

In AEP 38, skin protection against aerosol particles offered by CBRN protective equipment is recognized as an important issue that has to be verified. The work presented here focuses on material (i.e. swatch) tests; as opposed to whole system tests that use mannequins or human volunteers and which also include leakage and clothing design. No internationally accepted test standards exist to determine aerosol protection by clothing, but many swatch tests based on filter tests and some cylinder tests have been developed.

The filtration tests mainly differ in challenge aerosol, test flow rate, forced flow or free flow due to wind, and sampling strategy. Cylinder tests differ for example in the way the fabric is draped around the cylinder, the size of the cylinder and the direction of the air flow around the cylinder. TNO has developed a filtration method based on vapour penetration tests according to NATO AEP-38 and a cylinder test. We have identified the most important properties of the clothing and critical test procedure parameters in both tests.

Filtration efficiency and air flow resistance of the material appear to be the key parameters in a cylinder test. Air flow resistance determines the flow velocity through the cloth at a given wind speed and influences the deposition of aerosol onto the skin underneath the clothing thus influencing the protective performance of clothing materials. Filter efficiency improves, sometimes dramatically, with increasing particle size. Also the choice of flow rate, which can be set to a standard value or dependent on the cloth air flow resistance, is an important parameter, with efficiencies usually decreasing with increasing flow velocity through the cloth.

There is a strong correlation between the cylinder test and the filter test results.

**PREPARATION OF ANTIBACTERIAL AIR FILTER MATERIAL BY
IMMOBILIZING BIO-ANTIMICROBIAL ON FIBERGLASS AND
PROPERTY EVALUATION**

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Abstract: Use of an air filter material that has an antibacterial function is one of the most effective methods to resolve the problem of air filter contaminated by pathogenic microbes. ϵ -Polylysine and Natamycin are two biogenic food preservatives that have been widely applied in recent years, because of their high antibacterial efficiency, non toxicity, and environmental friendliness. In this paper, a novel antibacterial air filter material was prepared by immobilizing ϵ -Polylysine and Natamycin onto fiberglass high efficiency air filter media by acrylic ester bonding. The mechanical properties, aerosol filtration properties, and antibacterial properties were then evaluated. An improvement in the mechanical properties of the material prepared was seen compared to the untreated filter media. The filtration efficiency of the material prepared for particle aerosols and bioaerosols was 99.97% and 99.997% respectively. Antibacterial rates of the material prepared against *Staphylococcus aureus* and *Escherichia coli* in liquid were both greater than 99.99% compared to the untreated filter media. The bacteriostatic effect against *Aspergillus niger* in liquid was good compared to the untreated filter media. Antibacterial rates of the material prepared against bacteria in bioaerosols were greater than 99.99%, and bacteria observed with Scanning Electron Microscopy appeared to be dead. Thus, antibacterial air filter material prepared by immobilizing bio-antimicrobials on fiberglass had a strong inhibitory effect against gram-positive bacteria, gram-negative bacteria, and fungi, with no impairment of the intrinsic properties. This kind of material appears to be promising for applications in air cleaning and biological protection field.

Keywords: Air filter material, Bioaerosol, Antimicrobial, ϵ -Polylysine, Natamycin

WHAT FORMS AN ADEQUATE SKIN PROTECTION?

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The changes in the security environment over the past decades have resulted in other types of missions for military and first responders. Due to the effective Chemical Weapon Convention large stocks of CWA available in the previous century are or will be destroyed and what remain are relative small quantities in some rogue states or in the hands of terrorist. The threat of all out war with frequent, and massive use of CWA weapons changed to incidents which are highly likely but unpredictable in time and location. In addition casualty acceptance has reduced considerably. Consequently the permissible exposure levels to CBRN agents have been reduced. The most hazardous conclusion would be that passive Chemical defense is no longer required because this makes the opponents chemical weapons very effective and is an invitation to use them. Although changes regarding several aspects of the chemical defense posture could be discussed this paper focuses on the question "What forms an adequate skin protection"?

The required protection factor as well as the time the protection should last (capacity) will be discussed in relation to possible challenges with CB agents in the form of vapor, liquid drops and splashes or in the form of aerosols. Does one still need 24 hour protection against repeated attacks? Is it wise to reduce the challenge to lower levels say 1 gram/m²? Does one still need several protective garments per man or is in the light of the lower frequency of CW attacks a smaller surplus, a lower degree of sustainability, possible?

For first responders a quantitative threat analysis is missing so it is hard to define required protection factors and capacity. However the challenge levels are likely to be lower than those for the military. So what works for military will work even better for first responders. The commonly used level C and D type of air impermeable suits provide poor protection against vapor and aerosols but still might provide sufficient protection due to the lower challenge.

**SYSTEMS APPROACH TO RESTORATION OF AN AIRPORT FACILITY
FOLLOWING A CHEMICAL AGENT ATTACK**

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The U.S. Department of Homeland Security Science and Technology Directorate (DHS S&T) has the core mission to mobilize and leverage the nation's scientific capabilities to detect, protect against, respond to, and recover from high consequence events. An act of chemical terrorism that targets civilians and critical infrastructure (e.g., a transportation hub such as a major airport facility) represents an example of a high consequence event. In addition to the potential for numerous casualties and acute or chronic health effects upon other individuals impacted, the remediation and restoration of contaminated infrastructure can pose significant challenges. The closure of a facility for even a short time period can have a major economic impact, both locally and at the national level. Facilities typically possess a range of material types and large volumetric spaces that can make remediation a challenge. There are knowledge and technological capability gaps that can make efficient recovery difficult. Finally, there is a general lack of understanding among stakeholders of the time, cost, and process to restore a facility.

DHS S&T has applied a systems approach and has partnered with the Los Angeles International Airport (LAX), as a model critical facility, in a project which has led to the development of pre-planning procedures and templates, the filling of some fundamental knowledge gaps, remediation guidance, and various decision support tools that can be used to reduce and expedite the timeline to restore such a facility to normal operations following a chemical warfare agent attack. This presentation will describe the objective, goals, processes, and outcomes of this project.

FOI-R--2985--SE
Crisis Management

EUROPEAN RESEARCH AND INNOVATION AGENDA FOR CBRN (ESRIF)

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In 2007 the European Commission launched a strategic initiative called "European Security Research and Innovation Forum" (ESRIF). Its main objective was to develop a mid and long term strategy for civil security research and innovation for stakeholders from the demand and supply side. Late 2009 ESRIF proposed a long term Research&Innovation Agenda on a broad range of security topics. One of the 11 working groups had a focus on Chemical, Biological, and Radiological (CBRN) incidents and accidents. It addressed CBRN related threats and challenges, current capability gaps, and suggested means for closing those gaps through research. The scope included threat assessment, prevention, preparedness, response, mitigation, and recovery along the security cycle.

CBRN incidents, be they intentional or accidental, remain major threats to Member States for the coming decades. Although the scope of this threat still includes large-scale attacks by States, the pendulum is swinging more toward the use of small, improvised devices by terrorists. Of particular concern is the spread of technical knowledge and capabilities that could be misused in the form of CBRN weapons. Prevention is crucial and should receive particular attention by equipping intelligence agencies and policy makers with improved information analysis tools. Consequence management to overcome CBRN attacks and hoaxes requires networked warning and situational awareness systems with development of more effective and reliable detection and identification capabilities. Hands-on CBRN experience of response organizations is low and the awareness for the need to build capabilities is not always evident. Therefore, ESRIF advises not to seek for development of dedicated CBRN systems, but rather for integrating solutions into existing and developing security systems using an all hazard approach. Other important capability gaps involve broad-spectrum medical countermeasures, physical protection for first responders, and providing safe containment and decontamination procedures that work quickly without being harmful. Special focus must also be placed on understanding and measuring of psychological and sociological consequences of CBRN incidents.

A recommendation was made to establish dedicated CBRN expert-centres to gather and distribute information and experience. The EU commission is recommended to develop methodology and build infrastructure for intensified exchange of sensitive information like threat awareness, dual-use potential of emerging technology and trends in radicalization. The commission should promote a system-of-systems approach to CBRN(E) counterterrorism. Emphasis should be on integration of this approach into other hazard areas that the security community must cope with.

Building a Total Area CBRN Defence System

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In recent years, the worldwide safety situation has evidently changed. CBRN threats are now key factors which influence the national and military strategic safety. The NBC warfare threat has developed into increasingly multifold, complex and uncertain threats, such as CBRN proliferation, CBRN industrial accidents and CBRN terrorism. Furthermore, the potential of using emerging technologies to develop new CBRN weapons has intensified the complexity of CBRN threats. On the other hand, the current CBRN defence mechanism has exposed inadequacy and weakness, in which the national power, the different sources of intelligence, and the defence resources have not been fully integrated and sufficiently utilized. As a result, the various CBRN threats to the nation are in lack of effective monitor and control.

The total Area CBRN Defence System in this paper, refers to a national safety defence system built for guarding against CBRN threats. It can frighten, monitor, reconnoiter and warn in a real-time and effective manner against various CBRN threats on the homeland, airspace and marginal sea, and timely start up the CBRN defence measures so as to avoid or minimize the consequence of CBRN attacks.

Building a Total Area CBRN Defence System, programming the whole CBRN defence on the national level and forming a tiered defense network, will help to integrate and utilize all the involved departments' assets, fully share all sources of intelligence and defence resources, and ultimately realize the real -time and all-round monitor and control to all the CBRN threats, thus ensuring the whole nation's safety. The paper discusses the necessity to build such an system and develops an initial conception.

**INTERACTIVE EMERGENCY RESPONSE TRAINING AND EXERCISE
SYSTEM DECISION SUPPORT TOOLS, CURRENT SITUATION AND
FURTHER DEVELOPMENT/ REAL-TIME DECISION SUPPORT TOOLS**

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Abstract

New methods, models and tools in order to create services for societal risk management, including satellite support for prevention, preparations and operations, simple methods to quickly reduce the riskzone for liquefied toxic and corrosive gases, and an integrated decision support system. RIB - an Integrated Decision Support System - is a system for prevention and emergency management that combines a huge library, a chemical database with dispersion models, risk management tools and a command and control system. The system integrates into a user friendly GIS environment novelty features derived from the European project PREVIEW, with satellite data for prognosis on windstorms, floods, landslides and weather data for calculations of the dispersion of hazardous chemicals also in mobile operational situations.

WIS is a national, Internet-based information system, created to facilitate information sharing between, players in the Swedish emergency management system, before, during and after emergencies. With WIS, players can share management information with one another in journal form. When widely deployed, it creates the prerequisites for quickly obtaining comprehensive situational assessments during emergencies.

At the university in Karlstad, facilities for investigating new forms of user participation in the early phases of system design have been developed. The system is called Ozlab, and can be used in a wide range of activities from design concepts to human-computer interaction without the need of developing costly specific computer programs.

CRISIS MANAGEMENT DISPERSION MODELS

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Various dispersion models and hazard prediction and assessment software tools are used to assess consequences from dispersion of hazardous materials, such as toxic chemicals, both by military and civilian users. Such tools can be employed during and after an event to support crisis and consequence management, or to assess hypothetical scenarios for emergency preparedness planning, training and exercises. The dispersion modeling tools HPAC, ARGOS, DEGADIS and the fast hazard prediction and assessment tools ERGO, DSB Farlig gods, and NBC ANALYSIS are compared by performing calculations of the dispersions and predicted hazard areas on various constructed scenarios. The constructed scenarios are: dispersion of chlorine after a total rupture of a tank containing 20 tonnes of pressurized liquefied chlorine, dispersion of ammonia after a total rupture of a tank containing 10 tonnes of pressurized liquefied ammonia, and an attack with soman by bomber aircrafts. For the first two scenarios, several meteorological conditions are considered.

The motivations of this study are: to investigate the required input parameters and exemplify possible output of the various tools; to outline the assumptions and limitations of the programs; and to discuss the user friendliness and the required user knowledge and competence. It is found that the different models give quite different results for the same scenarios, and a fairly high competence level is required from the user in order to perform the dispersion modeling and to draw correct conclusions. One challenge with HPAC and ARGOS is to give proper meteorological inputs to the programs. If HPAC or ARGOS is to be used in an operation or a crisis situation, a connection to a meteorological service is highly desirable.

SOURCE ESTIMATION FROM SENSOR DATA

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To estimate risk areas and consequences when a hazardous gas or aerosol is released and dispersed in the atmosphere, we need to know the source location and release rate, as well as meteorological conditions in order to perform the necessary dispersion modeling. Source characteristics are usually unknown, at least partially; even if the source location is known, the release rate has to be determined. The emerging sensor network technology makes it possible to use data from a geographically distributed network of CB- and meteorological sensors to estimate the source characteristics. We will present a model system based on a Lagrangian stochastic particle model of Langevin type, suitable for short range dispersion (up to some km distance from the source), and a Monte Carlo estimation technique for the associated source estimation problem.

THERAPEUTIC RESEARCH WITHIN THE FRENCH CBRN PROGRAM

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The French Interministerial Research and Development Program against CBRN threats has launched a series of projects in search of inhibitors against various B agents including botulinum toxins, ricin, type three secretion systems (plague), large clostridial toxins. High-throughput screenings against these agents have been made using cellular or enzymatic assays. Three to 15 hits per agents were identified and confirmed. Mechanistic as well as structural studies have been conducted to progress on the characterization of the mechanism of action of these hits. One molecule was shown to protect mice from lethal nasal instillation of ricin. Two molecules bound to or near the catalytic site of Toxin B from *Clostridium difficile*. Co-crystallization with the toxin showed mechanism of inhibition. One molecule was shown to inhibit formation of the type three secretion system with which *Pseudomonas aeruginosa* and *Yersinia pestis* inject toxins inside cells.

The program develops also therapeutic antibodies against toxins and viruses. Mouse monoclonal antibodies, alone or in combinations of 2 or 3 were selected for their neutralizing action against 6 isotypes of botulinum toxin and ricin. Selected antibodies are chimerized in order to replace the mouse antibody framework by human sequences in order to avoid anaphylactic shock occurrence during treatment.

Finally, two projects propose novel vaccine approaches against plague and anthrax, intended to overcome the drawbacks of existing solutions.

Key words : toxin inhibitor, high throughput screening, therapeutic antibodies, vaccine

CHARACTERISATION OF IMMUNE RESPONSES AND PROTECTIVE EFFICACY IN MICE AFTER IMMUNISATION WITH RIFT VALLEY FEVER VIRUS VACCINES.

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Rift Valley Fever (RVF) is considered as one of the most important viral zoonoses in Africa. Since no effective vaccines or treatment is available, accurate and reliable diagnostics is required. As a possible biological agent, the virus could be introduced deliberately to humans and animals before further spread by local mosquito vectors. In nature, the virus has the feature to lay dormant in mosquito eggs for years before hatched and hence new areas may become endemic with long-term health consequences and large socio-economic effects.

To study the kinetics of the infection, a SYBR Green-based quantitative real-time RT-PCR assay was developed. Blood and organs of experimentally infected mice were sampled and high amounts of viral RNA were detected in blood, brain, and liver samples shortly after infection. The RVFV quantitative RT-PCR proved to be a valuable diagnostic tool during the first days of infection and even before detectable antibody levels and visual symptoms of RVF were observed.

Naked DNA vaccines is an interesting approach since the virus is highly infectious and existing RVFV vaccine strains display adverse effects in animal trials. In this study, gene-gun immunisations with cDNA encoding structural proteins of the RVFV were evaluated in mice. Even though complete protection was not achieved by genetic immunisation, 4/8 and 5/8 mice vaccinated with cDNA encoding the nucleocapsid protein or the glycoproteins, respectively, displayed no signs of infection. In contrast, all fourteen control animals showed clinical manifestations of RVF after challenge.

We have evidence that administration of RVF virus-like particles (VLPs) induces protective immunity in mice. After immunizations with VLP's, virus-neutralizing antibodies were detected, and 11 out of 12 mice were completely protected from challenge while only 1 out of 12 mice survived infection in control groups. Our study demonstrates that the RVFV VLPs confer protection against RVFV infection in mice.

Citations:

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PHAGE NANOBIOTECHNOLOGY, APPLICATIONS FOR DEFENSE AGAINST BIOAGENTS, NATURAL AND MAN-MADE

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The effective and rapid identification, treatment and prevention of bio-agents remain a complex issue. Drug-resistant pathogens are a growing menace to all people, effecting civilian and military operations and even causing problems in military field hospitals. From *Anthrax* to *Staphylococcus aureus*, and multidrug-resistant TB, the list is growing. The threat of newly emerging and engineered microorganisms further complicate the issue. The use of bacteriophages and their derivatives may provide a useful alternative to current approaches.

Phages are very small viruses that destroy by lysing or alter by DNA modification, select bacteria. The idea of using phage as a therapy for infectious bacterial diseases was first proposed around WWI and since then has been a key tool within Eastern Europe. More recently lytic and other components have been isolated. This has further broadened the potential of phage derived technologies with applications for treatments, preventatives, decontaminants and diagnostics – and has given rise to a growing interest in phage nanobiotechnology, or the use of natural or modified phage or their components for next generation technology development. The use of these enzymes has been further expanded to include replacement or enhancement of antibiotics to treat disease including those caused by various BW agents such as anthrax.

Examples and data of various applications of phage nanobiotechnology will be presented. These included applications of lytic enzymes as a spray or fogger for decontamination, incorporation on wounds or dressings, as well as a preventative for deployed forces. The utilization of this technology for selective lysing for identification and potential for replacement of monoclonal antibodies for improved detection will also be discussed. Other listed opportunities will include vaccine development, nanowire/nanotechnology and new bacteria evolution.

Key Words: Bacteriophage, nanotechnology, antibiotic resistance, phage therapy, lytic enzymes

PROTECTION OF AIRPORTS AGAINST CBR AGENTS

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In spite of the fact that terrorist organizations are known to be focused on aviation assets and to have acquired or seek to acquire biological agents, few of the world's airports currently have any level of detection or protection from terrorist attacks using CBR. An operational pilot project at an international US airport was initiated in 2008 to develop and demonstrate a practical detection capability that facilitates effective and affordable real-time CBR-threat detection in airports and other critical infrastructure. The pilot project has focused initially on bioaerosol threats since a bioaerosol release is the least likely to be detected and will likely have the most catastrophic impact on the aviation system. The detection system utilizes layers, or tiers of detection equipment to minimize cost and false positives. The sensors are networked into a command and control system similar to traditional security monitoring equipment.

This presentation will first present an overview of the equipment installed and the CONOPS developed. Results from tracer studies showing the spread of tracer aerosol will be shown. System performance is assessed against the following measures of effectiveness: 1) ability to detect the release; 2) ability to detect the release in time to mitigate the impact of the release, assuming HVAC mitigation measures are deployed; 3) ability to determine the exact nature of the agent; and 4) ability to detect the release in time to stop an agent released in one airport from spreading to other airports.

FOI-R--2985--SE
DIM Developing Techniques - Systems

PORTABLE RAMAN DETECTION INSTRUMENT FOR RAPID BIOLOGICAL AND
CHEMICAL IDENTIFICATION

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Built to quickly and accurately identify threats in the field, the StreetLab Mobile delivers breakthrough 2-in-1 chemical and biological identification capabilities in a go-anywhere, user-friendly handheld instrument.

Using Raman spectroscopy, the StreetLab Mobile can identify a broad range of chemical substances such as toxic industrial chemicals, toxic industrial materials, explosives, and narcotics. The StreetLab Mobile can also identify biological pathogens such as anthrax, E.coli, ricin, ... after concentration of the pathogen of interest with antibody-coated magnetic capture beads and sensitive Raman-based detection using antibody-coated nanometer sized SERS tags.

The chemical identification requires no sample preparation at all while the biological identification requires very little sample manipulation, thus minimizing hands-on time and maximizing the ease of use. In addition, the reagents used for biological identification are lyophilized to ensure long-term stability under a wide range of temperatures.

Rugged, with a 5-hour battery life for field operation, yet ergonomic and lightweight, the StreetLab Mobile reliably identifies liquids, powders, and solids. The StreetLab Mobile is also capable of analyzing unknown substances from a safe distance through extended-range wireless technology and hands-free remote operation.

Toward a new biological identification solutions

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Hostile use of chemical, CBRN agents is a constant threat on the battlefield and more and more in civilian environment. Today, advanced technologies provide the detection, identification, early warning and response capabilities in order to protect friendly forces and local populations. Modern armed forces systematically deploy assets such as CBRN reconnaissance vehicles, beacons and field laboratories on out-of-area operations, laboratories while advanced data management and network solutions help them to optimise the use of these assets. As adversaries and theatres of operations evolve, CBRN threat environments are harder to characterise. The emergence of asymmetric warfare has heightened the need to improve performance, basic functions (detection, identification...) and to optimise CBRN security network deployment and data management to mitigate risks for forces in the field.

Thales is building on its operational analysis capabilities to further enhance the performance, mobility and usability of future CBRN solutions. Thales is developing a new generation of miniaturised equipment, as the biological compact laboratory, as well as detection/identification payloads for UAVs. Expert systems are under development to provide context-sensitive support to operators and guide them through recommended procedures and protocols.

Furthermore the continuous biological exposure monitoring need to be investigate because it is one of the most critical gap in current warfighter capability. Existing biodetectors, when available, are still expensive and heavy, limiting both their availability and field deployment. There's thus a need for a small, robust and autonomous aerosol sampler able to record biological exposure of a soldier during the whole duration of a mission.

The presentation will focus on on-going developments for biological and chemical agents identification. Regarding the biological autonomous monitoring, a specific discussion will be put on the whole solution, from operational requirements, combat suit integration, sampling, post sampling treatment and analysis.

The lecture will be the opportunity to overview new concepts of compact systems allowing automated process from sample collection to analysis result.

REAL-TIME MALDI TOF MS ANALYSIS OF BIOLOGICAL AEROSOLS
AND CONTAMINATED PAPER DUST WITH THE BIOSPARQ™ DETECTOR

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In a continuing development, a demonstrator bioaerosol detector based upon MALDI TOF MS has been developed called BiosparQ™. BiosparQ™ is a unique detector that is able to analyze a biological aerosol on a particle by particle basis in real time, and provide special-level information for each particle. This opens the possibility to discriminate low concentrations (10-100 ACPLA typical) of harmful biological agents in high concentrations of biological and non-biological background particles. The single particle analysis approach solves the problem of high false alarm rates in existing bio aerosol trigger detectors. Since particles are analyzed in near real time (e.g., less than five minutes), reliable information is produced almost immediately. This is a big advantage for military commanders and for civil authorities, who need reliable information directly after a biological incident in order to take the right decisions in time.

Two examples of applications of BiosparQ™ will be presented. The first example is an example in a military context, a challenge of the BiosparQ™ detector with mixtures of biological and non-biological aerosol particles. The results of extensive tests with mixtures in a bio aerosol test chamber show that BiosparQ™ is able to analyze aerosolized mixtures of biological agents in real time.

The second example is an application in a civil context, mail screening for harmful bacillus spores. Trials with non-contaminated and contaminated paper dust samples clearly show that BiosparQ™ is able to identify bacillus spores in paper dust. The analysis of a dust sample takes less than 2 minutes.

SR-BIOSPECTRA: A HIGH-SENSITIVITY STANDOFF BIOLOGICAL DETECTOR FOR
FIRST RESPONDERS

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The development of the SR-Biospectra prototype was funded by the Chemical, Biological, Radiological-Nuclear, and Explosives Research and Technology Initiative, led by the Defense and Research Development Canada's (DRDC) Centre for Security Science. The prototype's goal is to fill a deficiency in standoff detection of airborne biological warfare agents (BWA) by enabling their efficient detection with a relatively small, low-cost sensor that can be used by first responders in the protection of critical infrastructures. The prototype is designed to work inside or outside, during day or night, and optimized for distances from 5-100 m which are of key interest for such applications, but detection can still be achieved at longer distances.

SR-Biospectra is a UV laser-induced fluorescence (LIF) lidar. A modular design was implemented allowing the development of a light optical head that can be placed on a pan/tilt unit and having a reduced footprint (63 x 30 x 40 cm). Most of the electronic components are housed in a remote enclosure the size of a desktop computer case. LIF from aerosols is measured over 32 channels to detect and classify BWA. The prototype includes a subsystem that maximizes the SNR in daylight conditions.

The prototype was tested during three evaluation campaigns organized by DRDC Valcartier and DRDC Suffield. Data was recorded over 150 releases of BG, EH, MS2, OV, naturally occurring spores, and products such as mace and pepper spray which are of importance for public security. The sensitivity threshold was less than 40 ACPLA at a distance of 100 m for all measured aerosols. Day-time measurements were also performed and the detection threshold was ~200 ACPLA. Blind tests were performed and the results were satisfactory, both for detection and classification of threats. Typical results will be presented and the classification and day-time measurement efficiency will be discussed.



Stand-Off Detection of C-Agents Parameters of Chemical Imaging and Application

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Stand Off detection principles follow the idea of analyzing hazardous gases from a safe distance. The ability of imaging analytical results additionally provide easy to understand information about location, distribution and movement of gases in the air. Military operations but also Civil Task Forces increasingly make use of these features while dealing with chemical accidents with the safety at large public events or simply environmental questions.

In Germany a novel solution has been developed in cooperation with the Office of population protection-and disaster prevention (BBK) for the use by specialized Fire Departments.

Challenges with stand-off detection are the way of dealing with environmental and atmospherically generated influences. With naturally given constraints, the most important features are sensitivity and distance that can be used in order to detect chemical compounds still at useful concentration levels. Analytical capability is requested when accurate identification of compounds in the gas phase is requested.

The detection system that has been developed to optimize these features is discussed in detail. Based on sensitive Fourier Transform Infrared (FTIR) Analysis the actual system is equipped with means providing pixelwise chemical analysis in visual format. The instruments design improves the sensitivity and optical resolution at large distances. The scanning operation principle allows full spectral analysis in combination with new algorithms for data evaluation. A couple of applications explain the use of the instrument with operational and environmental aspects.

MEDICAL COUNTERMEASURES AGAINST NERVE AGENT POISONING: DO WE
NEED NEW OXIMES?

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Presently available medical countermeasures are of limited effectiveness against poisoning by various nerve agents. This is mainly due to inadequate efficacy of oximes used as reactivators of nerve agent inhibited acetylcholinesterase (AChE). In an ongoing approach thousands of oximes have been synthesized in the past decades and their therapeutic effect was mainly tested in rather simple animal models since ethical considerations prohibit the testing of oximes in intentional human nerve agent poisoning. Due to marked species differences results from therapeutic animal models can hardly be extrapolated to humans. Hence, a substantiated evaluation of the value of new oximes as antidotes against nerve agent poisoning requires the ascertainment of comprehensive in vitro and in vivo data. Recently, our group developed a concept for the well-founded investigation of oximes. This includes the determination of kinetic interactions between human and animal AChE, nerve agents and oximes, the investigation of pharmac- and toxicokinetic parameters and the alliance of in vitro and in vivo human and animal data. At present, a complete data set is available for a number of nerve agents, oximes and species enabling the validation of the concept and the further assessment of novel oximes as antidotes against nerve agent poisoning. Now, a huge database is available giving a deepened insight into the potential and limitations of oxime treatment. This paper will provide an overview on the present status and will discuss the need for the search of new oximes in order to cover the whole range of organophosphorus compounds.

STRUCTURES OF OXIME-BOUND PHOSPHONYLATED-
ACETYLCHOLINESTERASE: NERVE AGENT REACTIVATOR
MECHANISM AND DESIGN

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Organophosphorus nerve agents and insecticides irreversibly inhibit the essential enzyme acetylcholinesterase (AChE) by a rapid phosphorylation of the catalytic serine residue. Structure based design of effective reactivators as antidotes to various organophosphonates requires detailed structural information on how the reactivators interact with the phosphorylated AChEs. Recent studies offering structural information suggests that carboxyaminopyridinium containing oximes and *bis*-pyridinium aldoximes have distinctively different interactions with AChE and that a hydrogen bonding network among Glu334, His447, (wat) and the oxime is necessary to deprotonate the oxime for reactivation. Based on these insights, we propose that a combination of protein crystallography, molecular dynamics simulation, high throughput screening of chemical libraries, enzyme kinetics and molecular docking can provide a venue for the rational design of novel nerve agent antidotes.

DEVELOPMENT OF CATALYTIC BIOSCAVENGERS FOR THE PREVENTION OF ORGANOPHOSPHORUS NERVE AGENT TOXICITY

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Organophosphorus (OP) nerve agents represent a risk to both military and civilian populations. A novel approach for counteracting the toxicity of these agents is the use of enzymes, both stoichiometric and catalytic, as bioscavengers. Human serum butyrylcholinesterase was granted Investigational New Drug status by the US Food and Drug Administration in 2006, as a first generation bioscavenger for the protection of humans against OP nerve agents. However, a major limitation of the first generation bioscavenger is the 1:1 stoichiometry between the enzyme and OP. Therefore, efforts are directed at developing catalytic bioscavengers, which will neutralize/hydrolyze multiple OP molecules. To avoid any complications related to adverse immune reactions, three enzymes from human sources are being considered as catalytic bioscavengers: (1) Hu prolidase; (2) Hu senescence marker protein-30 (SMP-30); and (3) Hu paraoxonase 1 (PON 1). Toward this effort, we isolated native prolidase from human erythrocytes, SMP-30 from mouse liver, and PON1 from human plasma. Recombinant Hu prolidase and Hu PON1 expressed in *E. coli* and insect larvae, respectively, were purified. The OP hydrolyzing properties of native and recombinant enzymes were characterized and compared with those of bacterial organophosphorus hydrolase (OPH) and organophosphorus acid anhydrolase (OPAA), two enzymes with well characterized OP hydrolyzing activities. At concentrations of 10 μ M, none of the mammalian enzymes were as efficient as bacterial OPAA and OPH in hydrolyzing G-type nerve agents. At concentrations of 1 mM, all three enzymes hydrolyzed soman, sarin, and cyclosarin; Hu prolidase hydrolyzed tabun also. These results suggest that wild-type Hu prolidase, Hu SMP-30, and Hu PON1, are not suitable as catalytic bioscavengers because they display high K_m values for nerve agents and hydrolyze the less toxic stereoisomer. Efforts are underway to improve the K_m and stereoselectivity of these enzymes using site-directed mutagenesis and/or directed evolution techniques.

**METABOTROPIC GLUTAMATE MODULATORS USED AS ANTICONVULSANTS
AGAINST SOMAN INTOXICATION: A MICROINFUSION STUDY IN RATS****Pål Aas, Siri Enger and Trond Myhrer****Norwegian Defence Research establishment, Protection Division, P O Box 25, NO-2027
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Examination of critical subreceptors in the seizure controlling perirhinal cortex of the brain has revealed that microinfusion of ionotropic glutamatergic antagonists can exert anticonvulsant efficacy against soman-induced seizures. Specification of receptors in seizure controlling brain areas would probably lead to a shorter list of potential anticonvulsants against nerve agent poisoning. The purpose of the present study was to investigate whether modulators of metabotropic glutamate receptors (mGluRs) may ensure anticonvulsant effects when microinfused into the perirhinal cortex. The results showed that the mGluR5 antagonist MPEP hydrochloride (2-Methyl-6-(phenylethynyl)pyridine hydrochloride) and the mGluR2/3 agonist DCG-IV ((2*S*,2'*R*,3'*R*)-2-(2',3'-dicarboxycyclopropyl)glycine) caused full protection against seizures or increased latency to onset of seizures, whereas the mGluR1 antagonist LY367385 ((*S*)-(+)- α -Amino-4-carboxy-2-methylbenzeneacetic acid) did not produce anticonvulsant efficacy in response to systemically administered soman (1.3 x LD₅₀). Low doses of the above modulators had no anticonvulsant effects, whereas too high dose of MPEP resulted in proconvulsant effects. The results suggest that the perirhinal cortex is a likely site of cholinergic recruitment of glutamatergic hyperactivity after exposure to a convulsant dose of soman. Modulators of mGluRs may represent an alternative or supplement to ionotropic glutamate antagonists as anticonvulsants against nerve agent-evoked seizures.

INHALATION OF ALKYLATING MUSTARDS CAUSE LONG-TERM DAMAGE IN
AIRWAYS INCLUDING PERSISTENT IMMUNE ACTIVATION AND GROWTH OF
CONNECTIVE TISSUE

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Alkylating compounds such as sulphur and nitrogen mustards cause severe injury to the respiratory tract when inhaled. At high-dose exposure this will lead to serious tissue damage, while at lower doses these chemicals rather act as lung irritants directly provoking the lung epithelium to produce pro-inflammatory mediators. We have previously demonstrated that non-lethal exposure of the alkylating mustard melphalan causes damage to the respiratory epithelium followed by an acute inflammatory response and lung edema. The acute phase is followed by long-term respiratory complications characterized by bronchitis and lung fibrosis. In this study, we utilized a mouse model for airway inflammation induced by intratracheal exposure of melphalan, in order to define early and late events in the pathogenesis such as early expression of pro-inflammatory cytokines, recruitment of inflammatory cells to airways and late-phase fibrosis. ELISA was used to measure cytokine levels of interleukin (IL)-1 β , IL-6 and IL-23 in bronchoalveolar lavage fluid and serum from mice. By using different types of knockout mice lacking either the predominant T lymphocyte subset expressing the $\alpha\beta$ T cell receptor (TCR) or the minor subset expressing the $\gamma\delta$ T cell receptor, we were able to investigate the roles of different lymphocyte subsets on the inflammatory response. Exposure to the alkylating agent induced an acute burst of pro-inflammatory cytokines and neutrophils in airways within 24 hours. The acute phase was followed by a sustained lymphocyte response that persisted for at least 14 days with resulting lung fibrosis. Engagement of T lymphocytes, particularly the $\gamma\delta$ T cell subset, was crucial both for the acute cytokine and neutrophil response, and for the late-phase lung fibrosis as indicated by the lack of response in T cell deficient mice. Our data demonstrate that T lymphocytes play a prominent role in the pathogenesis of long-term lung injuries caused by strong alkylating agents.

CHEMICAL IDENTIFICATION TO REDUCE FALSE ALARMS

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Warfighters and frontline emergency service workers need to ensure that they protect themselves against the possibility of a chemical agent attack. They need small light-weight equipment that they can carry on every deployment without hindrance, that will warn them when to take evasive action. However they need to be able to trust this equipment. The consequences of a false positive detection are very costly, both in terms of time and monetary value, having to deploy CBRN protective equipment and possible decontamination procedures. The consequence of a false negative detection could be fatal. Therefore improving the reliability of front-line detection equipment is of utmost importance.

This presentation will describe how one emerging technology FAIMS (Field Asymmetric Ion Mobility Spectrometry) is providing the sensitivity and discrimination required to dramatically improve performance, by allowing fast and reliable chemical identification. This paper will describe how this is being implemented and the benefits this approach will bring.

Selex Galileo is one of Europe's leading sensors solutions companies and the UK's foremost supplier of electronic systems for military platforms in the air, at sea and on land. Over the past few years we have been building on this legacy and applying our expertise to new sensor technologies for new markets. Working with our key technology partner Owlstone Ltd we believe we can offer a solution to this problem. This presentation will show how the teams have worked together to mature this solution and aims to discuss how, working together with the end-users, we believe its benefits can be delivered.

**INTEGRATION OF SAMPLING STRATEGIES WITH NEXT GENERATION FIELD
PORTABLE GC-TMS AND NEW METHODOLOGIES FOR RELIABLE
IDENTIFICATION**

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Accurate detection and identification of threat chemicals involves a methodology trilogy; sampling, analyzing and reporting. The first and arguably the most important step is getting a representative sample to the instrument. Varying concentrations, wide ranges in volatility and chemical reactivity compound the problem of sampling and sample transfer to the instrument. Non-volatile chemicals present even greater challenges in sampling due to the requirement to extract or remove the chemicals of interest from a wide variety of matrixes. Solid phase micro extraction (SPME) shows promise of providing a sampling medium that is easy to use, has broad based chemical affinity and can sample from a variety of matrixes. SPME comes close to the universal sampler; however it is limited in sensitivity and does not uniformly extract all compounds. A modular toolkit of sampling accessories with SPME as a primary screening tool can offer a path to effective sampling strategies.

The second step of the trilogy is high speed GC-MS with sample runs on the order of 3 minutes for volatile and semi-volatile organic compounds and total instrument cycle times of approximately 6 min. Optimizing field instrumentation for speed addresses the requirement to provide information as quickly as possible. While the single most prevalent error in defining chemical contamination is in sampling, the ability to process samples quickly allows for multiple samples/replicates to be taken.

The final step in the trilogy involves data handling and information reporting. In laboratory GC-MS a chemist creates a target compound method for a specific group of chemicals. The method uses GC retention times and mass spectra for identification, the combination of which provides the most reliable method of identification with GC-MS. It is not feasible to use this approach for thousands of chemicals and unknowns. Therefore innovative methods that narrow the possible candidates for reliable identification are required.

FOI-R--2985--SE
Commercial Developing Techniques

ADVANCES IN GAS/VAPOR DETECTION FOR CWA TIC DETECTION

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Before 2000 the military focused on Chemical Warfare Agent (CWA) threats and the First Responder community focused on Toxic Industrial Chemical releases. Since 2000 the needs of these two markets have approached each other with increasing speed. It is probable in the near future that the needs of what was once considered two separate markets will merge. This seminar will explore the following areas of convergence:

- **Broader protection:** Militaries are not only fielding CWA specific detectors but also Confined Space Entry (CSE) monitors to provide safe entry into caves, industrial plants and tunnels where common atmospheric conditions can kill. Conversely, since Aum Shinrikyo's 1995 Sarin attack, first responders have embraced CWA detection. Increasingly we are seeing "orthogonal" or multi-sensor products fielded to provide greater sensitivity and fewer false alarms for CWAs. Adding sensors for common CSE threats is the next logical step.
- **Greater gas/vapor selectivity:** Developments in both Differential Ion Mobility Spectroscopy (DMS) and pre-concentrators will potentially provide for much greater specificity from handheld technology for CWAs and TICs.
- **Reach-back and interoperability:** today's detectors increasingly feature wireless capability so that the sensor readings in the field can be fed back to incident command in real time. Some manufacturers have fielded their own proprietary portable wireless networks but the next shift will be TCP/IP internet based open platform networks open to all comers.
- **Intrinsic safety:** developers of what used to be just CWA detectors must embrace the intrinsic safety electronics safety precautions insisted upon by the users of industrial and first responder gas detection equipment.

We are certainly already beginning to see some of these changes hit the market. However, proactive end-user/manufacture partnerships will increase the speed of these paradigm shifts.

CURRENT AND FUTURE DEVELOPMENT OF MOBILE BIOLOGICAL
IDENTIFICATION SYSTEMS

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As especially the combination of high lethality and relative easy production of biological warfare agents (BWA) poses a challenging task for both civilian and military first responders world.wide, new and better systems capable in monitoring, detecting, sampling and identifying BWA are urgently required.

Rheinmetall Defence is a leading supplier of mobile CBRN reconnaissance systems on various vehicle platforms and deployable laboratories. Based on individual mission profiles of our customers we develop and integrate latest sensor system.

As an example of, Rheinmetall presents a new fully integrated Bio-Reconnaissance System recently launched to different customers. As the system also provides additional detection of nuclear and chemical warfare agents its main feature is the fully equipped Bio-Recce suite. Combined with a multifunctional external manipulator (remote controlled) and an aerosol detector/sampler, the glove box-integrated analysis system offers full sample processing capabilities. Subsequently immunological and real-time PCR technologies are used to identify up to a range of 21 different BWA – the necessary primers and antibodies being developed by Rheinmetall i.a.w. customer requirements. All CBRN sensors are integrated in a unique data processing system with appropriate system software, allowing for easy device and report management.

The modular approach allows for both CBRN and medical tasks by adapting the sensor settings accordingly. The open system architecture will facilitate future upgrades regarding e.g. improved sensors. Some of these topics will be discussed in detail.

FOI-R--2985--SE
Commercial Developing Techniques

CBRN – Alert Monitor

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CBRN – Alert Monitor is a common development of Bertin Technologies, CEA (French Atomic Energy Commission), Proengin and Saphymo SA.

The purpose of CBRN – Alert Monitor is to alert in case of Chemical, Biologic, Radiological, Nuclear risks.

For RN risks:

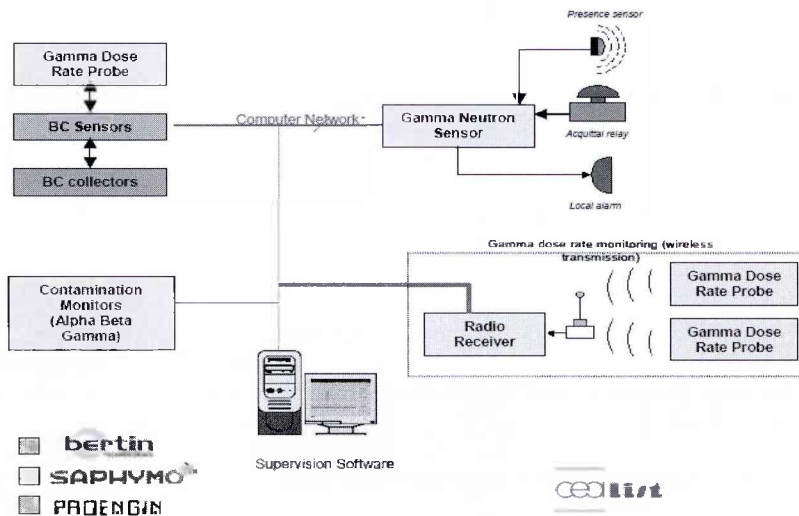
One monitor detects a gamma-neutron source being in/on humane body or vehicle when entering the protected site.

An other monitor represents an autonomous probe to detect any increase of the radioactive background.

A third one is an alpha, beta and gamma aerosol monitor to survey any radioactive contamination of the ambient air.

For CBRN risks:

This monitor is equipped with biological and chemical sensors + gamma probe to alert any potential risk related to any abnormal increase of biological, gamma background in the ambient air or in a presence of any chemical agent of terrorist origin.



This system is offering a global solution (CBRN monitors + supervision software) to alert dedicated agents in case of CBRN attack in critical infrastructures.
So, the detailed technologies uses for each monitor will be presented.

CLINICAL DEVELOPMENT STATUS OF THE THIRD GENERATION SMALLPOX VACCINE IMVAMUNE®

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Background

Currently developed as a stand-alone third generation smallpox vaccine, IMVAMUNE® (MVA-BN®) is a live, highly attenuated vaccinia strain vaccine which does not replicate in human cells.

Methods

Enrolling vaccinia-naïve and –experienced individuals 18 - 80 years of age, 14 clinical studies evaluating the safety and immunogenicity of IMVAMUNE® have been completed so far or are ongoing. Subjects are closely monitored for adverse reactions, particularly for cardiac events. Humoral and cellular immunogenicity are measured using vaccinia-specific ELISA, PRNT and ICS/ELISPOT.

Results

More than 2,700 subjects have been vaccinated with > 4700 doses of IMVAMUNE® including more than 1000 subjects from risk groups with contraindications for conventional smallpox vaccines, i.e. HIV infected and atopic dermatitis (AD) patients. IMVAMUNE® has proven to be safe in healthy individuals as well as in populations with impaired immune function. IMVAMUNE® induces a rapid and strong vaccinia-specific immune response, comparable between healthy subjects and at-risk groups, and is non-inferior to traditional vaccines like Dryvax®, used during the eradication of smallpox. A single vaccination with IMVAMUNE® induces a faster immune responses up to 100% seroconversion two weeks post vaccination, compared to similar levels four weeks post vaccination with Dryvax®. Furthermore, one or two vaccinations with IMVAMUNE® induce a long-lived immunity.

Conclusions

IMVAMUNE® is considered safe and immunogenic in healthy subjects, but also in persons with underlying diseases such as HIV infection and AD. Immune responses elicited by IMVAMUNE® are non-inferior to Dryvax®. This confirms that IMVAMUNE® is a suitable candidate for use in the general adult population including those with contraindications to conventional smallpox vaccines.

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Integrated Genomic Analysis System for Recognition of Known and Unknown Biothreats

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Abstract: The use of biological agents in warfare and acts of terrorism is facilitated because many pathogens occur naturally and can be obtained legally and illegally from multiple sources. This biosecurity risk is complicated because the increasing availability of technical information embedded in important scientific and technological discoveries related to synthetic and combinatorial biology. Microbial genomic information can aid the development of detection, prophylactic and therapeutic countermeasures, however, the exploitation of this data remains limited. Many of the computational techniques used for biothreat genomic analysis have specificity and sensitivity limitations that affect pathogen source attribution and classification resolution. Moreover, most recognition techniques are dependent of previous information to perform classification. Here, we present an innovative and powerful genomic and bioinformatic approach capable of identifying within a particular organism species- and strain-specific genome barcodes and motif fingerprints. These short genomic elements discriminate lethal vs. non lethal agents, natural vs. genetically manipulated and known vs. unknown threats. The application of this technique in the recognition of DNA viruses (*smallpox and monkeypox*), RNA viruses (*Ebola and Arenaviruses*) and bacteria (*Francisella tularensis*) will be presented. Our approach also includes an integrated management system to track the data generated during the classification process. A visualization tool allows represent genomic information into a tempo-spatial context and to track the source of origin of the sample. Our implementation represents a paradigm shift in the recognition of microbes and in the development of future integrated biodefense systems (diagnostics, surveillance, prophylactics and therapeutics). The implications of our approach in the enforcement of the Biological and Toxin Weapons Convention and forensics will be highlighted.

FOI-R--2985--SE
Commercial Developing Techniques
IDENTIFICATION OF VIRUSES, BACTERIA AND TOXINS USING AMPLIFIED
SINGLE MOLECULE DETECTION.

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Using an amplified single molecule detection platform based on padlock probe and proximity ligation, detection of very low amounts of nucleic acid or protein molecules is achieved. Protein and nucleic acid analyses are performed in a similar fashion using circularizing DNA probes, rolling circle amplification and fluorescence-based counting of single reporter molecules. This allows a single system to be used for detection and identification of different types of biological threats, such as bacteria, bacterial spores, viruses, and toxins and down to single viral or *Bacillus subtilis* particles or bacterial genomes can be detected.

The ability to accurately detect and identify microorganisms, or other potential agents of biological warfare, in field samples is an essential part of any biosecurity strategy. Any analysis platform should ideally fulfil a number of criteria such as high selectivity, a low limit of detection (LOD), a wide dynamic range, quantitative precision, multiplexing capability, high speed, high throughput, and low cost.

Our amplified single molecule detection (ASMD) platform is based on padlock probe and proximity ligation assays for targeting nucleic acids and proteins, respectively, with a high selectivity and low limit of detection. The digital readout format is based on single molecule counting, contrary to the average measurement strategies used by most biomolecular analysis methods. This yields high precision over a wide dynamic range.

Q-linea is developing fully automated instrumentation for ASMD identification readout and preparation. In the novel instrument, we use line illumination in conjunction with high-power lasers and multiple CCD line detectors to achieve an increased sampling rate. We expect this to provide a substantial improvement with regards to the time required per sample and to the limit of detection achievable with the detection instrument. Typically each sample is analyzed for 30 seconds and time to answer can be as low as 35 minutes.

**NEW BC VACUUM DECONTAMINATION TECHNOLOGIES
FOR MISSION CRITICAL SENSITIVE EQUIPMENT**

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Different kinds of sensitive equipment can be mission critical. The chemical and biological decontamination of optical and electronic equipment which cannot be treated using aggressive liquid decontamination chemicals can be carried out by special vacuum procedures. The overall objectives of the studies were to apply the vacuum technology to decontaminate CB contaminated sensitive subjects and to determine the important physical and chemical decontamination parameters.

The vapour pressure is the physical parameter which defines the volatility, persistency and mobility of the CWA. The conditions for removing CWA from sensitive equipment are established by treating the subjects to be decontaminated with vacuum and thermal energy. Complicated geometrical housing structures and surface sections that cannot be accessed by wet chemical decontamination are also reliably decontaminated in this way. Tests carried out show for C contaminated subjects (CWA challenge: 10 g/m²) after the vacuum decon procedure a residual content and a desorption rate of CWA that can be below the relevant NATO test criteria. The vacuum decontamination technology represents practically a chemical-free “dry” decontamination of CWA. After the vacuum chamber is being loaded with the relevant sensitive equipment the decontamination procedure runs practically automatically so that the personnel requirement is low in comparison.

The vacuum B decontamination is a new patented technology for this purpose. The procedure is carried out by degrading biological warfare agents by pressure induced impact of gaseous biological decontaminants.

For security reasons the removed gaseous products are immobilized in a special NBC-Filter unit.

The study describes the relevant B and C decontamination procedures and shows the new test results concerning the decontamination efficiency as well as the material compatibility.

The described vacuum process is fast becoming the decontamination technology of choice for sensitive equipment for many armed forces and civil defence organizations.

FOI-R--2985--SE
Commercial Developing Techniques

DESIGN AND EVALUATION OF ESCAPE AND CBRN RESPIRATOR CARTRIDGES
USING NANO GOLD CARBON MONOXIDE OXIDATION CATALYST

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Carbon monoxide (CO) is a toxic gas formed by incomplete burning of organic materials. CO may be present in an environment from a wide range of sources. These include uncontrolled sources such as fires and explosions or from intentional combustion sources such as engine exhausts and detonation of artillery shell propellants. Carbon monoxide is an odorless, colorless gas that can cause headaches within hours at 500 ppm and can be lethal in a few minutes at 10,000 to 40,000ppm.

3M has developed and scaled up a reproducible, economical method for preparing nanogold CO oxidation catalysts that are extremely active at ambient temperatures and humidity. Conventional ambient temperature CO oxidation catalysts are either poisoned by water vapor (such as hopcalite) or require large quantities of expensive platinum group metals to oxidize high CO concentrations.

This paper will present information regarding the design and evaluation of two Individual Protection respiratory cartridges that use 3M's nanogold CO oxidation catalyst. The first is for a carbon monoxide cartridge that will also comply with NIOSH CBRN requirements. The second cartridge is intended for escape from environments containing carbon monoxide and is designed to pass EN 403 requirements.

SX 34 SYSTEM FOR CBRN DECONTAMINATION OF SMALL AND LARGE SENSITIVE EQUIPMENTS

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Today, talking about equipment and instrument for an updated and efficient Army of our present time does not mean anymore listing all sort of weapons, guns and sophisticated sensitive equipment used as powerful means of offense. Talking of these systems today, it means talking about defense, life, budget and financial capability. It can be seen as solving a basic problem, finding a solution when some unexpected and unpredictable action takes place, as it happens in a terroristic attack. One of the major problems in today's tactics is the decontamination of sensitive equipment. This category of instruments includes cockpits, detectors, signal equipment and all the surfaces that, in case of contamination, should not be approached with water or with others corrosive solutions. Protecting them, as part of the mission, has got a fundamental value. Protecting them is equal to save the life of soldiers and to save the efficiency of the equipment.

The traditional way used since now is using solvent or other systems based on a heating /vacuum process in a dedicated shelter. This approach, not only makes this operation complicated and logistically unsuccessful, but is a serious attempt to the material itself. CRISTANINI, since 1972, devoted all its efforts to find out the best ways to solve the problem of decontamination. The company located in Italy, developed simple and intelligent solutions in the specific field of protection and CBRN Defence. No wonder that today Cristanini is considered the world market leader in development & production of systems and products for the decontamination and the detoxification of chemical, biological, radiological and nuclear agents (CBRN). Thanks to the continuous adherence to technological innovations and its R&D program, conducted in cooperation with university institutes and military labs, Cristanini has developed SX 34 SYSTEM, the only real solution available now in market, for the decontamination of sensible equipment. As in other sectors, the solution offered by Cristanini comes with a different approach and, in particular in this case, the difference has been made by studying the problem from another point of view: avoiding any reaction on the sensible part without losing the aim of decontaminating it. SX 34 is the result of a dedicated and creative work and an integrated approach searching for new solutions to highly complex scientific, technological and engineering problems. As part of Cristanini's Family of Decontaminants (FoD), this new SX 34 is a multispectral CBRN decontaminant which performs a thorough decontamination on sensitive material: it is non-corrosive, non-toxic and environmentally safe, suitable for a timely clean-up of CBRN agents on all materials and surfaces.

- SX34 acts by removing vertically substances with a wide range of polarity from sensitive surfaces without damaging them. The decontamination residues can be safely destroyed and disposed of afterwards.
- SX34 acts without spreading contamination or carry agents into gaps.
- SX34 is environmentally friendly (none of its components are prohibited or will be prohibited in the near future, no stratospheric ozone depletion substances are present or substances banned by WHO).

SX 34 has been tested following the French / German Protocol for decontamination testing (STANAG 4653 AEP-58) with positive results in the Military Institutes of Brno, Czech Republic, in the NBC Joint Logistic Technical Centre of Civitavecchia (Rome), Italy and in the CEB laboratory of Paris, France. These tests have been a big challenge since Cristanini decided to swift from the old to the new decontamination philosophy which does not foresee the use of water. Moreover, it was important to show, scientifically, that this decontaminant works effectively and this goal has been successfully achieved. The result is a close cooperation with important companies such as Eurofighter consortium in Civitavecchia and instrument manufacturers. SX 34 is now available in a complete tactical system including a transporting ruggedized case, special vacuum cleaner and 10 SX 34 spray canisters and all the necessary accessories.

As the Company philosophy SX 34 is the solution to a big problem before unsolved; in three words: SIMPLE, RELIABLE and ECONOMICAL.

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**REDUCED THERMAL BURDEN OF CB PROTECTIVE ENSEMBLES THROUGH
THE USE OF ADVANCED SELECTIVELY PERMEABLE BARRIERS**

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This talk will highlight and substantiate the breakthrough performance of two new CB protective ensembles which extend overall operational sustainability. Operational trials have demonstrated that well engineered selectively permeable materials deliver enhanced protection and improved thermal performance while still achieving wear durability objectives. These advancements enable a step change improvement to the thermal burden imposed on the warfighter or incident responder without compromising CB protection or durability.

A selectively permeable stretch laminate has been developed and constructed into a form-fitting CB protective undergarment. When worn with an appropriately selected combat uniform (CU), this layered clothing combination can deliver comfort equivalent to the CU currently worn by the U.S. Army. This thermal performance has been validated over several environmental conditions using a thermal manikin. In addition, end user trials have been conducted that validate the protective performance after 96 hours of wear. Material level live agent permeation test results and system level Man-In-Simulant-Test results confirm that no compromise has been made to the CB protection of the warfighter.

Civil incident responders have long been wearing impermeable chemical protective suits in warm zone operational areas (designated in the U.S. as below Immediately Dangerous to Life and Health levels). The development of a moisture vapor breathable protective suit for operations in these areas now affords responders safe levels of CB protection, as defined by third party standard compliance, while significantly reducing their discomfort and extending their overall mission times. Results from testing conducted to the National Fire Protection Association (NFPA) 1994 Standard on Protective Ensembles for First Responders to CBRN Terrorism Incidents, 2007 Edition and from a major US metropolitan law enforcement end-user wear trial evaluation will be presented.

These recent developments of GORE® CHEMPAK® Selectively Permeable Fabrics illustrate that end-users now have new capabilities available to them that specifically address their needs for reduced thermal burden and extended mission times without compromising specified levels of CB protection and durability.

The Missing Link – CBRN system integration

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End users require seamless integration of their CBRN equipment – particularly respiratory protection equipment, in order to meet their operational needs. The integration between two systems can fall between two companies leading to confusion on who owns the interface and more seriously, a potential compromise of the end user capability.

Further compromises may exist when military CBRN equipment is required to meet recognised safety standards directed predominantly at civilian requirements. Verification may include analysis of joints, seams and connections – but what happens when two individual systems need to integrate? The concept of use for military applications may also differ.

As the number and complexity of protection systems increase to meet customer operational requirements, links between these systems become more difficult to secure, compounding the issue of ownership; who owns the interface between a respirator and a protective suit?

Certification bodies are becoming more aware of the increased dependence of the interfaces and are driving legislation to ensure interfacing systems meet their individual and combined performance requirements.

Avon Protection Systems have recently taken control of a number of challenging interfaces formed where systems integrate with the new C50 respirator. This paper outlines some of the challenges and unique design aspects of those interfaces and the important learning points from the development.

A NEW DEVICE FOR TESTING RESPIRATOR INTEGRITY

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In fit testing, the assumption is that no leakage occurs through the respirator, and the aerosol measured in the respirator comes from face seal leakage. Measuring a high fit factor indicates that both the respirator fits the individual and there are no leaks in the respirator.

While rare to find leakages in new respirators, leaks may occur as respirators age and are maintained by the user. This is of particular concern with respirators that offer high levels of protection, where small leaks could be critical.

A new mask integrity test accessory (MITA) has been developed. The MITA tests critical components of the respirator and overall leakage, using a PORTACOUNT Respirator Fit Tester for the aerosol detector. Tests include drink tube leakage and flow, outlet valve leakage, and overall respirator leakage. A probe mode allows the user to determine the location of a leak using an aerosol probe.

The seal between the respirator and tester is critical for testing respirator leakage. Typically the respirator is mounted on a full or partial head form. Several existing designs were rejected due to the difficulty in mounting the mask or keeping a tight seal. A new head form was developed using an inflatable bladder for the sealing surface. The bladder shape and size were designed so most masks will fit and seal on a single size head form. The full head form design allows using the respirator's integral head harness for mounting.

Data will be presented showing the MITA's ability to validate respirator integrity.

NEW DEVELOPMENTS IN CBRN PROTECTIVE GARMENTS

Daguerre E.

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Military forces operating in CBRN situations can face extreme climatic conditions and particularly very high temperatures and humidity. Paul Boyé Technologies has developed a new generation of CBRN garment specially suited for such conditions.

The new CBRN protective garment was tested in laboratory regarding both chemical protection and comfort. All protection results were in compliance with NATO requirements. Comfort testing and simulation made on the suit showed no limitation of use due to thermal burden. The suit was then qualified for field experiment.

On the field (Djibouti), a real time monitoring system was recording the localization, physiological parameters and loss of individual physiological capacity, for each soldier. Comparing the New generation suit to the in service equipment, using a physiological stress index, showed that:

- Traditional materials are superseded by new technology such as knitted activated carbon fabric, associated with a very high permeable external layer.
- The safety time limits recorded for CBRN protective PPE, are strongly dependant on camouflage design chosen for the outershell. In the case of Djibouti climate (hot & dry) sand coloured version were found to be the best choice.
- Under sun load, it was proved that wearing the new generation CBRN garment lowers the used operational capacity of the wearer by more than 30%.

Field trials under extreme climatic conditions, together with laboratory tests, are needed to obtain a concrete and realistic evaluation of the physiological and operational capacity loss, for military units, engaged overseas, on CBRN scenarios.

New materials developed by Paul Boyé Technologies offer reliable NBC protective products with increased comfort that give the opportunity to face long term tropical military missions with minimal thermal stress.

FORCE PROTECTION - AN INCREASINGLY CHALLENGING TASK

Maj.General Dr. Jonathan Bailey CB MBE PhD (British Army ret., former Director General of Doctrine Development) c)

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To win quickly has always been seen as the acme of success in war. In traditional state-on-state wars, this was often achieved. Today's conflicts are less clear-cut, are conducted "amongst the people", and opponents are less likely to conform to Western measures of rationality. Today's conflicts are therefore likely to be more prolonged and their character less predictable. At the same time, sensitivity to casualties remains an important factor, and the legal requirements for the duty of care of troops become ever more demanding. The provision of adequate Force Protection in such complex situations becomes increasingly challenging, and new approaches are required.

Troops are now exposed to less predictable terrorist threats, which are diverse in nature, hence the traditional "fire door mentality" could be actually quite dangerous. The reason for this is that when being obliged to use traditional legacy protection systems, within an asymmetric theatre, crews will be sorely tempted if not required by SOP, to switch on their COLPRO only when the existence of a threat is known to exist (i.e. to save filter life and restrictions to operations), which, of course, could be too late to save the crew or the mission.

As in the asymmetric theatre, the crew doesn't actually know necessarily when the "fire-door protection" actually begun. This leads therefore to totally different COLPRO system design and SOP procedures.

A few examples of this new system approach will be shown, and the usage of it will be discussed in the presentation.

LABORATORY AND NATURALLY PROPAGATED FRANCISELLA TULARENSIS
SUBSPECIES TULARENSIS STRAINS WITH A COMMON ANCESTOR
DEMONSTRATE DIFFERENT MUTATIONAL SPECTRA.

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Next-generation sequencing generates high-quality data for genotyping bacteria at a pace never seen before. Growing repositories of genomic data offer improved strategies for the design of genetic assays for various applications, such as pathogen detection and microbial forensics. Demonstrating the utility of this technology, we here sequenced strains of the tularemia pathogen and biothreat agent *Francisella tularensis* subspecies *tularensis* (type A) in order to shed light on the origin of a set of unusual European type A strains.

The European type A strains, isolated in central Europe from ticks and mites in the late 1980s, represent to date the only exception to a North American restriction of this subspecies. A natural origin of these isolates has been questioned after a European type A isolate recently was sequenced and found to be nearly identical and derived from a North American prototypic type A strain, SCHU S4. One hypothesis is therefore that the European type A isolates may represent re-isolates of a previously released SCHU S4 strain. A competing possibility, however, is that the European type A strains also could be laboratory contaminants. These hypotheses were explored here by sequencing of another strain derivative of the SCHU S4, previously cultured extensively on artificial media in laboratories.

The data show distinct differences in patterns of insertion/deletions and single nucleotide polymorphism between the strains, supporting that propagation have occurred under different conditions. Thus, we find that the results favor the hypothesis that the European type A strains, rather than laboratory contaminants, indeed are natural re-isolates of a SCHU S4 strain previously released into nature. The results suggests that mutational spectra could be utilized in tracking of *F. tularensis* strains to provide clues to past history.

FORENSIC INVESTIGATION OF RICIN CASES IN THE NETHERLANDS

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Since a CBRN crime will always be followed by the question who is responsible for the crime, preparedness for a CBRN event also applies to the forensic world. To be able to perform a high quality investigation following a CBRN-event, the Netherlands Forensic Institute has started to prepare forensic experts to perform forensic investigations under CBRN-conditions and to develop methods for analysis of biological- and chemical agents.

This latter aspect deals with the development of analytical techniques to detect and identify CB agents as well as techniques to trace them back to their possible source. Besides attendance towards more general forensic and criminalistic aspects like the chain of custody, quality control of applied methods and a sound estimation of the evidential value, also a final forensic report which will hold in the courtroom has to be produced.

This poster presents these aspects based on a recent biocrime case involving an attempted poisoning with ricin, the phytotoxin that can be extracted from the beans of the castor plant (*Ricinus communis*). Ricin is included on many national and international selected agents lists, because of its very high toxicity, general availability and the ease with which the toxin can be extracted from beans.

The described forensic investigation follows various routes for detection and identification of ricin: morphological and microscopic analysis of pieces of evidence, DNA-techniques to detect traces of the castor beans and measurements of ricin biomarkers, all in various backgrounds including food and human blood.

For session:

Detection, identification and monitoring of CB agents

CHEMICAL PROFILING OF CHEMICAL WARFARE AGENTS FOR FORENSIC PURPOSES

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A program has been initiated towards the chemical profiling of chemical warfare agents, in order to support forensic investigations towards synthesis routes, production sites and suspect chemical suppliers. Within the first stage of the project various chemical warfare agents (VX, sulfur mustard, sarin) have been prepared according to well-known or more obscure quick-and-dirty synthesis protocols. After minimal work-up, the crude products were analyzed with GC-MS, affording highly useful mass spectral data sets of by-products. The intact agents were present at moderate to high levels in most crude products. In several cases it was possible to correlate the applied synthesis route to the formation of unique by-products, or to a characteristic ratio of by-products. It was also assessed that for the majority of the crude products the chemical profile remained more or less intact upon prolonged storage. This was remarkable because it was expected that the crude products would decompose rather quickly because of the presence of traces of acid. Finally, the stability of the chemical profile was assessed for the crude VX and mustard gas products in/on several matrices (soil, water, wood, textile and rubber). Dependent on the matrix, VX or sulfur mustard could be detected up to several weeks after liquid extraction or thermodesorption followed by GC-MS analysis, while for some of the crude products the characteristic by-products could still be detected.

For session:

Detection, identification and monitoring of chemical and biological agents

METABOLOMICS FOR CULTIVAR AND PROVENANCE DETERMINATION OF
RICINUS COMMUNIS EXTRACTS

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The castor bean plant *Ricinus communis* gained popularity in Australia in the 1960's as a garden ornamental. However, due to the nature of the plant to produce profuse amounts of progeny, it readily escaped gardens and has now become a common environmental weed in many localities within Australia. Importation of the seed into Australia is also banned. Therefore, the only available source of the seed – and hence ricin – is from specimens growing in the wild. This presents a unique problem to Australian law enforcement and forensic agencies, making techniques that can determine both cultivar and provenance a very useful tool.

To solve this problem, DSTO is applying metabolomic strategies to generated extracts *R. communis* seeds from different specimens collected around Australia. Metabolomics is the comprehensive characterisation of the small molecule metabolites (the metabolome) in biological systems. The metabolome of plants is strongly influenced by the type of soil the plant is grown in, and the type of climate that the plant is exposed to. As metabolomics is focused on small molecules, it is well suited to analysis using both ¹H NMR and LC-MS. Data collected from these analytical techniques were then subsequently analysed using multivariate statistical analysis.

The aim of this presentation is to discuss the concept of studying the metabolome of *R. communis* for cultivar and provenance determination. In particular, recently generated results from the study of the metabolome of both Australian and overseas specimens will be presented. Some of the important chemistry responsible for observed class separations in the statistical analysis will also be highlighted.

DEAMIDATION IN RICIN - A POTENTIAL SOURCE TO FORENSIC ATTRIBUTION PROFILE DATA

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Ricin is a protein toxin that originates from the seeds of the Castor plant (*Ricinus communis*). The history of terrorist and anarchist interest in the use of this toxin has increased the need for new strategies to link an extract (e.g. powder) to its source. It has previously been shown that different cultivars of ricin show different patterns when analysed by capillary isoelectric focusing, CIEF. We can now show that this diversity is due to deamidation at several positions in the toxin.

Deamidation is believed to be an aging process in proteins where glutamine and asparagine residues (Gln and Asn) are nonenzymatically converted into glutamic and aspartic acid (Glu and Asp). The rate of deamidation is structure dependent and each deamidation results in an increase of one unit in mass and introduces an extra negative charge at neutral pH. The extra negative charge changes the pI of the protein and several deamidation sites in ricin hence creates the diversity observed in CIEF. The change in charge also enables separation of deamidated peptides using capillary zone electrophoresis, CZE.

Both identification and quantification of deamidated sites in ricin were here achieved using enzymatic digestion followed by peptide analysis with capillary zone electrophoresis-mass spectrometry (CZE-MS). We have also investigated different cultivars and influence of toxin age to determine how it affects the degree of deamidation. This information is expected to be used as one of the forensic links between an analysed sample and the source of the ricin toxin, i.e. as attribution profile data.

Mass casualty management in Europe following chemical agent release

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The Mass Casualties and Health project (MASH) is a study financed by DG Health of the European Commission to investigate planning, preparedness and responses within Member States following release of chemical or radiological agents.

As part of the MASH project information was collected using an interactive website containing information about the level of preparedness in each EU Member State for the management of mass casualties following chemical release.

The chemical questionnaire covered planning and organisation together with operational aspects of health systems. The latter included detection, identification and monitoring, decontamination, clinical diagnosis, therapy and dedicated capacity for the management of chemical casualties.

Information received has revealed considerable variations in the management of mass chemical casualties around Europe. This presentation will present an overview of the findings and discuss models for training and response already produced in a small number of Member States which could provide a template for an integrated EU policy.

THE CBRN INCIDENT AS A MEDICAL EMERGENCY: LIFE-SAVING TREATMENT VS DECONTAMINATION.

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Background: CBRN incidents where exposed persons become acutely ill or injured represent a special challenge. In Norway, health personnel have previously been required not to deal with any CBRN exposed patients before decontamination. Delaying therapy in acute life-threatening situations must be avoided; the benefits of speedy interventions must be weighed against the risk of exposing first responders and medical personnel to potentially dangerous contaminating agents. Medical personnel experienced in acute medicine and with specialized knowledge about dangers of exposure to the assumed agent should give advice on priority decisions in such situations.

Methods: The Norwegian National Center for NBC Medicine is charged with establishing routines and offering guidance in connection with CBRN incidents in Norway, both pre- and in-hospital. Part of the funding and assignments comes from the Department of Health. It is a section within the department of Acute Medicine in a large university hospital, which is also a level 1 trauma centre and the main emergency hospital of the capital. The medical staff consists of four senior consultants with expertise within haematology, toxicology or infectious diseases. These also do calls and clinical work in their respective departments to maintain their medical competence and first-hand knowledge about changing routines and practices of the hospital. An ICU doctor, plus an emergency medicine nurse who also act as a liaison towards first responder organisations, complete the medical staff.

Results of evaluation: Due to the geography, population pattern and organisation of emergency services in Norway, it is not realistic to expect a full-fledged decontamination facility to be operative within the time constraints for life-saving interventions. After evacuation of patients from the hot zone, first responders will have to deal with medical emergencies on the spot. After removal of contaminated clothes, shoes and long hair the patients should be triaged and if necessary receive emergency treatment from ambulance personnel dressed in protective gear *before* decontamination. To facilitate such a paradigm shift, first responders should have the possibility to confer with physicians possessing specialized CBRN knowledge at all times. In Norway, the senior consultant on call at the department of Acute Medicine is available 24/365, has increased knowledge about CBRN medicine and act as a information/ communication hub in close contact with senior doctors within the hospital and the NBC center personnel.

Conclusion: In addition to written guidelines and active educational support, we believe that easy access to medical CBRN expertise for first responders can optimize medical treatment at the scene of a CBRN incident.

EXERCISE CARDS DEVELOPMENT FOR TRAINING OF PUBLIC HEALTH PERSONNEL
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One of the objectives of the EU-sponsored project *CIE (Chemical Incident Emergencies) Toolkit* is to develop a set of cards suitable for table-top training exercises with personnel from the public health sector. For that reason, we have now developed seven sets of training cards for one biological, one radiological, five chemical scenarios. The chemical scenarios originated in a previous EU project; GSCT (Generic Scenarios Chemical Terrorism). The common nominator of the seven scenarios is a hypothetical situation with an intentional, or unintentional, release of a C-, B-, or R-agent that the trainees shall discuss.

The table-top exercise is to be lead by a competent exercise director with adequate experience of handling emergency management situations. For trainees to benefit the most from the exercise, the director should be well prepared and comfortable with the situation. Trainees may come from different categories of public health personnel and it is probably most efficient to perform exercises in small teams (3-6 persons), but the scenarios *per se* do not set restrictions on the number of trainees. The importance of various issues and the number of topics will depend on category, experience and specific training requirements of trainees. It is recommended to reserve 4-8 hours for an exercise. For optimal gain, it is essential that the exercise is followed by an evaluation. Lessons identified in that process can later be used to improve emergency response plans and as input to subsequent training exercises. Exercises can be conducted with a strict focus on training of standard operating procedures, or be of explorative nature to identify strengths, shortcomings and bottlenecks in established plans, or as part of a process to define and develop plans and procedures. To avoid the risk of conflicting objectives, the number of aims should be limited. Too many exercises try to meet more purposes than they can deal with. In the worst case, this will result in extensive limitations on the evaluation of the exercise itself and the number of conclusions that are possible to draw from it. Thus, a well-prepared exercise is of utmost importance to achieve maximal benefit.

For one exercise, three sets of cards are used. Trainees get *Exercise Cards* which describe the development on the incident scene (the scenario) and provide topics to help the trainees assess and discuss their own actions in a real event. Each *Exercise Card* is related to one of the six different emergency response phases identified – Preparedness, Situation Assessment, Exposure Assessment, Acute Health Effects, Long-term Health Effects and Recovery. The trainees receive the *Exercise Cards* one by one and discuss proper actions with the game director after each card. In addition, the director has a set of *Director Cards* which are intended solely for the exercise leader and serve as a road map making sure that vital issues are not missed. The third set of cards is called *Assessment Cards* and is intended to be studied and discussed by the participants at the end of the exercise. The *Assessment Cards* give the calculated outcome of the scenario in distribution of injuries, number of persons injured, and different categories of injury. One of the *Assessment Cards* also suggests recommended best practices.

In the process of developing the exercise card concept, we have learned that at an overarching level it seems possible to handle chemical, biological or radiological incidents identical, i.e. the same set of generic topics for discussion can be used irrespectively of the type of agent involved. For planning purposes that may well suffice. From our limited experience of conducting exercises with the help of this concept, it seems that some information concerning scenario details has to be sparse whereas other information has to be rather detailed in order to drive the discussion forwards. Finding the right blend of information is a challenge for us in order to further develop the concept of exercise cards.

Issues and Dilemmas in Management of a CBRN Event at the Hospital Level.

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CBRN pose very complicated challenges for hospitals. Each CBRN component whether chemical, biological, radiological or nuclear, poses its own specific dilemmas and challenges. Hospital preparedness poses a series of challenges and many dilemmas concerning operational, ethical, medical and other issues.

The Israeli approach to CBRN, in general, is a generic universal "all hazard approach." The basic module for this is the conventional mass casualty incident. Based on this we have created protocols for various scenarios: toxicological (HAZMAT) event, chemical, biological and radiological. A top-down process from the national level, is introduced and integrated at the hospital level, where every hospital formulates its own specific protocol for the various emergency scenarios.

Every hospital in Israel has an Emergency Committee headed by the deputy director of the hospital or one of the heads of a clinical department who is experienced in emergency medicine and also has organizational capabilities. The Emergency Committee directly reports and is supervised by the CEO and Medical Director = the Hospital Director.

An outstanding challenge is to have the hospital staff familiar with all kinds of scenarios, agents, doctrines, standards of procedure and to achieve a state of constant alert and readiness. In order to meet that challenge a meticulously designed apparatus has been created that builds up capabilities and assesses readiness.

There are additional challenges that a hospital must face in a CBRN. In Israel, every acute care general hospital is designed to have a 20% surge capacity of its licensed number of beds. Decisions must be made regarding allocation of manpower, equipment and space. In addition, close cooperation and collaboration with external agencies such as police, fire department, EMS, the military, the media etc., is mandatory in order to maintain calm and ensure efficient and effective hospital functioning.

Finally, not all dilemmas or challenges have clear cut solutions or solutions that are good enough. There are numerous ethical, strategic and operational situations that require ongoing clarification and thought.

CBRN THEATRE MONITORING SYSTEM

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The proliferation of weapons of mass destruction is now considered as a permanent threat. Many nations and non state groups have now the ability to produce and use CBRN weapons. Even during peacekeeping operations, armed forces are threatened with nuclear, chemical or biological agents. Then it is essential for military forces to acquire an autonomous capability able to warn and identify all the CBRN threat on a theatre.

France initiates an extended acquisition process to improve the system capabilities against CBRN threat and mainly the biological.

The French Air Force has just received the Theater Biological Warning System - SABT (Système d'alerte biologique de théâtre) to support deployed French forces.

This unique integrated system is a complete set of field deployable system including a CBRN head quarter shelter, for data centralization and risk assessment, associated to a set of CBRN warning beacons and a fully automated laboratory for specific biological agent identification.

The SABT is the most advanced CBRN integrated system currently used by the French forces for the external military operations protection. This system benefits from the most successfully completed developments on the market in detection, warning and identification of biological threats. The SABT is aerotransportable, easily deployable, modular and can be configured according to the infrastructure to protect. Following an extended period of tests, the system is declared fully operational. It will offer a biological early warning, identification and dissemination capability during national and international operations.

This presentation will discuss the key design factors, the presentation of the main components and the operational concepts. It is an opportunity to have the overview of the first integrated CBRN concept and the associated operational advantages for the end users.

DEVELOPMENT OF BIOLOGICAL WARFARE AGENTS DETECTION SYSTEM USING
ELECTROCHEMICAL DNA CHIP

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Toshiba Corporation¹, National Research Institute of Police Science², Obihiro University of
Agriculture and Veterinary Medicine³

Objective: Rapid identification of biological warfare agents on site is very important to prevent and minimize damage when a criminal attack with biological threat happens. So we developed a mobile and automatic biological agent detection system using an electrochemical DNA chip.

Method: Nineteen biological agents including, *B. anthracis* were selected as the detection targets. A crude extract of biological agents were amplified by loop-mediated isothermal amplification (LAMP) method using target specific primers and *Bst* polymerase. The amplified DNAs were reacted with the electrochemical DNA chip which based on a DNA probe immobilized electrodes and electrochemical active intercalator Hoechst33258. Anodic current derived from Hoechst33258 was relative to the amount of the target DNA reacted with target specific probes on the electrode. All reactions and measurements were conducted using a disposable cassette which include the reagents for amplification and the DNA chip, and an automatic detection system GLF-M200W (Toshiba)

Result: For anthrax detection, pXO1, pXO2 and genomic DNA were used for target sequences in order to decrease a false positive signal and to speculate pathogenicity. A crude extract of nucleic acids from spore of anthrax was obtained using beads beating method. The target sequences of 10^3 to 10^4 copies could be easily amplified by LAMP, and identification using DNA chip was completed within 70min. We have demonstrated rapid, easy and highly sensitive detection for 19 biological warfare agents. This system will contribute for protection against biological threat.

SMARTDROP: AN INTEGRATED SYSTEM FROM SAMPLE PREPARATION TO ANALYSIS USING REAL-TIME PCR

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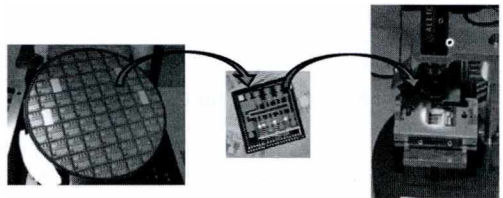
Development of fully integrated CBRN system for continuous monitoring of air or water attracts strong attention from worldwide research laboratories. In particular, there is a great need for integrated, compact and complete analytical systems with good performances in terms of analysis time and sensitivity [1]. The use of molecular biology methods such as real-time PCR (rtPCR) leads to very sensitive systems, with a detection limit of a few nucleic acids (NA) copies per sample. However, great challenges still remain regarding firstly the sample preparation process, and maybe more importantly the integration of sample preparation and rtPCR modules to create a transportable device, easy to use on the field.

The SMARTDROP system developed at CEA-Leti addresses this issue through the joint development and co-integration of:

- a sample preparation module dedicated to liquid sample (either from air collector or either from liquid sample such as blood, river water, industrial water, drink water, ...) with a concentration factor from biological pathogens to NA up to 3000
- a rtPCR module able to process a very small inlet volume (down to 0,5 μ L), divide it and perform a specific rtPCR reaction on each sub-volumes [2]



Integrated system



rtPCR chips and module

SMARTDROP features a purification yield around 50%, a simultaneous detection and quantification of 15 pathogens, a detection limit between 100 and 1000 CFU per sample and a time to result of less than 1 hour. Biological validations were performed with gram⁺ and gram⁻ bacteria, as well as viruses.

Compared with state of the art apparatus, SMARTDROP is more versatile, both on a biological standpoint through generic purification processes and on a multiplexing aspect through the use of microfluidics-based rtPCR devices, has a reduced overall size and weight, and allows to faster detect and identify the threats.

- [1] Lazcka O. et al., Biosensors and Bioelectronics, 2007, 22 (7), 1205-1217
[2] Fouillet Y. et al., Microfluidics and Nanofluidics, 2008, 4, 159-65

UTILIZING THE DATA FUSION OF ORTHOGONAL CBRN SENSOR GRID AND 4D MODELLING FOR EMERGENCY MANAGEMENT

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The requirements for features of emergency management tools both on military and civil sector has been increasing rapidly during past few years. Different systems are insisted to be compatible with each other, despite of the origin and technologies used. Other developing features of these systems are related to simulations, threat scenario analysis and dispersion modelling. As a result of feedback from the field and customers, Environics has developed and fielded 4D modelling features combined with real-time CBRN detection as part new technologies for emergency and CBRN incident management. Environics provides three methods for executing the data fusion and process the complex data into more practical and graphical format:

1. Automatic modelling method
2. Manual forward modelling
3. Manual backward modelling

The basis for the 4D modelling is called SILAM. It has been created to provide an environment capable of supporting various types of dispersion models and suitable for approaching a wide range of tasks. Currently, the dispersion tool in the framework is a Lagrangian dispersion model with a high-precision iterative advection algorithm and a Monte-Carlo random-walk representation of atmospheric diffusion. This modelling has been applied for various applications including toxic releases of forest fires and large scale nuclear fallouts. The model has been developed by Environics to cover large scale of CBRN incidents.

To successfully utilize the modelling system, sensor grid is needed to provide critical information to start actions related to the detected incident. The sensor grid consists of field devices communicating with data fusion servers. However, in practical field applications more sophisticated grid structures are used. Typically, a data fusion utilizing emergency management and monitoring system includes: Orthogonal chemical sensors, biological sensors, radiological sensors with nuclide identification capability and weather sensors with connection to a local/national meteorological authority. Orthogonal chemical sensors provide information about chemical releases, including detected chemical type (or exact compound) with its concentration. The maturity level of biological sensors is lower than with the chemical sensors but information can still be used to enable modelling and analysis. Most critical information for the modelling is meteorological information. Models, such as SILAM, requires forecast information which obviously not available from the field sensor grid. Additionally, information includes wide range of parameters that needs to be pre-processed for the model.

This complex information package will be refined through a data fusion engines into graphical animations and reports that enables the profitable use of system called EnviScreen.

**IPACT: IMPLEMENTATION PROGRAM AGAINST CBRNE
PROLIFERATION AND TERRORISM**

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The combined threat of the proliferation of weapons of mass destruction (WMD) and terrorism requires that all States implement fully their international non-proliferation and counter-terrorism obligations. Complementing that State action, other stakeholders, including industry, academia, non-governmental organisations and civic society need to become part of this evolving governance system. These obligations are framed in the Security Council Resolution 1540. IPACT is developed to implement 1540 and these obligations in Bosnia and Herzegovina.

The program aims at developing comprehensive national strategies to improve and sustain capacities to counter WMD proliferation and terrorism, and at promoting best international and national standards and practices in the implementation of their respective obligations. With Bosnia and Herzegovina as pilot country, the program builds on the experience and expertise in the region.

When approaching the danger of WMD proliferation, one often refers to the problem of safeguarding (non proliferation), security (the threat of terrorism) and safety (accidents). This program will take a holistic approach by integrating the efforts in all three areas.

The basis for IPACT is the IBC Functionality Approach, a methodology that is used in many different security areas including critical infrastructure protection, non proliferation, CBRN crisis management and airline security. Most international support programs lack a structured methodology. In IPACT, the scan is based on a concept of different functions a country needs to perform in order to achieve a certain objective.

The methodologies, models and tools to manage this multi-faceted and multi-stakeholder approach have been developed in one pilot country of the region (Bosnia Herzegovina), and conclusions and recommendations (generic or region-specific) will be extracted from the pilot, in cooperation with other regional and international partners. These tools can then be used by other countries elsewhere in their efforts to develop better-integrated non-proliferation systems (policies, infrastructure, mechanisms and procedures), and to further enhance regional coordination of these measures.

**THE FUTURE OF THE CHEMICAL WEAPONS CONVENTION: TOWARDS A
CONCEPTUALIZATION OF SELECTED MEDIUM- TO LONG-TERM PLANNING
FACTORS BY THE OPCW**

John Hart, SIPRI

The Organisation for the Prohibition of Chemical Weapons (OPCW) has four core objectives (or 'pillars'): (a) the elimination of chemical weapon (CW) stockpiles and CW production facilities, (b) the non-proliferation of CW, (c) assistance and protection against CW and (d) economic and technological development. Once CW stockpiles are destroyed, the parties will further consider the focus and balance of activities the OPCW should undertake. The organization could retain a strong non-proliferation focus that attempts to encompass threats posed by non-state actors and activities that differ from traditional state-run CW programmes. The OPCW might also evolve more towards a technological international assistance and cooperation regime which possesses a standby consultative capacity on chemical warfare issues and which continues to carry out baseline collection and inspections to confirm the consistency and accuracy of state declarations on national defence programmes and chemical industry.

General observations on implementation practice, including the role of institutional factors would be presented. The following topics would then be reviewed: (a) monitoring of chemical trade (e.g. reconciling data submissions), (b) routine inspections of the chemical industry and single small-scale facilities (SSSFs), (c) site selection methodology (including the possibility for nominations), (d) maintenance of Technical Secretariat expertise, (e) the implementation of CWC provisions on consultation, clarification and fact-finding and (f) the derivation and use of information for CWC implementation purposes. Each topic would be assessed from the perspective of confirming the non-production of CW against the following non-compliance scenarios: (a) traditional state CW development programmes; (b) standby capacity by states for either traditional military or non-traditional agents; (c) non-lethal and less-than-lethal agents developed by states for law enforcement, peacekeeping and the like which may also serve as a basis for a standby capacity for faster CW 'breakout'; and (d) non-state actor activity. Although there are numerous CWC implementation issues (most of which date from the 1993-97 Preparatory Commission), it is hoped that such a concept paper would provide a basis for better understanding political possibilities and technical requirements for maintaining and strengthening CWC implementation over the longer-term.

The Verification Dimension – Reporting on Verification Implementation

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When the Conference on Disarmament agreed, eighteen years ago, on a treaty to abolish chemical weapons under international verification, this was justifiably hailed as a major achievement of multilateral disarmament and non-proliferation, towards international peace and security. The Chemical Weapons Convention verification regime – perhaps more appropriately referred to as regimes – is the outcome of negotiations involving representatives of State Parties and their chemical industries, and subsequently representatives of the Technical Secretariat of the Organisation for the Prohibition of Chemical Weapons. It also reflects available inspection technology for verifying the demilitarisation and non-proliferation of chemical weapons.

The purpose of international verification is to contribute to confidence and trust between States Parties. An important aspect of this is the way in which the verification organisation reports on verification implementation. What degree of detail on declarations and inspection results should be reported to States Parties? How should assurances be expressed? How should verification issues be conveyed? What is the best way to describe the particulars of verification regimes? A key challenge of verification reporting is to provide an adequate level of transparency, while respecting the obligation to protect the confidentiality of certain information.

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TOXIC INDUSTRIAL CHEMICALS

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Toxic industrial chemicals (TICs) are compounds which as the name suggests have industrial applications but have a toxicity which nears that of chemical warfare agents (CWAs). A good example is phosgene – it is a precursor to polyurethanes which have a vast range of applications from flexible foam used in upholstery to hard plastics used in electronics equipment but phosgene has a toxicity of only around 50 times less than GA (in terms of ppm). TICs also do not have the same legal restrictions as CWAs and so can be stored and used in much larger quantities by many more companies. These factors make TICs a potentially more attractive material than conventional chemical warfare agents for use in a terrorist attack.

HOSDB (in conjunction with Dstl and the Police National CBRN Centre) have previously evaluated a range of chemical detection equipment for use by UK Police Forces. A range of the equipment which performed well and was available for testing was selected for TIC testing. An Owlstone vapour generator was procured and validated using thermal desorption gas chromatography-mass spectrometry (TD-GC-MS). The vapour generator was then used to produce a range of TIC vapours. The concentrations produced were based on short term exposure limits and the detection limits of the equipment. The results will go to inform further decisions on TIC capability and the testing will be encapsulated into the Blue Book testing in future editions.

DETECTION AND IDENTIFICATION OF TRACE SARIN HYDROLYSIS PRODUCTS BY SURFACE-ENHANCED RAMAN SPECTROSCOPY

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Abstract: Timely and accurate detection of chemical warfare agents (CWAs) is of great importance for national defense and anti-terrorism. Surface enhanced Raman spectroscopy (SERS) has gained a reputation as one of the most sensitive spectroscopic tools available for the trace detection of chemical species, even down to single-molecule sensitivity, therefore spurred substantial investigation interests. We have been investigating the ability of SERS to detect extremely low concentrations (e.g. part-per-billion) of chemical agents, as might be found in poisoned water. In this paper, a silver film substrate with highly reproducibility and SERS activity was fabricated based on a chemical-bath-deposition method for detecting trace sarin (GB) hydrolysis products in water. SERS spectrum of GB hydrolysis products, isopropyl methylphosphonic acid (IMPA) and methylphosphonic acid (MPA), were assigned according to their normal raman spectra. The limitation of detection (LOD) for IMPA and MPA were 27 ppm and 10 ppm, respectively. More importantly, the whole detection procedure was very quick and it can be done in several minutes. The detection sensitivity can be improved by devising and fabricating more active SERS substrates. All in all, SERS has an enormous potential both to detect and to identify trace chemicals such as CWAs.

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TRACE IDENTIFICATION OF RICIN AND OTHER RIP-II TOXINS BY AFFINITY ENRICHMENT AND LC-MSMS

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Ricin belongs to the family of ribosome-inactivating type 2 proteins (RIP-II toxins). Ricin is highly toxic and is easily prepared from the seeds of the Castor plant (*Ricinus communis*). Other plant toxins of the RIP-II family are abrin, volkensin, robin and viscum album toxin. RIP-II toxins are lectins (carbohydrate binding proteins) which bind to cell surface receptors terminated by galactose. After entry into the cell the toxin cleaves an adenine residue in the ribosomes which stops the protein synthesis. Entry of one ricin molecule is enough to kill the cell. The inhalation toxicity of ricin is high, estimated LD₅₀ of 1-10 µg/kg (orally 1-20 mg/kg). For abrin LD₅₀ is ca 0.04 µg/kg by inhalation.

We have utilised the lectin properties for class-specific affinity enrichment of RIP-II toxins from dilute and complex samples. A high-efficiency chromatographic material was modified by covalent attachment of a galactose-containing affinity ligand (p-4-aminophenyl-1-thio-β-D-galactopyranoside). The material was packed into miniaturised columns and parameters were optimised for recovery of RIP-II toxins from liquid samples and extracts. The enriched fraction was digested with trypsin and the resulting peptides were analysed by LC-MSMS for unambiguous identification of the toxin. The nonbinding fraction containing ricin biomarker peptides and proteins was collected for screening and to obtain attribution profile data. Limited volume (0.1 ml) samples could be processed at a rate of 1 sample/min. Large volume samples up to 10 ml were processed within 10 min and a concentration factor higher than 100x was obtained. The method was applied to dilute water samples containing ricin together with other masking proteins in an interlaboratory calibration test.

DETERMINATION OF CHEMICAL WARFARE AGENTS AND THEIR DEGRADATION PRODUCTS BY SOLID PHASE MICROEXTRACTION

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Norwegian Defence Research Establishment (FFI) is developing new analytical procedures for the identification of chemical warfare agents (CWA) and related compounds in different matrices. The laboratory is focusing on extraction methods which are based on low solvent consumption and little sample preparation. Solid phase microextraction (SPME) meet these requirements, as the technique both extracts and concentrates the analytes from the sample automatically without using solvents. The SPME technique uses a fused silica fiber coated with a polymer, where the fiber is either immersed or kept in the headspace of a sample.

The aim of the current study is to develop a fast screening method for CWA and its degradation products in water and polymer materials by use of SPME, coupled to a gas chromatograph (GC) with a mass spectrometer (MS). The work will be focused on analysis of CWA and its degradation products in one single run. Parameters such as SPME fiber selection, pH, extraction time and temperature, salt content and in-situ derivatisation conditions will be studied. The developed method for SPME will be compared with the existing analytical recommended procedures (ROP).

METHYLATION OF ALKYL METHYLPHOSPHONIC ACIDS FOR GC-MS ANALYSIS USING TRIMETHYLSILYL DIAZOMETHANE

–OPTIMIZATION OF REACTION CONDITIONS

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A method for the methylation of alkyl methylphosphonic acids for GC-MS analysis using the reagent trimethylsilyl diazomethane (TMSD) has been optimized by experimental design. The aim was to develop a method that can replace the traditional methylation based on diazomethane which is toxic, explosive and must be generated shortly before use.

Five different alkyl methylphosphonic acids (AMPA) were used in the evaluation; methylphosphonic acid (MPA), ethyl methylphosphonic acid (EMPA), isopropyl methylphosphonic acid (IMPA), pinacolyl methylphosphonic acid (PMPA) and cyclohexyl methylphosphonic acid (ChMPA). The parameters evaluated were; added amount of methanol, concentration of phosphonic acid, derivatization temperature and time, and choice of solvent.

The use of TMSD seems to be a very good alternative to diazomethane for derivatization of alkyl methylphosphonic acids (AMPA). Some of the different features found in this investigation are:

- The optimization resulted in a method that gives yields close to 100%.
- The reaction can be performed at room temperature.
- The reaction is completed within 90 minutes and for many applications the yield after 5 minutes is sufficient for identification/confirmation purposes.
- The carry-over from one GC-MS injection to another was negligible.
- The potential to optimize the experimental conditions towards high yield was good for the three solvents investigated: dichloromethane, acetonitrile, and toluene.
- The levels of methylated products are stable for more than 8 hours.
- A moisture content as high as 5 % in the samples only reduces the yield by 10-20 %.
- Derivatization with TMSD is well suited for use in mobile laboratories. TMSD is straightforward to use, stable, and commercially available. The reagent is relatively safe to handle and transportation of the reagent solution is not restricted.

Study on the speciation analysis of Lewisites in contaminated soils by chemical weapons abandoned by Japan in China

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Lewisite, developed as a chemical warfare agent in 1918 by Lewis and co-workers^[1,2], has never been proven to have been used in war, but remains of concern, as illustrated by its inclusion in Schedule One of the Chemical Weapons Convention^[3]. It is absorbed through the skin, producing painful blisters, and has high systemic toxicity^[4]. Yellow shells made by the Japan comprised 90% lewisite I (LI), 9% lewisite II (LII) and 1% lewisite III(LIII)^[5]. Extensive production and filling of munitions by several countries has led to environmental contamination. Destruction of chemical weapons abandoned by Japan in China(ACW)^[6] and polluted areas have renewed interest in the trace analysis of chemical warfare agents and their degradation products. Sometimes the original ACWs are hydrolyzed or oxidized. These degradation products cannot be detected and determined with gas chromatographic analysis. After derivatization with n-butanethiol, the degradation products can be identified and determined with gas chromatography –mass spectrometry detector^[7-8].

The speciation analysis of lewisites in contaminated soils classify two steps: firstly extracted the original agents with organic solvents; secondly extracted the degradation products with polar solvents and derivatizing with thiols, subsequent extracted derivatives with toluene. The concretely analytical procedure described as Fig.1.

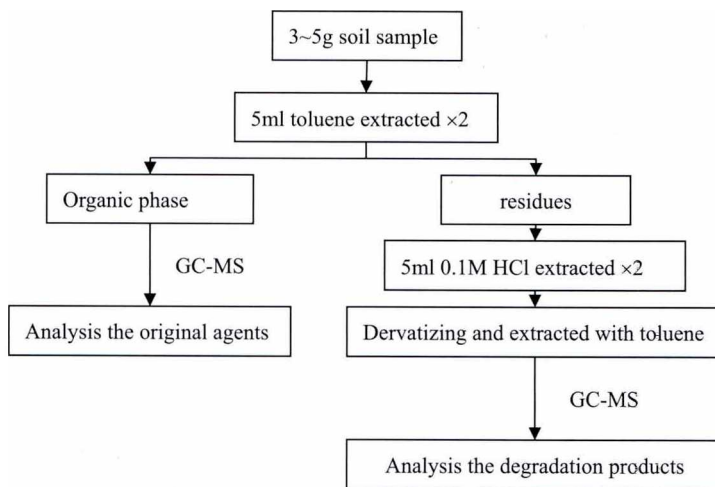


Fig.1. The process of speciation lewisites of sample preparation for GC/MS analysis
Fig.2. is the typical TIC of the lewisites speciation analysis by contaminated soil.

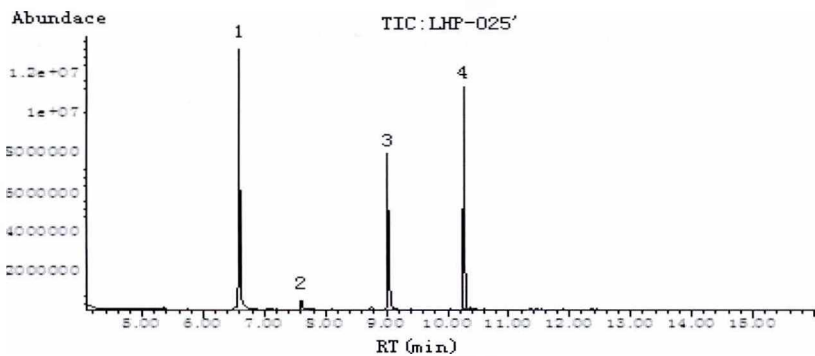


Fig.2. The typical TIC of the speciation of Lewisites for GC/MS analysis
1-dibutyldisulfide,2-Tris(2-chlorovinyl)arsine,3-Bis(2-chlorovinyl)arsine butylsulfide,
4-2-chlorovinyl arsine dibutyldisulfide

Tab.1 The mass spectrum data of the Lewisites and its derivatives.

Tab.1 The mass spectrum data of the Lewisites and its derivatives

| No. | Compounds | fomula/weight | The mainly fragment (abundance%) of mass spectra |
|-----|-----------------------------------------|--------------------------------------------------------|--------------------------------------------------|
| 1 | Tris(2-chlorovinyl)arsine | C ₆ H ₆ Cl ₃ As/258 | 258(6)、147(55)、145(78)、136(100)、110(66)、77(94)、 |
| 2 | Bis(2-chlorovinyl)arsine butylthioether | C ₈ H ₁₃ Cl ₂ AsS/286 | 286(49)、229(17)、164(27)、145(41)、107(22)、57(100) |
| 3 | 2-chlorovinyl arsine dibutyldisulfide | C ₁₀ H ₂₀ ClAsS/314 | 314(29)、225(20)、204(72)、164(49)、107(29)、57(100)、 |

Tab.2. shows the parameters of the speciation of Lewisites for quantitative analysis.

Tab.2 The quantitative analysis parameters of the Lewisites and its derivatives

| No. | compounds | Calibration curve and correlation coeffiecent | Linearity (mg/L) | LODs mg/kg |
|-----|-----------------------------------------|-----------------------------------------------|------------------|------------|
| 1 | Lewisite III | Y=2164.9x + 359.41,R ² =0.9993 | 0.10~40.0 | 0.02 |
| 2 | Bis(2-chlorovinyl)arsine butanthioether | Y=2450.2x - 50.11,R ² =0.9984 | 0.10~40.4 | 0.06 |
| 3 | 2-chlorovinyl arsine disulfide | Y=2450.2x - 50.11,R ² =0.9984 | 0.10~60.0 | 0.07 |

References

Omitted.

Development of an analytical methodology for sarin(GB) and its
mainly byproducts

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According to the right assigned by 《The Convention on the Prohibition of the Development, Production, Sstockpiling, and Use of Chemical Weapons and on Their Destruction》(CWC), in order to provide high purity chemical agents for purpose of chemical defence study, we had established the facility of the “Schedule 1 chemicals synthesis lab(10kg)”, which officially approved and put on record by our government and Organization for the Prohibition of Chemical Weapons (OPCW). The purity of the lab’s products is higher than 98%, Sarin(GB) is one of the lab’s products.

The synthetical pathways of GB have a great many catgoeries.This article adopted the method which consists of methylphosphonyl dichloride and methylphosphonyl difluoride and iso-propanol reacted under proper condition.The mainly byproducts of this synthyzing GB pathway are chlorosarin and diisopropyl methylphosphonate(DIMP) and diisopropyl dimethylpyrophosphonate,etc.. This study focuses on the quantification of the mainly byproducts of sarin.The analytical methodologies of GB and its relative compounds are a great many^[1-5].This paper, we developed an analytical procedure with gas chromatography-mass spectrometry(GC/MS) suitable for identification and for quantification of GB and its mainly byproducts.Fig.1.shows the TIC of the raw GB by GC/MS.

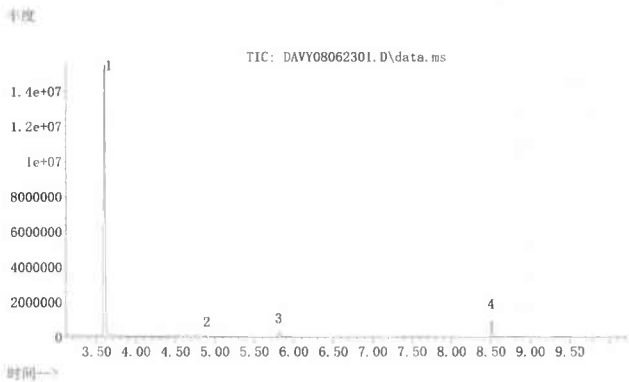


Fig. 1. The TIC of the raw GB by GC/MS

1- GB, 2- Chlorosarin, 3-Diisopropyl methylphosphonate, 4- Diisopropyl dimethylpyrophosphonate

Tab.1. describes the mass spectrum data of the GB and its mainly byproducts.

Tab.1 The mass spectrum data of the sarin and its mainly byproducts

| No. | Compounds | fomula/weight | The mainly fragment (abundance%) of mass spectra |
|-----|-------------|-------------------------------------------------------|---------------------------------------------------------|
| 1 | GB | C ₄ H ₁₀ FO ₂ P/140 | 125 (31), 99 (100), 81 (12), 47 (3), 43 (10), 41 (7) |
| 2 | Chlorosarin | C ₄ H ₁₀ ClO ₂ P/156 | 141 (42), 121 (17), 115 (100), |

| | | | |
|---|-------------------------------------|-----------------------|----------------------------------------------------------|
| | | | 97 (21), 79 (48), 43 (35) |
| 3 | DIMP | $C_7H_{17}O_3P/180$ | 165 (4), 139 (7), 123 (62), 97 (100), 79 (26), 43 (22) |
| 4 | diisopropyl dimethylpyrophosphonate | $C_8H_{20}O_5P_2/232$ | 217 (3), 201 (17), 175 (97), 157 (100), 97 (47), 79 (34) |

Owing to error of external standard mehtod is great than the internal standard^[6].This study selected the internal standard method as the quantitative.The internal standard n-nonane serves as GB and Chlorosarin for quantitative. For the internal standard n-nonane, the ions selected in SIM mode were 43 and 57and128.For determining GB,the ions selected in SIM mode were 99 and 125and 81. For determining Chlorosarin,the ions selected in SIM mode were 79 and 115 and 141. The internal standard triethylphosphate serves as DIMP for quantitative. For the internal standard triethylphosphate, the ions selected in SIM mode were 109and 127and 182.For determining DIMP,the ions selected in SIM mode were 79 and 97and 123. The internal standard tributylphosphate serves as diisopropyl dimethylpyrophosphonate for quantitative. For the internal standard tributylphosphate, the ions selected in SIM mode were 99and 155and 211.For determining diisopropyl dimethylpyrophosphonate,the ions selected in SIM mode were 157 and 175and 216. Tab.2.shows the quantitative analysis parameters of the sarin and its mainly byproducts.

Tab.2 The quantitative analysis parameters of the sarin and its mainly byproducts

| No. | compounds | Calibration curve and correlation coeffiecent | Linearity(ratios of area) | LODs |
|-----|-------------------------------------|-----------------------------------------------|---------------------------|---------|
| 1 | Sarin | $Y=1.8054x-0.0363$, $R^2=0.9995$ | 0.353~1.765 | 0.3mg/L |
| 2 | Chlorosarin | $Y=3.1875x-0.1379$, $R^2=0.9997$ | 0.217~2.173 | 0.5mg/L |
| 3 | diisopropyl methanephos-phionate | $Y=3.6457x-0.0771$, $R^2=0.9996$ | 0.193~1.934 | 1mg/L |
| 4 | diisopropyl dimethylpyrophosphonate | $Y=3.8869x-0.0228$, $R^2=0.9997$ | 0.177~1.773 | 0.8mg/L |

References

Omitted.

DETECTION OF FUMIGANTS IN AIR BY FOURIER TRANSFORM INFRARED SPECTROSCOPY USING THE PORTABLE GASMET DX-4030 FT-IR GAS ANALYZER

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Detection of fumigants in air by Fourier Transform Infrared Spectrometry (FT-IR) was investigated by the use of the Gasmet Dx-4030 FT-IR gas analyzer. Laboratory tests of the gas analyzer were performed using neat fumigant gas from gas cylinders or from evaporated liquids. The fumigants 1,2-dichloroethane, ammonia, chloropicrin, formaldehyde, hydrogen cyanide, methyl bromide, phosphine and sulfuryl fluoride were analyzed during varied humidity and exposure to a chemical background consisting of seven common solvents. The instrument did not face any problems when measuring ammonia, formaldehyde, hydrogen cyanide, phosphine or sulfuryl fluoride. While exposed to background chemicals, the gas analyzer overestimated the concentrations of 1,2-dichloroethane and methyl bromide, whereas the concentration of chloropicrin was underestimated. The obtained results indicate that the instrument's resolution (8 cm^{-1}) is not high enough to resolve these three fumigants from a background of common interfering chemicals.

A two day long measurement campaign was performed at the container terminal of the Port of Gothenburg in august 2009 to test the gas analyzer in the field. As reference, air was also sampled on Tenax tubes and analyzed with GC-MS to determine the level of background chemicals.

REAL-TIME MEDICAL SURVEILLANCE – THE FUTURE FOR BIOLOGICAL DETECTION

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Incidents involving exposure to biological warfare agents requires early medical intervention to potentially reduce the number of casualties. Speed of information gathering is critical to effective medical intervention. For example, if bio-weapon attacks are identified early it is possible to administer medical counter-measures to the vast majority of people exposed but not yet ill. In the case of anthrax for example, it is likely that by 24-36 hours after an attack, a small percentage of individuals – those with compromised immune systems or who have received a large dose of the organism due to proximity to the release point – will become ill with classical symptoms and signs. If such data can be collected and made available to medical officials in real time, most models of anthrax epidemics indicate that more than 80 per cent of an exposed population can receive antibiotic treatment before becoming symptomatic, and thus avoid the moderately high mortality of the disease. The military have long recognised the need for a system that will allow them to detect, in as near real time as possible, potential biological attacks from the symptoms presented by the personnel exposed to the attack. RMS (Real Time Medical Surveillance) is a computer-based system designed to pinpoint illness in real time and combines information on signs and symptoms with knowledge of the time and location of incidents. The system aids medical personnel identify outbreaks of disease more rapidly than hitherto, allowing treatment to be administered faster and more directly. The technology also has the potential to limit the size of outbreaks of other, non BWA, infectious diseases. This paper will outline the development of RMS, explain the advantages such a system offers and explore the various applications it can be adapted for.

Key Words: Real-time, Biological, Epidemiology, Surveillance, Intervention

**HYDROGEN PEROXIDE VAPOUR DECONTAMINATION: A VERSATILE
METHOD TO TACKLE BIOLOGICAL WARFARE AGENTS AND PATHOGENS
ASSOCIATED WITH HOSPITAL-ACQUIRED INFECTIONS**

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The challenges posed by biological warfare agents (“BWAs”) and pathogens associated with hospital acquired infection (“HAI”) share certain similarities. Anthrax (*Bacillus anthracis*) is a spore-forming bacterium that has been used as a BWA; deliberate release of anthrax has resulted in widespread and persistent environmental contamination. Similarly, *Clostridium difficile* (“*C.diff*”) is a spore forming bacteria which causes HAI. *C.diff* spores are shed by affected patients resulting in widespread environmental contamination that is difficult to eradicate and is thought to be an important factor in the transmission of *C.diff*. Other pathogens associated with HAI share the ability to cause widespread and persistent surface contamination. For example, *Acinetobacter* is a Gram-negative bacterium which caused significant complications in the treatment of injured warfighters in field hospitals in the Vietnam and Iraq wars – and is a major cause of HAI in intensive care units (“ICUs”) in North America and Europe. Norovirus causes significant problems for hospitals – infecting patients and healthcare workers – and has also caused outbreaks among warfighters on naval ships. BIOQUELL has developed hydrogen peroxide vapour (“HPV”) bio-decontamination technology which inactivates BWAs, including anthrax, and pathogens associated with HAI, including *Cdiff*. HPV has been used to eradicate pathogens associated with HAI from the inanimate hospital environment, including from sensitive electronics typically found in ICUs. BIOQUELL’s HPV technology is used in the US Department of Defense’s Joint Materials Decontamination System (JMDS) – which is used to decontaminate BWAs and chemical warfare agents. HPV bio-decontamination technology may be used to combat the threat posed by BWAs and also reduce the transmission of HAI in military hospitals.

MEMS PLATE RESONATORS FOR ULTRASENSITIVE MASS SENSING APPLICATIONS

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We present here a new concept of high-Q MEMS plate mass sensor. Indeed, in contrast to flexural mode NEMS resonators (either cantilever or bi-clamped beams), plate resonators feature higher Q [1]. In addition, a flexural mode resonator with the same fundamental resonant frequency as a bulk mode resonator would generally have a larger surface-to-volume ratio, and therefore more severely dominated by surface losses. The devices under study comprise square or disk plates excited according to a Lamé, or a “wine-glass” mode. The vibrations for this mode are characterised by the entire plate contracting and extending in the plane of motion. The resonators vibration is produced by electrostatic coupling across a 300nm transduction gap. As the structure is excited into vibration, the capacitance across the sense electrodes is modulated due to changes in the gap, which in turn produces a motional current. The devices transmission S_{21} response has been tested at atmospheric pressure featuring a sharp adsorption peak at the expected lamé mode (error less than 0.1% compared to the theoretical model).

To validate the mass sensor concept, typical experiments consist in local deposition of latex beads onto the plates using a microcantilever-based picoliter droplet dispenser [2]. The frequency shift arising from this spotting is recorded, and is varying according to the beads location. For a given beads quantity, the frequency shift is increased as soon as the deposition area is getting closer to the maximum amplitude of displacement for the corresponding mode. Repeating this protocol on our MEMS devices, we reached competitive performances at the atmospheric pressure compared to NEMS based approach, providing we are able to confine the grafting area close to the maximum amplitude of displacement for the Lamé (WG) mode.

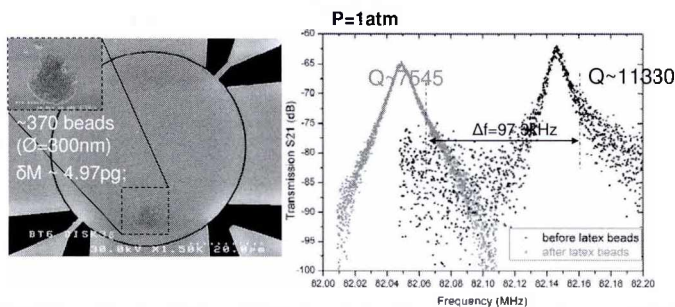


Figure 1: (a) SEM picture of a released 53µm wide disk plate after local deposition of latex beads on top of it. Fig. 2b shows the transmission response of the plate recorded at atmospheric pressure before and after the beads deposition

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**MOBILE GC/MS AND SAMPLING TOOLS FOR CONTINUOUS AIR
MONITORING**

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ICx manufactures a compact mobile GC/MS and handheld vapor sampler, which are used for on-site chemical identification applications from hot-zone exploration and forensics to industrial hygiene and environmental assessments. The proprietary CIT mass analyzer is housed in a small, internal vacuum system. Two integrated inlets accept liquid, vapor, and solid-phase samples: a split/splitless injector for direct syringe injections, SPME fibers, headspace sampler, and autosampler, and a universal sampling port for direct air intake via transfer line and thermal desorption from the handheld sampler.

Data will be presented for a variety of chemicals identified in lab and office air by the GC/MS. Characteristic signatures for isolated chemicals were obtained including hexane, cyclohexane, D5 (decamethylcyclopentasiloxane), and limonene. Relative concentration of D5, a common ingredient in personal care products, began to rise around 6:00 am and ebbed around 6:30 pm. Limonene signatures were present due to an employee eating an orange prior to entering the lab and hexane/cyclohexane when another employee sonicated instrument parts. External air analysis was accomplished by plumbing a line from the GC/MS to a window, where a different chemical signature was obtained, demonstrating the presence of naphthalene, methyl naphthalene, and acenaphthalene.

The handheld sampler was used for longer-term air monitoring of common volatile organic compounds, such as Toluene. Trace levels of toluene were identified by the GC/MS after desorption from the sampler. Air samples were collected from various locations inside and outside office buildings in a local research park, including in a brand new laboratory, which showed a toluene background concentration 100 times higher than other locations tested, presumably due to offgassing of new building materials. A calibration curve for toluene showed a detection limit of ~10 parts-per-trillion with a linear dynamic range of approximately three orders of magnitude for 40 minutes sampling time (~20 L).

FOI-R--2985--SE
Poster Session

DEVELOPMENT, VALIDATION AND COMMERCIALIZATION OF A NOVEL STAND-OFF DETECTOR FOR BIOTHRREATS USING LIDAR

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A promising new development in Light Detection and Ranging (LIDAR) has resulted in a highly precise and useful sensor for bio threat agents. Among the valuable characteristics of the device are eye-safety, compact size, optimization for daylight conditions, and spectral resolution of biological particles which results in levels of detection similar to state of the art point detectors used in current bio defense applications. This particular LIDAR is designed for short range operation (100 metres or longer) making it effective in an indoor or public security application such as an airport or other critical infrastructure building.

This poster describes the operational validation of the LIDAR in an indoor test facility, an outdoor chamber, and a real-life application. The comparison to other detection devices is presented and the degree of discrimination of certain threats and interferences is described. A discussion of the concept of operations for various applications is also presented. Finally, the commercialization program for the LIDAR is discussed, presenting the design trade-offs considered in order to offer a compact, rugged and portable device to suit the target applications.

DEVELOPMENT OF FIELD BIOLOGICAL DETECTION SYSTEM : KDTB GOLD®

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Biological warfare agents are inconspicuous, their detection is difficult and far from being instantaneous. This point is even more crucial because the effects of these agents, dramatically effective, are insidious. The development of powerful systems of detection and allowing identification of these agents, as early as their dispersion, is therefore crucial to be efficient. These systems will have to be easy to use, because they are susceptible to be used by non specialists. Among the various methods developed for detection purpose, immunochromatography test presents numerous advantages: fast response, ease of use, adapted to measurements on the ground and very low cost.

NBC-Sys, a subsidiary of Nexter, is a company whose mission is to develop, produce and market CBRN protective systems for forces and populations. The laboratory for immunological research (LERI/iBiTec-S/DSV/CEA) leads a research activity based on production, characterization and use of antibodies for analytical or therapeutic applications. NBC-Sys and CEA/DSV have collaborated to develop immunochromatographic test kits for the detection of pathogenic biological compounds. In a first step monoclonal antibodies directed against different toxins (botulinum toxins A, B and E, Enterotoxin B, ricin and microcystin) have been produced and characterized. In a second step, rapid tests using colloidal gold as label were set up using these antibodies.

KDTB Gold® field detection system is designed for a rapid collection, detection and identification of biological warfare agents. This system includes 25 gold immunochromatographic tests and 25 sample preparation kits. TBA, TBB, TBE, ricin and SEB are reliably detected within 15 minutes. Tests can be stored at room temperature for 24 months. Moreover a hand-held reader allowing gold signal measurement can be associated for greater accuracy.

Currently we have produced monoclonal antibodies against pathogens (*Bacillus anthracis* and *Yersinia pestis*). The development of the corresponding immunochromatographic tests is already started and tests are expected to be available in 2010.

**AN INNOVATIVE APPROACH IN THE QUANTIFICATION OF FREE VX IN
PLASMA USING LC-MS/MS TECHNIQUE**

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A novel analytical method has been developed to detect and quantify an organophosphorus nerve agent, VX (O-ethyl S-(2(diisopropylamino)ethyl) (methylphosphonothioate)), in plasma using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) technique. Our distinctive methodology was implemented to evaluate the presence of VX in very small quantities of plasma (between 10 to 1000 µL) as in small rodents.

Next optimization of liquid-liquid extraction of VX from HBSS environment was achieved with excellent yields (>99%), extraction from plasma was performed and it generated a recovery rate of approximately sixty five percent (65%) followed by an LC-MS/MS analysis in a 100% organic phase. An Allure biphenyl column (Restek) was tested with detection limit at 0.5 pg/mL in plasma (5 µl injected).

Our study is now focusing on the separation of VX enantiomers and hopeful results are provided using a Lux cellulose-1 column (Phenomenex).

FOI-R--2985--SE
Poster Session

SYSTEM OF SYSTEMS FOR PROTECTION AGAINST CHEMICAL WEAPONS:
DETECTION, DECONTAMINATION AND DECONTAMINATION ASSURANCE

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ICx Technologies will present a system of systems for protection against chemical weapons including detection, decontamination and decontamination assurance utilizing enzymes.

Our detection systems use enzyme-based sensing chemistries to allow for the detection of acids, bases, strong oxidizers and aldehydes plus nerve, blood and blister agents in vapor, water and on surfaces. We will describe in detail an efficient dynamic buffering method to control pH in sensing reactions catalyzed by hydrolytic enzymes and the successful application to the development of a positive-response inhibition-based sensor. We will discuss enzyme-based sensing approaches for detection of chemical agents on surfaces and describe two new enzymatic devices for continuous use to detect low levels of chemicals in air and water. All systems have been challenged in methodical interference tests, used continuously for extended periods, and validated by 3rd parties.

Our decontamination system is a broad-spectrum, enzyme-based spray that combines oxidation and hydrolysis to decontaminate both chemical and biological warfare agents with little to no negative impact on a variety of materials. It has been demonstrated to be effective against V-, H- and G-series agents and anthrax spores.

Our decontamination assurance system is an enzyme based formula that is fast, extremely sensitive and easy-to-use. It has been validated in live agent tests. The formulation operates via a color change that occurs when the solution is sprayed onto areas of residual contamination. We will describe in detail our systematic protein stabilization techniques for extended robustness in shelf-life at elevated temperatures and interference resistance.

Finally we will briefly discuss other CBRNE detection techniques of ICx Technologies.

INVESTIGATING THE BENEFITS OF INFORMATION MANAGEMENT SYSTEMS FOR INCIDENT SUPPORT

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With increasing concern of hazardous materials being maliciously released to cause mass casualties and panic, there is a growing interest in the use of Information Management (IM) systems for incident support. These IM systems facilitate collaborative planning, aid decision support and provide an accurate and up-to-date picture of the evolving hazard. However, little has been done to investigate the benefits and effectiveness of these systems, particularly when used prior to an event or while the immediate hazard is still present. These benefits need to be evaluated against the aim of the system, i.e. to minimise the number of casualties and level of damage and disruption caused.

We have developed a Prototype Response and Information Management Engine (PRIME) which enables us to investigate the effectiveness and potential benefits of IM systems for incident support and to evaluate which future capabilities could be incorporated. PRIME integrates optimised sensor placement, sensor management, sensor data fusion, data assimilation, rapid hazard modelling, recommended mitigation, hazard “templating”, and links to scientific reachback. Using metrics and measures of effectiveness, combined with a CB synthetic environment to produce realistic simulated challenge data, we evaluate the effectiveness of each component or combination of components. We also investigate whether an increasing level of automation and sophistication can provide real benefits.

In the presentation, we will provide a brief overview of the PRIME capability and its constituent components, as well as the approach taken in our study and the metrics used. We will present the results of the study, including what benefits CBR information systems can provide over current procedures, what the constraints are and how to maximise the benefits offered. We hope to be able to provide some insights into the development of future systems, what can be achieved and what challenges may be faced.

DEVELOPMENT OF A NEXT GENERATION WET/DRY AUTO- INJECTOR DELIVERY SYSTEM FOR HI-6

Robert Hill

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Meridian Medical Technologies has been supplying nerve agent antidotes in auto-injectors for over 40 years. These auto-injectors are effective and extremely easy to use. Meridian's auto-injectors require a simple, two-step operation. First, remove the safety cap. Second press the end of the auto-injector against the outer thigh to activate the unit and deliver the drug.

HI-6 is a desirable oxime that is effective against many nerve agents; however, it is unstable in solution making an auto-injector delivery system very difficult. Previous wet/dry auto-injectors were difficult to use requiring many extra steps including reconstitution of the HI-6 by shaking the auto-injector for up to one minute.

Meridian's goal in designing a next generation auto-injector for HI-6 was to make it operate the same as our existing auto-injectors. This would be more suitable in a battlefield scenario as it is quicker and more efficient than current wet/dry auto-injectors in delivering a much needed antidote to the soldier. This requires automatic reconstitution of the HI-6 during the delivery of the drug. Meridian has designed and patented an auto-injector that accomplishes this goal and delivers 636 mg of HI-6 dimethanesulfonate (DMS) and 2 mg of atropine sulfate within 5 seconds. Furthermore, Meridian has completed an R&D project in cooperation with an allied government that included: completing the auto-injector design and development, establishing pilot production manufacturing capability, synthesizing 30+ kg of HI-6 DMS via a non-carcinogenic method, and producing an initial quantity of pilot production auto-injectors. Further manufacturing scale-up is required. All specifications have been met up through 6 months for normal 25°C / 60% RH storage conditions in the on-going stability study.

**AN INTEGRATED INFORMATION SYSTEM (LIMS) FOR DETECTION OF
THREAT SUBSTANCES IN URBAN SOCIETY**

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European Community's FP7 framework programme with grant agreement No:
FP7-SEC-2007-1 LOTUS no.217925

Lotus (Localisation of Threat Substances in Urban Society) project is a collaborative project funded by the EU FP7 framework programme. The consortium consists of three research institutes, two industries, three SMEs, one university and a group of end-users. The objective of the project is to create a system by which illicit production of explosives and drugs can be detected during preparation and production. The LOTUS system covers a whole functional chain from sensors, mobile/WAN/LAN communication, centre system and final client system.

This presentation focuses mainly on the description of LOTUS information management system (LIMS) and its final uses. The LIMS system consists of a central system and unlimited client systems. It is used in real-time to conduct and analyze the information flow from wide distributed sensors in relation to supplementary information such navigation data and weather information. It has the functions such as Alarm Confidence Evaluation Function (ACEF), Data Fusion for various information sources and decision support like mission and alarm management, warning and reporting representing all detected results to a decision maker. The aim is to improve situation awareness including visualisation of detection locations on a map, threats detected, their nature and timing, alarm/warning representation, meteorology, urban structure, facilities legally using at threat substance and presence of local security forces. The architecture and its developing process are designed to integrate different subsystem into the system. Finally this LIMS system will be tested and verified through demonstration and in field by the end-users and the future potential users.

PROVIDING A DEFENSIBLE CBRE SAMPLING KIT

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Sample collection introduces the most error in the entire sample collection, handling and analysis process. While some of this error relates to the selection of the area or item sampled, other errors can be linked to the tools used to collect the sample. Elimination of these errors can be simplified by taking adequate steps to prepare the tools prior to use. QuickSilver Analytics, a SDVOB and ISO 9001:2008 registered company, with more than 10 years of experience with WMD sampling and analysis, provides customized CBRE sampling kits that contribute to the reduction of errors by providing the precise sample collection tools, with the documentation and testing required to support forensic and litigation investigations. .

The QS Sampling kit was designed to address any sampling problem by designing and creating a kit that contains all the tools and consumables required to collect a defensible sample. The tools that touch the sample are cleaned by a specialized process, the effectiveness of this cleaning is verified by testing, and the tools sealed in protective material ensuring the cleanliness of those sampling tools. Likewise, sterility may be needed for sampling tools for biological applications. Two options are available- the tools can be cleaned as described above, and sterilized, with verification of sterility (dual use tools); or if purchased sterile, a number of them can be reserved for sterility testing (bio only sampling tools). Training kits are also available.

Numerous customer recommendations have provided continuous improvement to the kits. In 2001, biological sampling capability was added (QSA 102 Full FAC Sampling kit), and in 2006, explosive and radiological sampling capability (QSA 102 Full FAC CBRE Sampling Kit). Our customer list includes 20th Support Command, Coast Guard Strike Teams, Special Ops Teams, and all of the NG Civil Support Teams.

**INTERNATIONAL BIOLOGICAL PREPAREDNESS:
THE DEVELOPMENT AND STOCKPILING OF MEDICAL COUNTERMEASURES**

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Bioterrorism has been referred to as the ideal terrorist weapon. It is difficult to predict, relatively simple to execute, challenging to prepare for, and likely to produce mass casualties. For the unsuspecting, target jurisdiction, a large-scale biological attack will strain all preparedness sectors and capacities. The fact that the damage is intentionally caused will add unparalleled dimensions of panic and disruption. The preparedness and response system in the affected region will have to function at maximum efficiency to mitigate loss of life, and to maintain order within the citizenry.

In parts of the world such as the United States, where a preparedness and response plan to combat bioterrorism has been developed, a sophisticated set of necessary resources have been identified, developed, procured, and, where appropriate, stockpiled. Much like more traditional defense assets, such as military aircraft and warships, medical countermeasures (MCMs) involve a substantial investment in an asset that will hopefully never be used. Nevertheless, intelligent government policy points to the fact that MCMs should constitute a major component of any nation's defense arsenal. MCMs represent the best known opportunity to afford preventative protection against biological attack and may be necessary as a part of the post-exposure medical response.

Biological weapon release brings with it an extreme timing challenge in that casualties can result in a matter of days or even hours. MCMs, if not used in a preventative manner, such as through vaccination, must be stockpiled in order to ensure a readily available resource to be used in the post-attack medical response. Recognizing that every nation has its own legal regime for researching and developing MCMs, approving their use, procuring them for government stockpiles, detecting disease, authorizing the use of those stockpiles, transporting the MCMs to points of delivery, dispensing the MCMs to potential victims, and dealing with the consequences of those medical interventions, the time to develop and execute a comprehensive preparedness and response plan is well in advance of an actual biological incident. The time is now!

The proposed talk will highlight the bioterrorism preparedness approach being pursued in the United States (including the use of stockpiling), discuss the international quandary of MCM supply and demand, and offer suggestions for advancing a meaningful bioterrorism preparedness and response strategy that will replace random regional approaches with a workable global plan.

Negative Pressure, Rapid Set-up, Portable Isolation Systems for Collective Protection in case of Radiological and Biological Pollutions

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The Threat of Non-Conventional Terrorism, Bioterrorism and Pandemic emphasizes the importance of Rapid Setup Isolation capability

The rapidly rising threat of the use of non-conventional agents for terrorist purposes, together with the possibility of bio-attack, highlights the importance of rapid set-up isolation capability.

Emerging infectious diseases and bioterrorism are health threats that should raise our awareness of global vulnerability. Highly infectious agents like aspergillosis and tuberculosis are serious health threats both for the general public and the healthcare providers.

New life threats call for on-spot protection. This is especially applicable in places where mass population is present in public places today. Airborne Infection Isolation Rooms (AIIR) are part of the arsenal of containment and infection control measures for airborne transmissible agents. The negative pressure portable isolation chamber is a complete rapid setup solution for infection control and pandemic prevention.

Keywords: bioterrorism, pandemic, negative pressure, portable isolation

The revised Israeli Standard SI 4570 – Regulating the performance and testing of collective NBC protection systems

Samuel Koeger – Member of Standard Committee SI 4570 ^{a)}

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Due to the rising threat of nuclear, biological and chemical attack, the Israeli Standard SI 4570 had to be revised and enhanced

The Israeli Standard SI 4570 regulates the performance requirements and test methods for collective NBC-protection systems for use in bomb shelters and infrastructures. The SI 4570 was created in June 2002 when it replaced the MAFMACH 388 from 1995.

Due to the change and increase of the threat in Israel a revision and enhancement of the SI 4570 became necessary and after more than 1 year of intensive work the revision was approved in November 2009.

Part 5 of SI 4570 which regulates the requirements of large NBC-protection systems was generated and part 1 to part 4 were revised.

Part 1: Blower for use in collective NBC-protection system.

Part 2: Blast Protection Valves and Overpressure Valves for use in collective NBC-protection systems

Part 3: NBC-filters for use in collective NBC-protection systems

Part 4: Installation, testing and commissioning and service of collective NBC-protection systems

The key-requirements and main modifications will be presented. Some test methods and test set-ups will be shown and explained.

Keywords: collective protection, NBC protection, Israeli Standard, SI 4570,

**CHEMICAL AGENT DETECTION AND IDENTIFICATION WITH A
HYPERSPSCTRAL IMAGING INFRARED SENSOR**

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Chamberland and Jean Giroux

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Standoff detection, identification and quantification of chemical agents are fundamental needs in several fields of applications. Additional required sensor characteristics include high sensitivity, low false alarms and high-speed (ideally real-time) operation, all in a compact and robust package. The thermal infrared portion of the electromagnetic spectrum has been utilized to implement such chemical sensors, either with spectrometers (with none or moderate imaging capability) or with imagers (with moderate spectral capability). Only with the recent emergence of high-speed, large format infrared imaging arrays, has it been possible to design chemical sensors offering uncompromising performance in the spectral, spatial, as well as the temporal domain.

Telops has developed an innovative instrument that can not only provide an early warning for chemical agents and toxic chemicals, but also one that provides a “Chemical Map” in the field of view. To provide to best field imaging spectroscopy instrument, Telops has developed the Hyper-Cam. This instrument is based on a modular design that includes: a high-performance infrared FPA and data acquisition electronics, onboard data processing electronics, a high-performance Fourier transform modulator, dual integrated radiometric calibration targets and a visible boresight camera. These modules, assembled together in an environmentally robust structure, used in combination with Telops’ proven radiometric and spectral calibration algorithms make this instrument a world-class passive standoff detection system for chemical imaging. This paper presents chemical detection and identification results obtained with the Hyper-Cam sensor.

DEVELOPING WEARABLE SENSOR TAGS FOR REAL-TIME MONITORING OF CWAS AND TICS WITHIN INDIVIDUAL PROTECTION EQUIPMENT

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Sensor Research and Development Corporation (SRD) has developed wearable sensor tags that are capable of real-time detection, identification, and quantification of chemical warfare agents, sarin and mustard, and toxic industrial chemicals, methyl salicylate (MeS) to support evaluating and monitoring individual protection equipment (IPE). These sensor tags operate continuously and autonomously while storing or sending raw sensor array response signals, at 1 Hz interval, to a laptop for real-time algorithm processing. Each thumb-size sensor tag consists of an array of four chemically tailored metal oxide sensors (MOS) supported on a MEMS platform, temperature and relative humidity sensors, gas-sampling system with miniature diaphragm pump or fan, measurement and control electronics, rechargeable battery power, data storage via micro SD card, communications via I²C bus, and real-time clock/calendar for time-stamping data.

SRD's sensor tags have demonstrated capabilities for real-time detection, identification, and quantification of MeS between 25 ng/m³ and 5 mg/m³ in 30-80% relative humidities at temperatures ranging from 10 °C - 50 °C. The sensor tags were also successful in measuring low levels of MeS within interferent backgrounds including simulated human sweat and/or body odor along with JP-8. SRD will present the sensor tag gas-testing results including the raw data along with the autonomously processed outputs. These results demonstrate the success and advantages of SRD's nano-clustered MOS technology coupled with the suite of algorithms to provide real-time measurement capabilities needed to evaluate and improve IPE.

SRD's sensor tags have been developed with a focus on IPE applications including Man In Simulant Test (MIST) and Individual Protection Equipment Mannequin System (IPEMS). However, these sensor tags can be adapted toward a broad range of applications, such as collective protection, facility monitoring, plume tracking and analysis. SRD's thumb-size sensor tag has demonstrated near analytical grade chemical detection capabilities with robust, accurate performance at low levels.

**WEARABLE BIOLOGICAL AEROSOL SAMPLER FOR INDIVIDUAL
DOSIMETRY**

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Exposure to biological threats is a constant concern for military troops, police forces and first responders around the world. Low level concentrations of aerosols can go undetected using existing methods, but still present a real threat. A wearable bioaerosol sampler, called the BioBadge was developed to perform the critical function of constant, unobtrusive sampling for aerosols at the individual level. This small, battery-powered sampler is the equivalent of a bio-dosimeter.

This presentation will first include a detailed overview of the technology embedded in the sampler, which is based on the principle of rotating impaction. Rotating impactors consume much less power per liter of air sampled than traditional impactors or cyclones. The sampler operates in a dry collection mode allowing it to be used over a wide range of temperatures, consume little power and have a high collection efficiency/concentration factor. A procedure to extract the sample into a buffered saline solution that is compatible with existing PCR analyzers will be presented. Finally, a concept of operations (CONOPS), including the sample analysis function will be described. Third-party independent testing has been completed, and results from these tests will be presented.

PHARMACOKINETIC EVIDENCE SUPPORTING THE ADDITION OF MIDAZOLAM
TO THE THERAPY FOR NERVE AGENT TOXICITY

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The combination of atropine (AT), pralidoxime (PAM) and pyridostigmine (PYR) is the standard U.S. regimen against the peripheral effects of nerve agent toxicity; however, the drugs are only modestly effective in treating convulsions produced by the warfare agents. It has been suggested that the addition of midazolam (MDZ) for seizure treatment may be more effective than diazepam, which is the current standard treatment. Therefore, a series of human trials were conducted to evaluate the safety of MDZ given by auto-injector and to verify the approved regimen does not alter the pharmacokinetics (PK) and pharmacodynamics (PD) of MDZ. Two 10 mg I.M. injections of MDZ were given to 24 subjects in a crossover design either <1 or 10 minutes apart. No differences were observed in the PK parameters: C_{max}, T_{max}, AUC, K_e, Clearance and half-life. In a series of three successive PK and PD crossover studies, each with an n=24, MDZ was given alone and in combination with either AT, AT+PAM and AT+PAM+PYR. AT+PAM were given 10 minutes and PYR was given 2 hours prior to MDZ. No PK or PD interactions were noted. Target blood levels greater than 190 ng/ml were achieved in 15-20 minutes. Safety data are available from the 96 subjects described above, as well as 250 subjects who participated in a safety study in which two 10 mg I.M. MDZ injections were given within <1 minute, with 30 days follow-up. Common adverse events observed in most subjects included: mild somnolence and injection site pain, with mild headache in approximately 10%. Approximately 5% had mild transient decrease in oxygen saturation; however, none had episodes of respiratory depression requiring oxygen. Therefore, I.M. auto-injection of MDZ is well tolerated and PD and PK characteristics are not altered by co-administration of the approved 3-drug regimen used to treat nerve agent toxicity.

**RAPID MULTIPLEXED NUCLEIC ACID AND ANTIBODY BASED SENSOR FOR
BIOTHREAT DETECTION**

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RapidPlex is a fully automated system designed for detection and identification of bacteria, viruses, and toxins from environmental samples in as little as 10-15 minutes. The RapidPlex system provides simultaneous, multiplexed detection of protein and DNA/RNA markers through parallel antibody and nucleic acid-based assays. Immunoassays for toxin detection are performed in under 10 minutes. Nucleic acid-based assays include automated sample prep and multiplexed DNA/RNA amplification for detection of viruses, vegetative cells, and bacterial spores in under 15 minutes. Prototype versions of the RapidPlex system have been developed under the United States Department of Homeland Security (DHS) Detect-to-Protect program for rapid bioterror detection in aerosols. In addition, the RapidPlex identifier technology has also been extended to other biodetection applications such as clinical diagnostics, food/water testing, and environmental monitoring.

DEVELOPMENT OF A TACTICAL BIOLOGICAL DETECTOR WITH THE PERFORMANCE CAPABILITIES OF POINT DETECTORS

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A bio-aerosol trigger fulfils two functions: detect-to-warn, and detect-to-treat. The trigger must be able to operate continuously with minimal maintenance in a wide range of environments, while providing a good level of detection sensitivity for non-specific threat bio-aerosols in addition to a low false alarm rate.

A number of groups have been developing tactical bio-aerosol triggers. Such detectors would be deployed in higher numbers in potential threat areas. As such, requirements include lower costs, battery-powered operation, ruggedness, and light weight for portability. The requirements of low power for battery operation, small size, and lower costs have resulted in instruments with reduced sensitivity.

Work by Dr. Jim Ho at DRDC-Suffield showed the feasibility of using a 405 nm laser diode as the fluorescence excitation source. This break-through and the need for smaller, less expensive instruments with lower maintenance requirements led to the development of the TSI Model 3317. This instrument uses a 30mW CW 405nm laser diode for extended lifetime. Threat determination is based on regions in particle size and 2-D fluorescence intensity space using optical sizing data for particle size. The size, weight, and cost are greatly reduced from the previous generation.

To increase sensitivity and reduce response time, the instrument uses an aerosol concentrator. This has been a limiting factor for TSI to develop tactical based systems due to power and size. A recent technology development, the Circumferential Slot Virtual Impactor (CSVI), enabled TSI to break through the limitation. The CSVI incorporates a circumferential slit. This reduces end effects and pressure drop associated with circular jets or slits. The result is that power requirements drop from 150 watts to 10 watts. This makes battery operation a possibility.

The FLAPS LT is the culmination of over 12 years of TSI fluorescence trigger technology development and 3 years of CSVI particle concentration technology development conducted with Dr. Andy McFarland of Texas A&M University. Using proven single-particle fluorescence technology at 405 nm in conjunction with a low-power CSVI, a high performance, battery-operated, portable tactical bio-aerosol detector is now feasible.

OPERATION OF AN INTEGRATED BIOLOGICAL DETECTION SYSTEM IN AN
ARMORED PERSONNEL VEHICLE WHILE UNDER DRIVING CONDITIONS

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A Reconnaissance Vehicle has been developed that can accomplish the complex task of performing detection, sampling and in identification of potential chemical, biological and radiological threats in support of troops.

The biological detection subsystem, the “bio-suite”, is an internally mounted workstation incorporating a glovebox that allows operator safety and sample security. In respect to aerosol samples, the system acquires an external air sample into a real-time bio detector. Background measurements are compared to advanced algorithms so that, in the event of detection of a real threat, an alarm signal can be given. Another external air sampling stack delivers the sample into a liquid medium inside the glovebox for further analysis and identification.

This configuration for detection , identification and monitoring is novel and practical, but there have been few observations taken while driving a vehicle of this kind, that is, usually the system are operated with the vehicle stationary. The reconnaissance vehicle in question was operated while in a normal driving mode. A discussion of the system operation, its validation and the bio-background are presented in this poster in addition to comments on its operation under driving conditions.

ELECTRET-BASED AEROSOL SAMPLING

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SystemA dry filter-based aerosol sampling system is described that will function over a temperature range of -40°C to $+70^{\circ}\text{C}$. This energy efficient system is suitable for both military and public health applications, and is designed around a fibrous electret media that displays a high capture efficiency, but which also exhibits an unusually low pressure drop and the ability to tolerate high particulate loadings.

An electrostatic field has been frozen into each filter fiber at the time of manufacture. These fields extend outside the fibers and induce a charge in particulates passing through the filter. This in turn creates an electrostatic attraction between the particles and the fiber matrix, resulting in particle capture. Filters based on electrostatic capture are typically much more energy-efficient than traditional direct impaction filters and may be less harmful to delicate organisms.

The electrostatic forces that are so effective in collecting aerosol particles also make their extraction from the filters difficult. To overcome this issue, a simple wet extraction process has been developed. In tests performed at the U.S. Army's Edgewood Chemical/Biological Center in 2008, this collection/extraction system exhibited the best overall collection efficiency out of often systems evaluated for transition into the Joint Biological Tactical Detection System (JBTDS).

**IMPROVED CHEMICAL PROCESS FOR DECONTAMINATING
CHEMICAL AND BIOLOGICAL AGENTS ON SENSITIVE EQUIPMENT &
PRESENTATION OF THE JMDS**

E. William Sarver, Program Manager, Joint Material Decontamination System,
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Joseph Huber, Director CB Defense Development, Program Manager, Joint Material
Decontamination System, Teledyne Brown Engineering, Belcamp, MD
Nicholas Adams, CEO, Bioquell, Andover, UK

To date studies of hydrogen peroxide chemistry have established it as a highly effective decontaminant for biological warfare agents with significant potential for the decontamination of chemical warfare agents. Under the Joint Materials Decontamination System (JMDS) development work led by the Joint Project Manager for Decontamination, the JMDS hydrogen peroxide and ammonia decontamination system has been demonstrated as an effective process for rapid and thorough decontamination of biological agents, such as, *Bacillus anthracis* and other spore formers, and the chemical warfare agents; HD, GD, and VX. Material compatibility results for a number of materials used in the construction of aircraft and other interiors where sensitive equipment might be installed and needs to be decontaminated will also be presented. The JMDS, targeted for ground, air, sea, and amphibious operational use, provides a multi-role decontamination capability with attractive transportability and supportability features. Information on the new JMDS will be presented as well as a discussion of the equipment used to achieve these results on material compatibility.

DEVELOPMENT OF FULL RANGE NBC HYDRATION PERSONAL SYSTEM

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It has been established, that in hot conditions, approximately 150-200 ml (and no more than 1 liter) of drinking fluids should be consumed by the user each hour.¹ A soldier wearing full protective gear in a contaminated environment generally copes with two vulnerabilities:

1) Insufficient thirst drive and 2) Threat stress.

Drinking procedures in contaminated environments incorporate several safety measures, including personal decontamination before attaching the mask to the standard canteen, safe disconnection etc². These measures can enable the soldier to safely hydrate himself.

Flexible NBC hydration systems with continuous drinking connections can provide the soldier with hands-free drinking and required water volume. Continuous hydration flow also negates the need to exit the environment, which would prolong the individual mission.

The Source NBC hydration system product line was developed to provide the soldier with a wide range of hydration options. The requirements list was built by focusing on the multi-operation view (standard/special combat conditions, up-scaling NBC scenario, logistics support, etc.) and on personal operational characters (ease of mission preparedness, high flow rate, taste-free even under contaminated conditions, etc.)

Our exclusive multilayer film utilizes a proprietary Glass-Like™ liner in combination with a unique NBC barrier layer. This exceptional technology ensures a high level of protection against chemical and biological threats while maintaining the taste-free and easy maintenance qualities.

The novel Source NBC filler cap (Indicap™) provides a simple indicator to ensure the filling port seal.

The novel Source NBC hydration system provides two basic concepts:

1. NBC Maximum Hydration: highest level of protection for hydration with minimum detachable parts and with taste-free qualities.
2. Convertible NBC Hydration: standard hydration system functionality with quick transition to NBC Hydration mode and other features (water pump, quick refill accessory, etc.)

¹US Army, FM 4-02.7. Multiservice Tactics, Techniques, and Procedures for Health Service Support in a Chemical, Biological, Radiological, and Nuclear Environment, July 2009, pp. V-(16-17)

²US Army TM 3-4240-346-10. Operator's manual for chemical-biological mask: field, M40. August 1998.

FUNCTIONALITY BASED RISK ASSESSMENT FOR AIRPORTS AND AIRLINES

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Airports and airlines need protection against terrorist attacks. These attacks may consist of explosives or suicide bombers, armed attacks but also attacks using CBRN agents. Recent examples of attacks are the Christmas 2009 attack in on a Delta flight from Amsterdam in Detroit, the attack on the airport of Glasgow and 9/11. The increase in lethality and brutality over the past 20 years, makes it more likely that a future attack may use CBRNe agents.

Current approaches and systems use a long list of measures, technologies and equipment that is either used, applied or installed in or around the object to protect. Applying such a system or approach results in a long list of items, some of which are marked as 'done' while some are not. This list will tell the owner or user of an object not whether or not the object is sufficiently protected, nor what to do to improve the resilience of the object.

Functionality in our approach has two angles. First, the approach focuses on the functions a terrorist must execute to succeed in an attack and on the counter terrorism functions of an airport or airline owner to counter these functions. Each physical measure, emergency plan, piece of installed equipment, training or applied technology, contributes to a certain extent to some or all of these functional combinations. The resilience of an airport or airline is determined by the extent to which each of these combinations is sufficiently present in an object. Scoring very well on one of the combinations (like detection), could in an old 'list approach' make it seem that an airport or airline is well protected when it is not. Our functional approach will show that improvement is needed in the other areas.

The second functionality angle in our approach is the functionality of an airport or airline, in a 'normal' sense. What is the function of an airport or airline and its components such as a departure hall, office space etc and to what extent has a terrorist attack a negative effect on this functionality? The effect of a terrorist attack is therefore not measured or estimated based on blast profiles or dispersion modeling outcomes but on the effect on the 'real' function of an airport or airline and its components.

THE FABIOLA PROJECT : AN ENHANCED EARLY WARNING SYSTEM FOR BIO-AEROSOL DETECTION

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Defence against biological warfare agents is primary requirement for military operations as well as for civilian security and safety. Timely and accurate warning of biological attack is essential to ensure personnel can take protective countermeasures. Monitoring of hazards also enables protection to be at the earliest opportunity, thus reducing the time spent in protective equipment and minimising performance degradation.

Nowadays, there is an urgent need, recognized by EDA, to develop and provide the forces with early warning systems. In order to fulfil the requirements, European project FABIOLA (Fluorescence Appplied to BIOlogical Agents detection) as been signed in January 2005 between FIN, FRA (lead nation), GRE, ITA, POL and SWE. The main objectives for the consortium were to demonstrate the feasibility of improved LIF detection of BW agents, with some more capabilities, allowed by current technologies such as the spectral absorption profiles, the spectral response (emission), and the time evolution of the emission.

After aerosol sampling and concentration, the optical detection system collects the fluorescence radiation emitted by the aerosol particle under test (hit by a sequence of two UV laser pulses with 50ns delay), splits it into four wavebands covering the 350-600nm range, and acquires the time decay shape by means of 4 ultra-fast MCP-PMTs that are read by a fast electronics. A fifth PMT is devoted to the acquisition of the elastically scattered signal for data normalization.

The requirements are discussed as well as the design and development of the different components including laser source, aerosol sampling, concentration and aerodynamics, optical chamber and detection system

Signal processing is described and the results discussed. Also described are the different test campaign for calibration and field experiment. At the end, some potential future extensions and/or linkages to more complete detection/identification systems are considered.

Keywords: Optical Detection, Laser Induced Fluorescence, decay time, spectral analysis, PCA analysis

SECOND SIGHT[®] MS: A CWA IMAGING SYSTEM

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BERTIN Technologies, specialized in the design and supply of innovating systems for industry, defense and health, has developed a stand-off gas detection system using a multispectral infrared imaging technology. With this system, the gas cloud size, localization and evolution can be seen in real time.

This technology has been developed several years ago in partnership with the “Centre d’Etudes du Bouchet” (CEB) a French MOD organization. The goal was to meet the requirement for an early warning caused by a chemical threat. This led to the patented TACIT process. With a night & day efficiency of up to 2 km, this process is able to detect Chemical Warfare Agents (CWA) critical Toxic Industrial Compounds (TIC) and flammables gases.

Then the process has been adapted to industrial security issues, using off the shelf uncooled infrared cameras, allowing 24 hours a day surveillance of petrochemical plant without costly recurrent maintenance. The adaptations achieved in order to be compliant with Militaries Specifications (MS) have been focused in particular on the HMI and the evolution of signal processing for classifying the detected products.

Second Sight[®] MS is the only, in series produced, passive stand-off CWA imaging system with a wide field of view, already ordered by two regulars armies.

The originality and theory of the gas detection and classification functions have already been described elsewhere. The main topic of this paper is to describe the Second Sight[®] MS.

We will present the results of official acceptance tests on CWA done in the French CEB facilities. The sensitivity threshold of the system in closed chamber for some TICs (like ammonia, phosgene, sulfur dioxide...) and CWAs (G and H agents...) will be given. A video file of the detection of a gas cloud of sulfur hexafluoride at a huge distance of 3 km will be also shown.

TEST AND EVALUATION OF BIOLOGICAL DETECTION, IDENTIFICATION
AND MONITORING EQUIPMENTS

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In various national defence organisations, protocols exist for test and evaluation (T&E) of equipment in order to provide expertise to decision makers or purchasers rendering it available to the defence forces. Biological Detection, Identification and Monitoring (DIM) Equipment T&E programs are not coordinated across national boundaries, thereby limiting response capability by gaps in knowledge and causing duplication of effort in defining a common and improved European BIODIM capability. The aim of this project is to define joint standards for test and evaluation (T&E) regarding biological detection, identification and monitoring capabilities (BIODIM). This project is approved as an *ad hoc* European Defence Agency (EDA) Category B project and will establish a set of agreed T&E protocols strongly suggested to be applied by the acquirers. Thus, this constitutes the reference for the EDA BIOEDP (Biological Detection Identification Monitoring Equipment Development and Enhancement Programme), supports the EU CREATIF and TWOBIAS projects and builds upon results obtained in the EDA project "Database of B agents".

The T&E project consists of two phases, where Phase 1, initiating in 2010, defines T&E criteria. The later Phase 2 is a phase of experimentation crucial for verifying the T&E standards. In Phase 1, T&E criteria will be defined for i) relevant simulants for bacteria, virus and toxins, ii) bioaerosol challenge (simulants) protocols to evaluate biological collection, point and stand-off detection equipments for chamber and outdoor trials, iii) reference methods for T&E of biological identification technologies and sample preparation, iv) identification reagents (T&E protocols), and v) software-based simulation tools to modelling BIODIM system responses in the field.

The presentation will provide an overview of the T&E BIODIM *ad hoc* Category B EDA project and encourage European defence institutes and agencies to participate in this work in order to obtain joint criteria and standards improving BIODIM test systems for End Users.

TWO STAGE RAPID BIOLOGICAL SURVEILLANCE AND
ALARM SYSTEM FOR AIRBORNE THREATS
-TWOBias-

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Biological preparedness includes implementing safety measurements to reduce the number of individuals exposed to biological pathogenic threat agents, whether exposure is caused by bioterrorist attack, an accidental release of biological threat agents or by naturally occurring disease-causing pathogens. Current biodetectors and surveillance systems for continuous monitoring airborne biological agents do not necessarily fulfill performance and User requirements to instill confidence. Realistic trials proving the operational concept of biodetectors and biological surveillance systems at different environments are generally lacking.

The EU Seventh Framework Programme, Theme Security, has financed a three year project, which will initiate in 2010. The aim of this project is to develop a modular demonstrator of a stationary, reliable, vehicle-portable, low false alarm rate Two Stage Rapid Biological Surveillance and Alarm System for Airborne Threats (TWOBias) for use at indoor or outdoor public sites regarded as targets for bioterrorist attacks. Ten partners from six European nations, in addition to End Users are taking part in the TWOBias project which is coordinated by Norway. The TWOBias system includes a two stages scheme, to both provide a detection alarm and identify the biodetected bioaerosols. It will improve the state-of-the-art regarding biological detection by using orthogonal detector techniques (laser induced fluorescence, laser induced breakdown spectroscopy and mass spectroscopy) together with classification and data fusion algorithms. It will also advance the identification technologies by using padlock probe analysis.

The presentation will include an overview of the TWOBias project and outline the scheme of the Consortium's work in order to obtain a successful biological surveillance and alarm system for biological airborne threats.

**PIBBDT: A PORTABLE BIOLOGICAL DETECTION
AND IDENTIFICATION SYSTEM**

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The Portable Integrated Battlespace Biological Detection Technology (PIBBDT) is a technical demonstrator programme for the next generation of biological detectors for the UK armed forces. The contract was awarded to Biral in April 2008 and the design, system development and construction have now all been successfully completed. The final phase in which the system will be rigorously tested in laboratory and field trials is about to begin at the Dstl laboratories.

The objective of the programme is to build a biological detection and agent identification system that has high sensitivity with a very low incidence of false alarms but one that is also portable, adaptable and capable of autonomous operation. The intention was that the system would make use of techniques developed at Dstl but would also use commercial expertise to both develop and supplement these techniques.

During Phase I of the project all the individual elements of the system including the collector, the sensors, the fluidics, the control systems, etc. have been separately developed and final designs produced. Two sensors with competing merits have been developed to determine which offers the best overall performance. The assays for both sensors were initially developed during Phase I but these developments have continued during Phase II to optimise performance.

In Phase II the separately developed elements have been integrated into a single engineered system suitable for both indoor and outdoor trials. As both sensors continued to show desirable performance qualities during Phase II they will both be included in the final phase of testing.

Comprehensive trials in the laboratory, in test chambers and in the field will be carried out by Dstl during Phase III of the project. Separate sensor elements are being built for containment trials with live agent material.

RAPID DETECTION OF BACILLUS ANTHRACIS USING DNA PROBE FUNCTIONALIZED QCM BIOSENSOR

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Abstract: The rapid and accurate detection of *Bacillus anthracis* has drawn great attention for countering bioterrorism. Herein, a DNA probe functionalized quartz crystal microbalance (QCM) biosensor was developed to detect *Bacillus anthracis* based on the recognition of its specific DNA fragment in chromosomal and plasmid PXO1. Firstly, primers were designed towards the specific DNA sequences, i.e., 168 bp Ba813 gene in chromosomal and 340 bp fragment of *pag* gene in pXO1. Then, the upward primer, also as probe with additional 10 bases of T, was modified with mercapto-group at its 5' end, and subsequently immobilized onto the Au electrode of QCM through self-assembly for the hybridization with target ss-DNA sequence. The hybridization between the target DNA and probe would lead to the increase of mass and result in the decrease of resonance frequency of QCM as signal read out. Moreover, in order to amplify the response signal, a thiol-DNA fragment complementary to the other end of the target DNA was functionalized with gold nanoparticles at its 3' end. The results indicated that target DNA fragment could be detectable within 30 min with the functionalized biosensor at the level of 3.5×10^2 CFU/mL of *Bacillus anthracis* cell just after asymmetry PCR amplification but without culture enrichment. This QCM biosensor, coupling with electrochemical, was also used to characterize the procedures of decoration and detection in cyclic voltammogram (CV) and electrochemical impedance spectroscopy (EIS), and the results indicated that the electron-transfer resistance over the QCM surface was increased along with these procedures. Comparing with conventional immuno-biosensor, this DNA functionalized QCM biosensor features the advantage of real-time, stable, label-free, and easily manipulate in the rapid detection for *Bacillus anthracis*.

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**SELF-ASSEMBLED MONOLAYER WITH
HEXAFLUOROISOPROPANOL TAIL GROUP FUNCTIONALIZED
QCM SENSOR FOR TRACE SARIN VAPOR DETECTION**

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Abstract: Sensing layer is quite essential to functionalize a chemical sensor for specific and sensitive detection. Herein, two mecapto compounds with hexafluoropropanol (HFIP) as the tail group were synthesized. The obtained monomer was then modified on the gold nano-particles and deposited on the quartz crystal microbalance (QCM) as the sensing layer to sarin vapor. The sensing experimental results indicated that the functionalized QCM sensor exhibit rapid and reproducible detection to trace sarin. The response time the sensor was less than 2s, and the signal-to-noise resolution of the sensor to sarin vapor can reach $6.3 \times 10^{-2} \text{ mg/m}^3$. The interaction of the tail group HFIP with sarin vapor was characterized with FT-IR. It indicated that the both monomer adsorbed with GB can lead to the shift of the absorption peak of —OH in terminal group, and also that of P-F and P=O bond in GB molecule. This suggested that there was a strong hydrogen bonding interaction between the tail group and sarin molecule.

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DESIGN AND PERFORMANCE EVALUATION OF A 3-STAGE AEROSOL CONCENTRATOR

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A 3-stage aerosol concentrator with one trap impactor and two virtual impactors in a series was designed and fabricated. For separation and concentration of biological aerosols in the air, the first stage trap impactor whose cutoff diameter is 15 μm was designed to remove large particles such as coarse particles, insects, and pollen. Also, the cutoff diameter of the secondary and the third stage virtual impactors was set at 1.5 μm to concentrate biological aerosols. The concentrated particles passed through the third stage virtual impactor were introduced into a liquid collector, cyclone, to collect particles more than 1.5 μm . The total inlet flow rate of the trap impactor was set at 1,000 l/min. The flow passed through the trap impactor was introduced into the secondary virtual impactor and the major and the minor flow rates were 940 l/min and 60 l/min respectively. The flow passed through the secondary virtual impactor was introduced into the third virtual impactor and the major and the minor flow rates were 55 l/min and 5 l/min respectively. The particles passed through the third virtual impactor were accumulated in a liquid collector. Collection efficacies of particle sizes which are 1 μm to 15 μm were tested at wind tunnel. The total separation efficacies for each particle size in 1 μm , 2 μm , and 10 μm were 21%, 71%, and 48%. Also, the collection efficacy for particle size of 2 μm in a liquid collector was 98%. The 3-stage aerosol concentrator was manufactured to operate in harsh environment and was controlled automatically. It is expected that application of the 3-stage aerosol concentrator can vary widely from concentration of biological agents at low aerosol concentration in military field to a monitoring device of respiratory aerosols in atmospheric environment.

DESIGN OF OPTICAL SYSTEM FOR BIOLOGICAL AEROSOL MONITORING SYSTEM

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The biological aerosol monitoring system simultaneously measures the elastic scattered light intensity and the fluorescence emissions for airborne particles and distinguishes man-made biological threat particles from inanimate interferents using the obtained information in the real time. For this purpose, the optical system of the biological aerosol monitoring system is constructed using a laser beam shaping system, a nozzle to focus particles into a small-diameter jet, two reflectors to efficiently collect scattered light and fluorescence signals, and two dichroic beam splitters to separate fluorescence from a collected light signal. Scattered light and fluorescence signals are excited using a laser diode. Geometrical optics has been applied to the design of the laser beam shaping system and a 600 nm wide and 50 nm high uniform beam profile has been obtained. A nozzle focuses aerosol particles into a small-diameter flow to interact with a laser beam and individual particles are illuminated by a continuous and well defined laser beam. As a result of an interaction between a laser beam and focused particles, the light scattering and/or laser-induced fluorescence spectra can be measured by three sensors. These signals provide correlated size distribution for measured particles between 0.5 μm and 15 μm and fluorescence signals every second. Fluorescence signals and scattered light signals are measured using two channel photomultiplieres and an avalanche photo diode. The Optical system of the biological aerosol monitoring system has been designed and developed to discriminate biological aerosols from non-biological aerosols using a size and fluorescence intensity of individual airborne particles. The well defined signals of the simulants of the biological threat agents are obtained in the lab environment from the fabricated system. Real-time data analysis using alarm algorithm will provide interference rejection for biological threat detection in the future research.

DEVELOPMENT OF A SHORT-RANGE FLUORESCENCE LIDAR FOR INTERNAL SECURITY APPLICATIONS

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The Preparatory Action for Security Research (PASR) initiative from the EU invited proposals for research projects in areas that included the development of improved techniques for the detection of dispersed biological agent material. A consortium led by Cilas of France proposed investigating the development of a short-range fluorescence lidar for the detection and discrimination of biological aerosols at stand-off distances of a few hundred metres. The proposal was successful and became the Biological Optical Detection Equipment (BODE) project. In total there were nine participating organisations with Biral (UK) responsible for the lidar receiver and data acquisition systems, DLR (Germany) the laser system, Cilas (France) the integration and software, FOI (Sweden) the system trials and CEB (France) the data analysis. Other members of the consortium provided support and advice based on their experience in related fields.

The principles of the system design were established by agreement between all the consortium members with the detailed design of the components and other aspects of the project the responsibility of the individual organisation. The governing objective was to extend the technology of fluorescence lidar to give the best possible discrimination between deliberately generated biological material and the particulate background of the atmosphere.

A prototype system was designed, built and assembled by the consortium members responsible for the hardware. It was then shipped to the FOI trials ground in Sweden for field trials. This poster will describe the principles of the system design with particular emphasis on the receiver and data acquisition systems. It will also show results from the trials carried out at the Swedish test ground with the integrated system.

THE TOOLS FOR AN OPERATIONAL BIO DIM CAPABILITY

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The problem of biological security represents recently a challenge to both the new technology development and the crisis situation management, in particular of the risk assessment area. Due to the enormous diversity of biological agents, the biological risk is hardly to estimate. Thus the need for rapid, sensitive and selective tools for detection, identification, and finally unambiguous typing of biological agents still exists. The BIO DIM tools represent the substantial methodology for resolution of biological incidents in the frame of biological crisis management (see fig. 1).

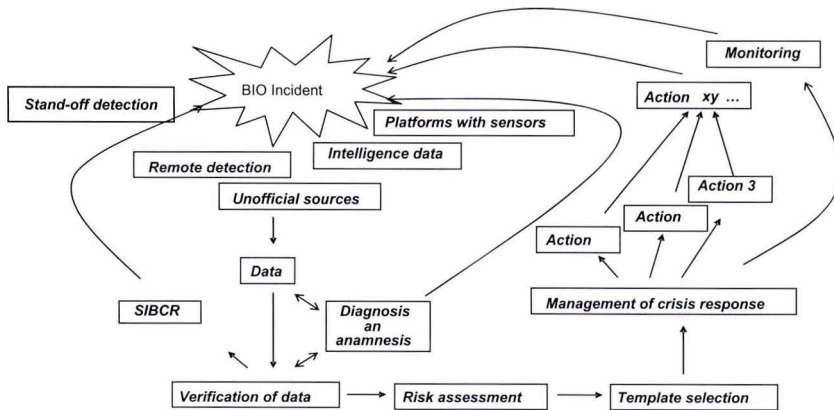


Fig. 1: The scheme of reaction to a biological incident - Bio DIM and management of crisis response

The bioinformatics and epidemiological characteristics of B-agents as well as genomic and proteomic biomarkers as prove of exposure constitute the main tools in Bio DIM and biological risk assessment. There is a wide range of methods available for the purpose. They differ in physical principles and many other aspects making them suitable for the utilization at certain stages of the process of resolution of biological incidents. Selectivity, sensitivity, cost, speed, instrumentation etc., all has to be taken into account when putting the methods onto the map of potential application in BIO DIM. DNA has been main focus of majority of recent development in methods and techniques targeting the need to differentiate and discriminate all B-agents. The potential of those methods is dramatic, but it also has certain limits. Protein analysis has gone through revolutionary phase mainly due to tremendous advances in mass spectrometry instrumentation. However, no single technology can fill up the required operational Bio DIM capabilities. Here we summarize the possibilities of the current methods and their characteristics that could be exploited for creation of functional Bio DIM system.

RESULTS FROM BIOLOGICAL AEROSOL STAND-OFF DETECTION AT A FIELD TRIAL

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We have performed a field trial to evaluate technologies for stand-off detection of biological aerosols, both in daytime and at night. Several lidar (light detection and ranging) systems were tested in parallel (see left in Figure 1). We present the results from two different lidar systems; one system for detection and localization of aerosol clouds using elastic backscattering at 1.57 μm , and one system for detection and classification of aerosol using spectral detection of ultraviolet laser-induced fluorescence (UV LIF) excited at 355 nm. The UV lidar system was utilizing a photomultiplier tube (PMT) array for the spectral detection. During the first week of the field trial, the lidar systems were measuring towards a semi-closed chamber at a distance of 230 m. The chamber was built from two docked standard 20-foot containers with air curtains in the short sides to contain the aerosol inside the chamber. Aerosol was generated inside the semi-closed chamber and monitored by reference equipments, e.g. slit sampler and particle counters. Signatures from several biological warfare agent simulants and interferents were measured at different aerosol concentrations. During the second week the aerosol was released in the air and the reference equipments were located in the centre of the test site. The lidar systems were measuring towards the test site centre at distances of either 230 m or approximately 1 km. In this paper we are presenting results of the lidar systems from both the semi-closed chamber tests and the free releases. The graph in Figure 1 shows a result of spectral fluorescence detection of *Bacillus thuringiensis* spores.

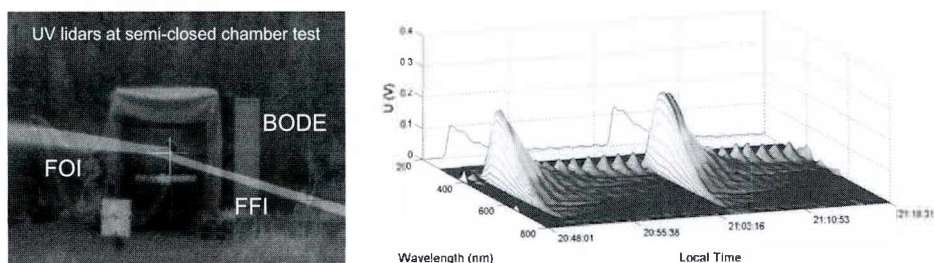


Figure 1. To the left a UV picture of three UV lidar systems in action. To the right is an example of results from spectral fluorescence detection of a *Bacillus thuringiensis* release in the semi-closed chamber.

APPLICATION OF TWO DIFFERENT FTIR TECHNIQUES FOR
DISCRIMINATION OF BIOLOGICAL MATERIALS

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Rapid detection and discrimination of dangerous biological materials such as bacteria and their spores has become a security aim of considerable importance. Various analytical methods, including FTIR spectroscopy combined with PCA (Principal Component Analysis), have been used to identify vegetative bacteria, bacterial spores and background interferants. The present work discusses the application of two different FTIR techniques for discrimination of biological materials, including: KBr pellets technique using transmission mode and Horizontal Attenuated Total Reflectance (HATR) technique. Both techniques provide rapid analysis for materials identification, but HATR (in comparison with KBr pellets) is non-destructive technology that can be used for bacterial cells without damaging of their biological structure.

Several kinds of biological material was analysed in this study. The IR spectra of bacteria and spores have been measured using HATR technique. Spectra of fungi which belong to the background interferants have also been measured. KBr pellets have been used to measure the spectra in transmission mode of another group of interferants - pollens and smut spores.

It has been demonstrated that each kind of biological material have unique infrared signatures in the IR spectrum. It can be used to preliminary classification of bacteria and spores. PCA showed the ability to reliable discrimination of different biological materials.

MID-RANGE AND SHORT-RANGE STANDOFF SYSTEMS FOR B-AGENTS
DETECTION

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Two lidar-type systems for real-time standoff bio-agents detections are presented. Both devices are based on Laser Induced Fluorescence (LIF) phenomenon, however they differ in size and operational destination.

The short-range system is a hand-held, small device for a single person to keep and perform detection analysis, particularly in confined spaces of buildings, airports, etc. The mid-range system was designed to provide the stand-off detection capability at ranges above 300-500 m. It is a mobile platform designed to be installed on a vehicle or on a building. Additionally, it's combined with a scanning mechanics and advanced software, which enable to conduct the semi-automatic monitoring of a specified space sector. The system is equipped with an eye-safe (1,5 μm) laser for elastic backscattering detection and a depolarization detection component to serve as the particle-shape analyzer, which is very valuable in case of non-spherical bio-aerosols sensing.

The difference in working range, weight and overall dimensions of the two systems come from laser light sources and detection optics applied. In case of the short-range device, laser diodes and LEDs at wavelengths 375 nm, 405 nm, 445 nm have been applied. They operate in a *cw* (continuous-wave) regime and are coded within 5 ms length sequences for our sub-noise detection technique requirements. The mid-range system on the other side, takes the advantage of high power solid state Nd:YAG lasers, namely its 3-rd (355 nm) and 4-th (266 nm) harmonics, emitted in the form of short (~ 6 ns) pulses with the repetition rate of 20 Hz.

***DIFFERENTIAL MULTI-PIXEL IMAGING STANDOFF CHEMICAL DETECTION,
iCATSI***

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ABSTRACT:

Defence Research and Development Canada (DRDC) – Valcartier and ABB Bomem are currently developing, the iCATSI, a new member of the CATSI (Compact ATmospheric Sounding Interferometer) family. This passive standoff sensor developed for the detection of chemical warfare agents (CWAs) is based on the differential multi-pixel Fourier-transform infrared (FTIR) radiometry technology. This novel hyperspectral instrument uses a proven double-beam FTIR modulator optimized for optical subtraction. The iCATSI is optimised for the VLWIR (cut-off near 14 μm) and generates three images of 24 by 16 pixels per seconds with a total field of view of 24 x 16 mrad. The multi-pixels configuration allows the acquisition in differential mode. The instrument design and initial results from field trials will be presented. This includes detection of chemical warfare simulants released in reconnaissance and survey type scenarios. The analysis focuses on establishing the capability of the iCATSI sensor for the detection and identification of chemical vapor plumes.

KEYWORDS:

CATSI, FTS, hyperspectral imaging, Spectrometer, FTIR, standoff detection

FIELD PORTABLE AUTOMATED BIOSENSOR 4000 FOR RAPID MULTI-BIOAGENT THREAT DETECTION

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With the ever emerging threat of biological agents the need for rapid accurate detection is paramount to first responders. The original launch of the Biosensor 2200 provided first responders a highly sensitive field portable rapid detection system for a variety of single biological agent threats. The system was light weight, easy to use and boasted a false positive rate of 1 in 1,000,000. The Biosensor 2200 allowed for the determination of a single agent threat in less than 5 minutes. The single agent tests included a wide arrange of bacterial targets (*Anthrax, Plague etc.*) and toxins (*BotA, Ricin, SEB etc.*). These single agent tests enabled the user to quickly determine the presence or absence of a specific biological agent. One limitation of the first generation Biosensor 2200 is the single test nature of the system, the user must run multiple tests to evaluate the presence of multiple agents. The next generation biosensor was developed to directly address this concern. The Biosensor 4000 will enable the first responder to test specifically for three pathogens while employing a built-in negative control. The Biosensor 4000 is fully automated and requires no other user interaction other than sample addition. The Biosensor 4000 platform, like its predecessor, provides a rapid, highly sensitive, reliable field portable unit for biological agent detection.

TEST OF BIOLOGICAL AGENTS DNA PREPARATION METHODS

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The crucial phase of proper B-agents detection and identification by molecular genetics methods is DNA preparation. DNA preparation from a sample with very low concentration of tested B-agent is the main problem. Even the highest quality identification methods failed without enough quantity of DNA in prepared sample.

We tested a ten commercial available methods for a bacterial DNA preparation suitable for system of biological defence: boiling in water, Maxwell 16 DNA Purification Kits (Promega Corporation), Maxwell 16 DNA Purification Kits with lysozyme incubation (Promega Corporation), IT 1-2-3- SWIPE Sample Purification Kit (Idaho Technology Inc.), PrepMan Ultra Sample Preparation Reagent (Applied Biosystems Corporation), GenElute Bacterial Genomic DNA kit (Sigma-Aldrich Corporation), microwave, combination of liquid nitrogen and boiling in water, lysis and lysis with lysozyme incubation.

All these tests were performed with group of bacterial species. Species were chosen so that cover wide spectrum of bacteria. Representatives are from G⁻ as well as G⁺ coccus and rods: *Acinetobacter haemolyticus*, *Nocardia asteroides*, *Corynebacterium diphtheriae*, *Actinomyces israelii*, *Staphylococcus epidermidis*, *Bacillus subtilis* (vegetative), *Bacillus subtilis* (sporulating) and *Escherichia coli*. Suspensions of all bacteria were prepared in 3 copies of each and two types of concentrations: 10^5 a 10^8 .

Quantity of a total result DNA in the final samples was controlled by the Qubit fluorometer instrument (Invitrogen). Different results were measured for all methods used for the group of samples with 10^8 concentrations.

We have found a problem with DNA preparation from samples of 10^5 concentrations. Only PrepMan Ultra Sample Preparation Reagent method has shown positive result for all samples. It was probably due to "nonspecific background" since all measured values are very similar. Positive result was shown sporadically in case of other method.

We tested quality of DNA samples and their capability to be used for identification procedures in the second phase. We have used the Real time PCR method with the primers for bacterial 16S rRNA.

Our general conclusion of this study is that boiling in water is the most suitable method for a mobile biological team. This method was identified as very simple, fast, as well as reliable in case of all bacteria. This method can be used under the field condition even by team without wide experience in laboratory working.

Supported by the Ministry of Defence of the Czech Republic project No.: OVUOFVZ200901 "Biodefence".

**AN APPROACH TO SUSPICIOUS BWA POWDER INCIDENTS USING A
COMBINATION OF ORTHOGONAL FIELD PORTABLE TECHNOLOGIES**

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The number of suspicious powder incidents has increased in the past decade since the anthrax mailings 2001. While the vast majority of these incidents turn out to be hoaxes, they still call for a systematic approach to efficiently assess the threat in the field while collecting the bulk sample for confirmatory testing at a public health laboratory.

This poster presents an approach to suspicious BWA powder incidents using a combination of orthogonal field deployable technologies. This includes preliminary screening using a field ruggedized FTIR spectrometer suitable for identifying most common 'false alarm' powders. This is followed by a broad spectrum microbe assay that flags the presence of DNA containing bacteria and bacterial spores, followed by a multi-toxin assay that flags the presence of ricin, botulinum or SEB toxin. If any of these screening tests are positive or if other credible threat evidence exists, a Field Presumptive Identification test is performed using a field portable PCR instrument with highly sensitive and selective assays suitable to identify *B. anthracis*, *Y. pestis*, *F. tularensis* and Pan-Orthopox. Efficient use of this combination of technologies by well trained public safety personnel allows rapid threat assessment and public safety action to be taken pending final testing.

**ANALYSIS OF SAMPLES CONTAINING A MIXTURE OF BIOLOGICAL,
CHEMICAL AND RADIOLOGICAL AGENTS ("MIXED SAMPLES")**

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Efficient response and national preparedness against biological, chemical and radiological (CBR) threat agents is dependent on reliable detection and identification methods of *any* sample to be analysed. The analysis of several CBR agents simultaneously and/or immediately in sequence is more challenging compared to analyses of a sample solely containing one agent. NATO has recognized this challenge and has taken effort to strengthen the alliance's competence on handling samples containing a mixture of CBR agents (mixed samples) by organizing exercises among the participating nations of the NATO SIBCRA Subgroup (Sampling and Identification of Biological, Chemical and Radiological Agents). These exercises were initiated in 2007. The Norwegian Defence Research Establishment (FFI) takes part in these exercises and has now developed and implemented a set of procedures and routines *in house* for handling and analysis of "CBR mixed samples". FFIs CBR laboratory capability is unique in Norway and thus, also improves national defence against CBR agents. There are several interdisciplinary challenges for efficient analysis of CBR mixed samples, also requiring well-defined routines and excellent collaboration among laboratory personnel as well as safety issues to protect all personnel against the threat agents.

As, no adequate real-time detectors for screening of biological threat agents are available, the sample must be handled as contaminated of such. This requires that the sample is opened in a BSL-3 laboratory (Biological Safety Laboratory). Our procedures outlines, in sequential order; screening of the sample for C and R at arrival, transport to the BSL-3 laboratory, opening of the sample and further C and R screening and then aliquoting of the sample. Further preparation of the aliquoted subsamples includes sterilization/disinfection prior to transport out of the BSL-3 laboratory. Our work suggests that sterilization (autoclave) could be sufficient preparation of the subsample for radiological analyses while samples prepared for identification of chemical agents must be filtered prior to analysis to eliminate bacterial agents from the sample. The radiological hazard during chemical and biological analyses is alleviated by minimizing personnel contact time and/or by analysing smaller aliquots. Chemical and radiological analyses are performed in respective laboratory facilities.

Biological Threat Detection on a Lab-On-Chip Platform

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A Lab-on Chip (LOC) platform designed for early detection of Biological agents namely *Bacillus anthracis*, *Francisella Tularensis*, *Y. Pestis* and Vaccinia Virus has been evaluated in this study. The LOC platform combines the use of two powerful molecular diagnostic techniques: Polymerase Chain Reaction (PCR) and Micro-array. The polymerase chain reactions allow the amplification of a few copies of specific DNA/RNA sequence into large enough numbers for detection. The Micro-array enables specific identification and differentiation of a large number of targets from DNA pool in a single test. The single LOC platform evaluated in this study is able to concurrently detect the 4 biological agents in one single diagnostic time of 2 hours.

The laboratory study has shown that the LOC platform is able to achieve a high level of detection sensitivity and is able to differentiate between the target species and close neighbors and unrelated strain.

CHEMICAL SENSOR TRIAL FOR NERVE AGENT DIFFERENTIATION: IMPACT OF
HYDROGEN BONDS ON DETECTION

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Chemical warfare agents (CWAs) are a growing concern to many countries. The uses of CWAs by terrorist, organizations or even states is significant as they can be readily synthesized by simple chemical reactions and often have an extremely high toxicity. Unfortunately, there is incidence of CWAs being used in human history and many countries.

Conventional analytical techniques for detection of nerve agents from environmental and biological samples includes such as gas chromatography, liquid chromatography, gas chromatography–mass spectrometry, ion chromatography, atomic emission detection, capillary electrophoresis, etc... These methods have very high sensitivity, reliability and precision. However, in spite of these advantages, these techniques require expensive instrumentation and highly trained personnel, as well as time consuming and unsuitable for field analysis. To meet these prerequisites of rapid warning and field deployment, more compact low-cost instruments are highly desirable for facilitating the task of on-site monitoring of nerve agents. quartz crystal microbalance (QCM) sensor could be a reliable and promising alternative to routine methods because of their simplicity, ease of use and high sensitivity and selectivity [1,2].

In this study, we prepared QCM sensors functionalized with $-NH_2$ and $-COOH$ groups for differentiate diethyl ester phosphonic acid (DEHP) from diethyl phthalate (DEP), which are known as G and VX agent stimulants respectively. Infrared spectroscopy (FT-IR) was performed in order to characterize the surface of the sensor after modification and the detection. Furthermore, impact of hydrogen bonds on detection was discussed.

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**EVALUATION OF THE REAL TIME PCR FOR DIAGNOSIS OF *BRUCELLA SPP.*:
DIFFERENTIATION BETWEEN *BRUCELLA ABORTUS* AND *BRUCELLA
MELITENSIS***

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Brucellosis is considered to be an emerging infection in some regions of the world, including Portugal. Some epidemiological, microbiological and clinical parameters of *Brucella spp.*, as for example the small inoculum needed to induce human disease, traditionally described in the levels of 10-100 microorganisms, and its significance as a potential agent of bioterrorism was acknowledged early, reason why it remains on the category B biodefense research list of the CDC.

The important means of transmission in the context of bioterrorism importance is airborne transmission. *Brucella* can be easily aerolized, and when in air, can be easily transmitted through the airways and induce disease while staying for a protracted period in this virulent form.

In Portugal, brucellosis is endemic and became a notifiable disease in 1949. Surprisingly, probably due to the underreporting problem, already detected in other similar diseases, only a few cases are reported each year, which does not allow consistent analysis of risk factors and the impact on public health. According with the data, between 2003-2007, 590 cases were notified in Portugal.

To be able to identify the *Brucella* species present in a biological sample, using fast and reliable molecular methods is crucial to differentiate between endemic cases, caused endogenous strains and clinical cases due to the deliberate dissemination of a highly pathogenic variant.

In the present study, we describe the evaluation of the recently established real-time PCR assay that allows not only the rapid detection of the *Brucella* genus (IS711) but also the differentiation between two species, *B. melitensis* and *B. abortus*.

We tested several bacterial genera involved in bioterrorism as controls and determine a 100% of specificity. Amplification of 10 fold serial dilution of each strain revealed linearity from 10^7 to 10 copies for both the genus corresponding an sensitivity of 10 copies/ml.

We conclude that this protocol could be a valuable tool for the detection of *B. abortus* and *B. melitensis* in biological samples because it allows a rapid and accurate diagnosis and could be used as a valid method for laboratory diagnosis of Brucellosis in endemic cases and bioterrorism situations.

THE PORTABLE MINI-LAB DEFI FOR RAPID DETECTION OF TOXINS

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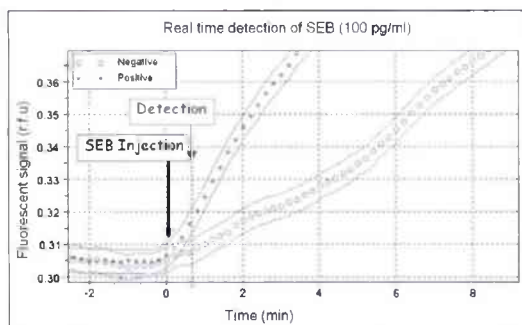
We have recently designed a Planar Optical Waveguide Sensor (POWS) which was integrated in a compact, portable and real-time mini-laboratory named DEFI well adapted for outside biodetection.

The first use of this mini-lab DEFI is the multiplex detection of low molecular weight proteins without labelling step thanks to a fluorescent renewable immunoassay.^[1] The first application demonstrated was the continuously monitoring of Aflatoxin in drink water during 3 weeks without the intervention of any operator.^[2]

The small size (10 L) and low weight (4 kg) of DEFI are also very interesting characteristics for emergency diagnosis and area control applications. In the mini-lab DEFI, we have designed a three steps sandwich immuno-assay protocol (baseline, reaction, optional washing) with only two reagents (1 negative buffer, 1 labelling reagent mixed with the sample).

Interestingly, the mini-lab DEFI allows real time detection during the reaction with the sample without the need of a washing step. *Botulinum neurotoxins B* (BoNT/B) and *Staphylococcus Aureus Enterotoxin B* (SEB) were respectively detected in 20 min and 2 min. Limits of quantification after 30 min are respectively estimated to 1.5 ng/ml and 30 pg/ml. The limit of detection obtained for SEB in the mini-lab DEFI was comparable to the one of ELISA test using the same antibodies (20 pg/ml after 90 min). if detection performances are comparable to the ELISA, the protocol is simplest (no enzyme, no washing, applicable by non-specialists) and gives a first response more quickly.

So, the DEFI mini-lab is well suited for outside uses, emergency diagnostic on area.



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**DESIGN OF A HAND PORTABLE GC-TMS FOR DETECTION AND
IDENTIFICATION OF HIGH THREAT CHEMICALS**

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Explicit in the design of field portable instrumentation are the requirements to produce products that provide the sensitivity/accuracy necessary to accomplish mission objectives. At the same time users are requesting tools that are smaller, lighter and faster than previous units. Smiths Detection has partnered with Torion Technologies to produce a hardened ruggedized version of their GUARDION[®]-7 GC-TMS.

The complete GC-MS system weighing approximately 13 kilograms is housed in a magnesium enclosure. The enclosure is sealed, capable of operation in the hot zone and of withstanding standard decon procedures. The system is 44.7 x 38.2 x 22.9 cm with a touch screen/keypad user interface. The system is battery operated and capable of 2 – 3 hours operation from a single rechargeable battery. The carrier gas is supplied by a 250 cc cylinder of helium that provides 100 – 200 runs.

The mass spectrometer is an Ion Trap with a unique toroidal (TMS) design. The Toroidal design allows for miniaturization of the Ion trap while maintaining the trapping volume. The use of mechanical pumps provides for extremely fast startup times, less than 5 minutes. The GC is a low thermal mass with optimized geometries for fast heating and cooling cycles. The capillary GC system contains a 5 m x 0.1 mm x 0.4 µm df capillary column coated with 5% phenylmethylpolysiloxane stationary phase. Primary sample introduction is achieved by a novel solid phase micro-extraction (SPME) syringe. The GC-TMS has a universal sample interface that includes provision for air sampling and future expansion of sampling capabilities. The high speed GC supplies excellent separation of complex mixtures in minimal time (3 minute run 6 minute cycle typical) with identification by the mass spectrometry using on-board or reference libraries.

ION MOBILITY SPECTROMETRY EQUIPMENT INTERFERENT EVALUATIONS

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Ion mobility spectrometry (IMS) chemical detection, identification and monitoring (DIM) equipment often suffers from false positive readings, caused by non-threat substances present in an environment. These false positive readings are due to the interferents possessing similar chemical characteristics to the target molecules resulting in similar drift times. This can cause a false positive alarm in the absence of a target molecule. Such interferents are present in many common household and industrial products for example personal hygiene and cleaning products. To aid in identification of interferents HOSDB have developed a bespoke air sampling kit that enables first responders to take an air sample when the DIM equipment false alarms. These air samples are analysed by Gas Chromatography-Mass Spectrometry and enable interferents present in the environment to be monitored and a known interferent list compiled. This interferent list is then utilised as part of the interferent testing for the Manual of Search and Detection equipment: Part B CBRN – commonly known as the ‘Blue Book’. The Blue Book is distributed to first responders as a reference guide for purchasing new equipment. Commercially available equipment is evaluated prior to entry into the Blue Book, and the equipments performance when challenged with interferents is tested as part of this process. Vapour interferent chemicals are presented to the equipment in a controlled manner and the response of the equipment is observed. If the equipment performs to a standard suitable for first responders then it is entered into the Blue Book.

Automatic Chem/Bio BMD post intercept ground prediction

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During the international Ballistic Missile Defence (BMD) exercise (JPOW) in 2008 , TNO presented a prototype of a post intercept ground prediction tool (HAPPIE/RIOT) that was directly connected to the Air and Missile Defence Datalink network (Link16), e.g. a Patriot unit. By doing so, consequence calculations from Chem/Bio BMD intercepts could automatically be calculated based on the intercept messages reported on the Link16 network, effectively decreasing the time for warning and reporting of Chem/Bio incidents from more than 45 minutes to 30 seconds.

At JPOW 2008 it was difficult to integrate the new predictions into the NATO Warning and Reporting network. Since then, NATO has developed the ATP-45(D) standard with reports and templates which enables to share information on BMD consequences.

The NBC-Analysis Warning and Reporting software developed by Bruhn NewTech is being modified to become CBRN-Analysis and cover ATP-45(D). A BMD module based on HAPPIE/RIOT is being developed jointly by TNO and Bruhn NewTech.

Implementing this functionality improves the CBRN passive defence capabilities, and raises some new questions:

- Ground effects can be predicted very fast, in some cases even before they reach the ground;
- How can a consequence calculation system help during BMD planning where more information is available to minimise contamination of vulnerable targets by suggesting alternative launch locations? ;
- How will an operator manage several (suspected, but not confirmed NBC) missile intercepts simultaneously and ground consequences can (theoretically) emerge every minute? The speed of processing might probably exceed the capabilities of the NBC organization when multiple consequence areas are calculated within less than a minute;
- Which intercepts can be handled automatically and which events need operator interaction? How will the NBC cell and the Patriot operators work together to achieve the most effective organisation and procedures?

This presentation will describe the system, as well as lessons learned and solutions for some of the challenges described above.

DEVELOPMENT OF AIR SAMPLING STRATEGIES FOR MONITORING COMMON AIR POLLUTANTS IN MISSION AREAS

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It can be problematic to collect representative samples in the occupational environment of deployed soldiers using personal samplers. In the present study several air sampling strategies were examined to identify the most efficient method for collecting air samples that were representative of the soldiers' exposure profiles at the Swedish KFOR Camp Victoria outside Pristina, Kosovo. Stationary monitoring was performed during two five-day sampling campaigns, one in summer and one in winter, then the acquired data were related to measurements obtained by personal monitoring of three and four subgroups, respectively. Patrolling soldiers, mechanics and indoor workers were selected to represent groups with different exposure profiles, and particles, metals associated with particles, nitrogen dioxide, sulfur dioxide, polycyclic aromatic hydrocarbons, aldehydes and volatile organic compounds they were exposed to were measured.

Generally, low concentrations of the analytes were found in both sampling periods, but the variability was greater in the winter campaign. Samples collected by stationary samplers captured most of the variation in the personnel's exposure, according to Principal Component Analysis (PCA). However, the results also indicate that personal exposure to most potential pollutants would be underestimated if a single outdoor station was used to monitor them. Nevertheless, combined data from stations located outdoors and in relevant microenvironments (in a workshop, an office and a guard station), provided substantially better representation. Thus, it may be possible to obtain monitoring data without using personal samplers in these inherently problematic situations.

COMPARISON OF FLUORESCENCE OF BIOLOGICAL MATERIALS WITH DEEP UV
EXCITATION (225 NM, 266 NM AND 280 NM)

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In this study we analyze possibility of application of laser induced fluorescence for discrimination of BWA from various interferents. Fluorescence of 30 materials, belonging to 8 groups (vegetative bacteria, bacterial spores, pollens, fungi, amino acids, proteins and native bacterial fluorophores) was measured using Edinburgh Instruments fluorescence spectrometer. The samples were analyzed in water solutions/suspensions. Three excitation wavelengths were chosen for analysis: 266 nm (for quadrupled Nd:YAG application), 225 nm and 280 nm (excitation wavelengths optimal for maximum native fluorescence intensity). Emission spectra were analyzed using Principal Components Analysis (PCA).

PCA was performed for individual excitation wavelengths and also for all excitations together. In all cases bacteria and bacterial spores form one group. Bacterial spores (BTs and BGs) of technical grade form distinctly separate group from clean spores.

Excitation with 225 nm results in merging of albumins with bacteria-spore group, but simultaneously that wavelength gives best discrimination between bacteria and spores. Pollens are spread over broad graph area but generally can be separated from bacteria and spores. Excitation wavelength 225 nm is only one that excluded completely pollens from bacteria-spore group.

Presented results show possibility of discrimination bacteria/spore BWA simulants from interferants like fungi and pollens. Application of excitation at 225 nm, which until now was not often considered, opens possibility to discriminate between bacteria and spores. Measured database is a starting point for development of stand-off and remote detection of BWA.

**Application of two dimensional ^1H - ^{31}P NMR spectroscopy
to the determination of chemical warfare agent related compounds**

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Abstract Two-dimensional ^1H - ^{31}P heteronuclear multiple quantum coherence (HMQC) NMR method was used for determination of organophosphorus compounds related to the Chemical Weapons Convention. The HMQC method provided a means for associating a phosphorus resonance with the resonance of a proton or even many protons simultaneously, demonstrated high sensitivity and selectivity. It could also produce a clean spectrum from a sample containing intense background resonances. It was shown in the 24st and 26st OPCW proficiency test that the ^1H - ^{31}P HMQC method is the key technique to identify organophosphorus compounds of chemical warfare agents in environmental samples with intense background signals and huge interfering matrix.

Keywords ^1H - ^{31}P heteronuclear multiple quantum coherence, NMR, chemical warfare agent

HYPERBRANCHED POLYMER LAYER-BY-LAYER SELF-ASSEMBLED ON RESONANT MICRO-CANTILEVER FOR DETECTION OF SARIN IN TRACE-LEVEL

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Abstract: Sensitive detection of nerve agents is of great essential and has drawn great attention from the entire world. In this work, a hyperbranched polymer with terminal sensing groups is grafted layer-by-layer on a resonant micro-cantilever for specific detection of trace sarin vapor. The hyperbranched polymer with function-groups is directly constructed onto cantilever surface via a layer-by-layer routine of A_2+B_4 composition, where A_2 and B_4 are complementary interaction groups for coupled linking. This hyperbranched structure provides a very high specific-surface-area to specifically load enough mass of the targeted chemical small molecules for resonance detection. In addition, the “more branches but fewer roots” polymer structure is expected to depress the cross-talk signal from adsorption induced cantilever spring-stiffening effect. Experimental results indicated that the functionalized resonant cantilever sensor exhibit rapid and reproducible detection to trace GB, with the detection limit as low as 5.0 ppb. Based on the FT-IR analysis results, the interaction between terminal group and GB molecule is investigated. It indicated that the hyperbranched polymer adsorbed with GB can lead to the shift of the absorption peak of —OH in terminal group, and also that of P-F and P=O bond in GB molecule. The hyperbranched sensing polymer and its construction on the cantilever surface feature effective elimination to the cross-talks from water, other organic solution and the cantilever spring-stiffening induced parasitic frequency-shift. Therefore, the developed technique of sensing-group-functionalized hyperbranched polymer directly growing on a cantilever can become a micro/nano combined sensing-platform for broad applications of trace chemicals detection.

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Poster Session

**HEMP AND CYBER WARFARE: UNRECOGNIZED THREATS TO MODERN
MEDICAL CARE**

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We rely on high technology every day. Cell phones, pagers, two-way radio, computers, palm pilots...even our vehicles are computerized. Few people recognize the vulnerability of both the power grid and modern electronic devices to the high-altitude nuclear electromagnetic pulse (HEMP) generated from a detonation. Furthermore, non-nuclear sources of EMP can be generated for a few hundred dollars and a little know-how. It is an effective force multiplier to disrupt critical infrastructure, including medical technology, at a critical time.

Cyber-warfare has already been used in Europe, the Middle East, and against the USA—it can disrupt whole economies by disabling critical infrastructure. It can also be used directly or indirectly to target medical systems—both hardware and software-- down to the individual patient with a pacemaker.

Prevention and Mitigation of HEMP and Cyber Attacks

| | Prevention and Preparation | Mitigation |
|---------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|
| Malware Attacks | Firewall, updates and patches, antivirus | Back-up image of OS, optical copy of alternate OS |
| Hardware Attacks | Disconnect critical systems from the internet, use strong wireless encryption | Off-line, redundant hardware (processor and drives) |
| Electronics Damage | Filters, shielding, disconnect cables on units not in service | Offline, back-up systems held in reserve, non-electronic alternatives, alternative communications |
| Long-term Power Disruption | Educate medical leaders about the threat and lobby politicians for infrastructure protection | Extreme power conservation, alternative local power sources, austere medicine techniques |
| Data Loss or Alteration | Disconnect critical systems from the internet, use encryption | Off-line, back-up copies of data on non-rewritable optical media |
| Medical Response to Austere Conditions due to Infrastructure Degradation | Practice Total Intravenous Anesthesia (TIVA), learn regional anesthesia techniques, have a personal (family) survival plan, practice the plan! | TIVA, regional anesthesia, heat and chemical disinfection techniques, stockpile medications, simple surgical techniques |

**ESTIMATION OF ATMOSPHERIC GAS-PHASE OH RADICAL REACTION
RATE FOR THE ABANDONED CHEMICAL WEAPONS AGENTS IN CHINA BY
JAPAN**

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abstract: A structure-activity relationship developed for the estimation of rate constants for the gas-reactions of OH radical with organic compounds is described and extended to several abandoned chemical weapons agents (CWAs) in China by Japan. The use of this estimation method is illustrated. The kinetic data we got have been used to derive atmospheric lifetimes for these species. Our results we obtained demonstrate that this technique will contribute to the environment impact assessment in the course of excavating and destroying these CWAs.

Keyword: OH radical; structure-activity relationship; abandoned chemical weapons agents in China by Japan ; rate constants

CHEMICAL WARFARE IS THE LEAST DANGEROUS WAR

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Chemical agents are substances intended for use in military operations to kill, seriously injure, or incapacitate humans because of their toxic properties. A model is developed to simulate the spread of chemical warfare agents. It is found that the spread of chemical warfare agent is greatly influenced by the nature of explosion and thus the area covered in the explosion poses greater hazard compared to the downwind spread area. The model gives the immediate impact area and the affected area. The dispersion of the gas is also presented based on the wind direction and speed. It uses the data; type of chemical warfare agent, amount of agent, weapon type, wind direction and wind speed. After providing the details, the model predicts the speed and the direction of the spread of the chemical warfare agent. It was concluded that the spread of chemical agents makes there concentration below the lethal dose, except for the area of immediate explosion which is more deadly in conventional weapons.

The ORCHIDS Project: evaluation, optimisation, trialling and modelling procedures for mass casualty decontamination

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Effective decontamination will be crucial to the outcome of any incident involving the deliberate release of potentially hazardous substances and large numbers of contaminated casualties. This presentation will communicate the interim outcomes of the ORCHIDS project, which aims to investigate fundamental aspects of mass casualty decontamination which have not been subject to prior scientific scrutiny. The project will enhance response capabilities via the identification of ways in which decontamination processes for emergencies can be optimised.

Project activities currently underway are exploring mass casualty decontamination from 'first principles' in laboratory and field trials, which will inform a large scale mass casualty decontamination field exercise and the development of simulation models of decontamination emergency response. A review of EU civilian decontamination provision is also underway, incorporating the development of guidelines for best practice with vulnerable groups in mass casualty decontamination.

The ORCHIDS project will deliver quantitative evidence on the optimum techniques for dealing with a range of potential contaminants and scenarios requiring emergency decontamination. This applied research will generate evidence-based guidelines on the optimum techniques for effective mass casualty decontamination, which will be disseminated by the project team through a network of EU partners and stakeholders. The project will also consider the provision for minority and vulnerable groups in emergency decontamination, and will produce educational tools designed to increase knowledge, trust and confidence in emergency decontamination provision.

The ORCHIDS project partners include the UK Health Protection Agency; Faculty of Military Health Sciences, University of Defence, Czech Republic; Swedish Defence Research Agency and the Army Biomedical Research Institute, France. The project is supported by the European Commission via the Health Threats Strand of the Community Action in the Field of Public Health Work Plan for 2007, with additional support from the UK Department of Health and Home Office. For more information visit: <http://www.orchidsproject.eu>

INFLUENCE OF SUBSTRATE AND SURFACTANT LIPOPHILICITY ON
HYDROLYTIC REACTIONS OF P-NITROPHENYLALKANOATE IN
ALKYLPYRIDINIUM BROMIDE ENVIRONMENT

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Micellar catalysis is process significantly influencing hydrolytic degradation of nerve agents. Efficacy of this process depends mostly on ability of surfactant to create micelles, on affinity of substrate to the core of micelle and on the oriented localisation of substrate in surface layers of micelles. Set of alkyipyridinium bromides (from C10 to C18) and p-nitrophenylalkanoates were tested for hydrolytic efficacy to evaluate these effects. Hydrolytic reactions were monitored photometrically by pH10 and temperature 50°C.

Influence of micellar environment on changes in rate of substrate hydrolysis was evaluated as ratio of velocity of reaction in micellar and nonmicellar environment. Obtained results show that efficient hydrolysis proceeds only with alkyipyridinium bromides with alkyls C14, C16 and C18. If substrate lipophilicity is considered, the best hydrolysed esters are derived from caproic and caprylic acid.

EDA PROJECT DEVELOPMENT OF A CBRN COUNTER MEASURES CONCEPT FOR ESDP OPERATIONS

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The EDA Capability Development Plan (CDP) identifies that the proliferation of Chemical, Biological, Radiological and Nuclear Explosives (CBRNE) weapons and their associated technologies persist despite recent treaties, conventions and protocols attempting to limit or eliminate them. The attempts by certain States to acquire and/or to field ever more capable CBRNE weapons remain an imminent threat in the global security environment. In order to address this issue CBRNE Defence is included as an EDA priority action.

The project requirement was for a scenario driven study to provide supporting operational analysis for a threat based capability orientated CBRN Defence functional concept for ESDP operations. The study has analysed current and future threats up to 2030 taking into account the findings of the CDP and identify appropriate counter measures based on the 5 stages of countering a CBRN Incident; deter, prevent, protect, respond and recover and identify the capability requirements for individual soldier protection, non-specialist unit protection and protection provided by specialist CBRN defence: Detection, Identification, Monitoring (DIM); Physical Protection; CBRN Advice and Assessment (including CBRN Intelligence, CBRN Information Management, CBRN Warning and Reporting and CBRN Reach Back); Hazard Management (including Decontamination); CBRN Consequence Management; CBRN hardening and CBRN Medical Counter Measures.

This abstract is submitted pending permission from EDA to present on this study.

MASS CASUALTY DECONTAMINATION OF VULNERABLE AND MINORITY GROUPS

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In the event of a terrorist attack involving the release of biological or chemical agents, it is probable that those affected will include members of vulnerable and minority groups. These groups include children, the elderly, people with physical, sensory or cognitive impairments, non-native language speakers, the homeless, tourists and members of travelling communities. Vulnerable persons have different needs in emergencies and it is unclear whether existing response plans are capable of meeting these needs.

To explore current evidence and best practice around the management of vulnerable and minority groups in mass casualty incidents involving emergency decontamination, a systematic review of the research literature, existing emergency plans, preparedness exercises and incident reports was conducted. Specific aims of this review were to explore issues associated with the definition of vulnerability in the context of emergency planning, the characteristics of particular groups which are likely to make them vulnerable and the extent to which existing preparedness plans account for the needs of these groups. Where they existed, recommendations for meeting the needs of vulnerable groups in mass casualty incidents were identified and evaluated.

There is widespread agreement that the needs of vulnerable and minority groups must be incorporated into emergency plans for mass casualty incidents, particularly those involving emergency decontamination. Progress has been made toward identifying likely vulnerable populations, the aspects of their vulnerability that may present a challenge to emergency responders and potential recommendations for countering these difficulties. However, gaps exist in the implementation of these recommendations, and current emergency planning appears to fall short in preparing responders to deal with mass casualty incidents that involve significant numbers of vulnerable persons.

This review was conducted as part of the EU co-funded ORCHIDS project (www.orchidsproject.eu), which seeks to strengthen the preparedness of European countries to respond to incidents which require mass casualty decontamination.

**THE HEALTH PROTECTION AGENCY ACUTE INCIDENT RESPONSE
HANDBOOK VOLUME 1: CHEMICALS**

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The HPA Incident Response Handbook was developed to assist emergency response organisations in the management of incidents involving the accidental or deliberate release of a toxic chemical. It is intended as quick look-up-guide for first responders and can also be used as a useful tool in emergency planning and training exercises.

The handbook is divided into three main sections. Section I comprises Emergency Guidance Monographs; a collection of information sheets for a range of hazardous chemicals. Each monograph provides information on various aspects of incident management including patient management, decontamination and evacuation distances. To provide further guidance and ease of use the information presented in the monographs is categorised using a traffic light system.

Section II contains Indicative Toxicity Graphs. These provide a visual indication of the acute inhalation toxicity of various hazardous chemicals for exposure durations of up to eight hours. The data for the graphs were collated from a number of occupational and emergency reference sources including Acute Exposure Guideline Levels (AEGs), Workplace Exposure Limits (WELs) and Emergency Response Planning Guideline values (ERPGs).

Section III is the Precursor Guide; a table that lists precursors and solvents that may be used in the preparation of hazardous substances. This list provides the class of chemical that may potentially be synthesised from a given precursor, not the specific product and so does not disclose sensitive information.

Representative extracts of information available in the handbook will be presented at the poster exhibition.

ARGOS DISPERSION MODELLING

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This poster shows some results from a case study using ARGOS (Accident Reporting and Guiding Operational System) in risk management analysis on storage of hazardous chemicals. The results from the case study show how much the actual weather conditions influence on the simulated dispersion of hazardous chemical in the atmosphere.

Denmark has a long tradition for using computer technology in its nuclear emergency preparedness. The first version of the ARGOS decision support system was launched by Risø National Laboratory in the early eighties. During the recent years the ARGOS system has been developed in a close cooperation between DEMA, Risø, PDC and the Danish Meteorological Institute (DMI). Today ARGOS is an easy-to-use PC / Lap-top/ Workstation based system using the Microsoft Windows 2000/XP operating systems.

In 2005 a chemical capacity was added to ARGOS and since then DEMA has used and constantly developed ARGOS as a decision support tool for chemical emergency preparedness. Later ARGOS has been developed to a decision support system with CBRN capacity.

Additional the poster presents a new web based "Forecast service", and introduces how ARGOS is to be used in "Distributed information acquisition and decision-making for environmental management" (Diadem) which is a research project funded by the European Commission under the Seventh Framework Programme (FP7).

**THE SKIN PENETRATION OF ORGANOPHOSPHOROUS COMPOUNDS IS
HIGHLY DEPENDENT ON LIPOPHILIC PROPERTIES AND AGENT
CONCENTRATION**

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The penetration of neat and diluted organophosphorous compounds through human epidermis was studied at different penetration conditions in order to examine factors affecting the skin penetration properties. Evaluated agents were: O-ethyl S-[2-(diisopropylamino)ethyl] methylphosphonothioate (VX), triethyl phosphonoacetate (LT12), tripropyl phosphate (TPP), triisopropyl phosphate (TIPP) and triethyl phosphate (TEP).

All tests were performed *in vitro* using human epidermal disks mounted in a diffusion cell, with a flow-through receptor compartment. The agents were applied neat or diluted in water at different concentrations on the external side of the membrane. The agent flux was monitored in 10 minutes batches during 320 minutes exposure, by analysis of the receptor fluid with GC-FID or LC-MS.

The penetration rate was dependent on both the agent concentration and on the lipophilic properties of the compounds. A common property of all tested agents was that the penetration rates were highest in the concentration interval between 10 and 75 %, whereas relative low penetration rate was observed both at high (>75%) and at low concentrations (<10%). The three lipophilic compounds (VX, TPP and TIPP) had almost symmetrical bell-shaped curves when the cumulative penetration was plotted against the agent concentration. The hydrophilic compounds (TEP and LT12), however, had their maximal penetration rates when applied as 10 or 20 % solutions, showing more than 20 times increase in penetration rate in this concentration interval.

These results clearly demonstrate that both lipophilic properties of organophosphorous compounds and agent concentration when deposited on the skin should be regarded in toxicity assessment. The findings should also be considered when procedures for skin decontamination are evaluated. Thus, if the penetration rate is more than tenfold increased due to dilution of a neat agent, for example by extensive sweating, the time for efficient decontamination of the exposed persons is significantly shortened.

**OXIME-ASSISTED REACTIVATION OF PHOSPHORYLATED
ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE**

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Phosphorylation of serine in the active site of acetylcholinesterase (AChE) and of butyrylcholinesterase (BChE) inactivates both enzymes. AChE is essential enzyme in neurotransmission, while BChE is an endogenous stoichiometric bioscavenger of organophosphorus compounds (OP), including insecticides and nerve agents. Oximes are reactivators of both phosphorylated cholinesterases, but none of the newly synthesised or currently used oximes like 2-PAM, HI-6, TMB-4, and obidoxime, have shown satisfactory reactivation potency in tabun poisoning. This is especially true for reactivation of tabun-inhibited BChE. Reactivation efficiency is primarily attributed to the nucleophilic displacement rate of organophosphate, but varies with the structure of the oxime. In this study we tested reactivation of tabun-inhibited AChE and BChE with bispyridinium oximes varying in the length and type of the linker between rings, and in the position of the oxime group on the ring.

Tabun-inhibited AChE was completely reactivated by K203 [(*E*)-1-(4-carbamoylpyridinium)-4-(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide] with the overall reactivation rate constants of $1806 \text{ L mol}^{-1} \text{ min}^{-1}$, which singles out this oxime as a very potent reactivator of tabun-inhibited AChE. Among the tested oximes, the most potent reactivators of tabun inhibited BChE were K117 [1,1'-(2,2'-oxybis(ethane-2,1-diyl))bis(4-hydroxyiminomethylpyridinium) bromide] and K127 [4-carbamoyl-1-(2-(2-(4-(hydroxyiminomethyl)pyridinium-1-yl)ethoxy)ethyl)pyridinium bromide]. The maximum reactivation of BChE of 70 % was obtained in 50 min, which is the shortest time so far according to literature and our previous data. This is no surprise, since all selected oximes were designed as reactivators of phosphorylated AChE. Our findings may provide a platform for further modifications and development of more potent antidotes in OP poisoning.

Keywords: Antidote, Bioscavenger; Cholinesterase; Nerve agents; Reactivation; Tabun;

CZECH DEVELOPMENT OF NOVEL ACETYLCHOLINESTERASE REACTIVATORS

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Efficacy of currently clinically available acetylcholinesterase (AChE) reactivators (pralidoxime, obidoxime, trimedoxime, MMB-4 and HI-6) is limited. None of them is able to serve as universal antidote in case of nerve agent poisoning. Due to this, there are still efforts to develop novel universal oxime with broader reactivation efficacy.

During last seven years, we have synthesized and tested more than five hundred of structurally different AChE reactivators. Unfortunately, none of them appears as broad-spectrum antidote. On the contrary, two of them are currently considered as the best antidotes against specific toxins – oxime K027 (against pesticides) and oxime K203 (against tabun).

In this contribution, we would like to present our recent results in the development of novel oximes, especially oximes K027, K203 and DMS salt of the oxime HI-6 (including our novel autoinjector).

UK RECOVERY HANDBOOK FOR CHEMICAL INCIDENTS

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Major chemical incidents have occurred in developed and developing countries, occurring both accidentally and deliberately. Although rare, they have caused many casualties, fatalities and mass disruption resulting in long-lasting effects on communities involved.

The scientific knowledge surrounding the response to the acute phase of such emergencies is extensive. However, the same cannot be said for the recovery phase. This knowledge gap has been of concern and issues surrounding 'how clean is clean' have been identified as complex and difficult to address. The Health Protection Agency was tasked by the Department for Environment Food and Rural Affairs, Food Standards Agency, Environment Agency, Northern Ireland Environment Agency, Home Office and Scottish Government, to produce a tool to aid those involved in the recovery phase.

The project team is following a life-cycle approach of a select list of chemicals which in the past have been of concern. By studying these chemicals and specific incidents in which they have been involved, considerations about a range of risks in different environmental scenarios can be assessed and their management discussed with various stakeholders. This approach, already substantiated by the UK Recovery Handbook for Radiation Incidents, which was well received on both a national and European platform, will be used to develop a structured decision aiding process. This will provide 'Recovery Working Groups' with information on what is achievable, based on science, taking into account what is acceptable and practicable.

Development of the UK Recovery Handbook for Chemical Incidents will take three years, the results of which will be released in May 2012. On completion, the product is intended to provide a user-friendly online reference handbook, which will aid relevant stakeholders involved in the recovery phase of a chemical incident.

AN INTEGRATED APPROACH TO DEAL WITH THE CBRNE THREAT WITHIN THE EU

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Within the 7th Framework Programme Security the EU is heading for a demonstration project on CBRNE counterterrorism. The scope of this project will be the demonstration of a consistent portfolio of counter measures for CBRNE along the chain from prevention to response and recovery. The call for this demonstration project (phase 2) will be issued during Summer 2011. As a preparation the EU tasked two consortia to develop a roadmap for this demonstration project (phase 1).

The first project is led by TNO, the Netherlands, and is called DECOTESSC1. The basic idea is an analysis and prioritization of the gaps between the current situation and the ideal situation of CBRNE system-of-systems counterterrorism. The strategic roadmap is aimed at filling the identified gaps and will address the full concept of an EU counterterrorism system-of-systems against CBRNE and outlines all the necessary missions, tasks, capabilities, systems, technologies, etc. to be considered. As a result the focus will be on the enhancement of the integrated operational competences.

The second project is led by CBRNE Centre, Sweden, and is called CBRNEmap. The CBRNE Demonstrator roadmap for development of technologies and systems will take into consideration all present and past activities and search for generic solutions of technologies relevant to the full European setting at both the societal and individual level. CBRNEmap will prioritise demonstration tasks based on systematic analysis of end-user requirement and comprehensive reviews of available CBRNE Science & Technology investments. The final roadmap will be developed for an optimised demonstration programme based on a Concept Development & Experimentation approach.

This presentation will give insight into the objectives of the intended CBRNE demonstration programme of the EU, it addresses the development of the two roadmaps, the differences between the approaches, but also on the collaboration between the projects.

**SEQUENTIAL PROCEDURE FOR THE ANALYSES OF PRINCIPAL
BIOMARKERS OF SULPHUR MUSTARD EXPOSURE**

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Proficiency tests to designate laboratories for detection and identification of chemicals related to chemical weapons in environment and industrial media keep going since 1996 regularly. Testing schemes and protocols for the analysis of such type of samples thoroughly developed and documented. Reliable establishment of humane exposure to warfare agents should be based on chemical analytical procedures for identification of biomarkers of the acting agent and determination of exposure level. The analytical strategy in meeting the objective of identification and quantitative assessment of biological markers of exposure to chemical warfare agents is not similar to the recommended procedures developed by OPCW for proficiency tests. Our institute has accumulated abundant experience in this area. We developed a number of procedures for identification of biomarkers of CW agents in biomedical samples. Some procedures were employed in International Confidence Building Exercise on Biomedical Sample Analysis recently. In this connection the sequential procedure for the analysis of principal biomarkers of sulphur mustard exposure was developed and applied to spiked urinary samples. For thiodiglycol and thiodiglycol sulphoxide as major urinary metabolites derived from the hydrolysis of sulphur mustard GCMS after reduction and derivatization can be considered as a method of choice. Two β -lyase metabolites: 1,1 - sulphonylbis[2-methylsulphinyl]-ethane and 1 - methylsulphinyl-2-[2-methylthio]ethylsulphonyl-ethane were identified by LC-ESMS individually with no sample preparation. The known procedure involving reduction of the two β -lyase metabolites into a single analyte 1,1'-Sulphonylbis[2-(methylthio)ethane] and its GCMS analysis is much more time and labor consuming.

BIOLOGICAL DEFENCE SYSTEM IN THE ARMED FORCES OF THE CZECH REPUBLIC

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The Central Military Health Institute (CMHI) is the main subject of the Armed forces of the Czech Republic which is responsible for bio-defence. This institute was founded in 1957. The importance of this institute raised especially after September 2001 when new goals related to the bio-defence issues appeared. The Central Military Health Institute consists of the headquarters located in Prague and the branches situated in strategically chosen places of the Czech Republic.

The Biological defence system is based on Doctrine of the Czech Army called "Biological Warfare Agents Defence". This document generally provides preventive and repressive epidemiological countermeasures, activities connected with vaccination and prophylaxis, detection, identification of biological agents and transportation of infected or contaminated persons and their isolation and treatment.

The basic components of the Czech Army Biological Defence System are the Medical Intelligence Service, the Biological Event Response Teams, the equipment for detection and identification of biological agents, the stationary and mobile microbiological laboratories and the Centre of Biological Defence. Medical Intelligence Service belongs to preventive countermeasures while the rest of components of the bio-defence system are intended for elimination of damages and casualties after attack, so these components belong to repressive countermeasures. Biological Event Response Teams are the first component of the bio-defence system which realizes immediate response to bio events. These teams are prepared 24 hours every day. Their main function consists in the presence in the place of biological agents attack. The assignment includes the reconnaissance of the place of attack and situation assessment, taking samples and their rapid identification.

There are two different types of the team car, the city version Bio-Master and the field version Bio-Rover. The stationary military microbiological laboratories give us the capability for identification and verification of the biological agents by different types of method. We can use Mobile biological laboratory (PHEL-2) or Mobile field microbiological laboratory (MPML) for identification of biological agents. Mobile hospital unit (MHJ) is determined for field hospitalization of infected people. The Centre of Biological Defence is the final component of the bio-defence system. Centre is intended for an isolation and treatment of seriously infected people.

The members of the CMHI took a part in Kuwait during the Operation "Enduring Freedom" (2002-2003) and "Summit South American and Caribbean states with European Union" in Lima, Peru (2008). Nowadays they are component of Chemical unit within ISAF KAIA mission in Afghanistan (from 2008). CMHI regularly participate in the structure of CBRN Defence Battalions NRF, they also have worked in CBRN Defence Battalion NRF 3 in Athens, Greece (2004).

MASS CASUALTIES AND HEALTH CARE FOLLOWING THE RELEASE OF TOXIC CHEMICALS AND RADIOACTIVE MATERIAL

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Exposures to toxic chemicals or to radioactive materials following mass emergencies may develop at a rate and reach a magnitude sufficient to create a major crisis. The MASH project addresses the problem of mass casualties and adheres to the idea expressed by the Commission that generic preparedness planning and interoperability are key elements in mitigating the impact of mass emergencies

MASH has been using scenario-derived discussions, surveys, interviews, reviews, systems analysis, forecasting and critical seminars. Specifically MASH has sought to define the level of knowledge, preparedness and treatment in the Member States. Mash has also in a foresight suggested improvements to the primary medical care process. Furthermore, Mash will totally organize four seminars where a reference group representing EU 27 will analyze, discuss and participate in formulating recommendations on the best generic preparedness. The final report and its recommendation will, accordingly, reflect the situation of a majority of the Member States.

Mash will introduce technical tools and suggest organizational measures that increase the competence and capability of the health care system. Such measures are well in line with and should be of strategic value to the Commission's ambitions in making our society more resilient and therefore more secure.

The Mash project will be presented at a work shop during the present CBW Conference, Thursday afternoon the 10th MASH. A short presentation of each work package will be given and challenged by some relevant invited speakers. The topics are:

- MASH project introduction
- CR(E) Scenarios
- Best practice of today - C mass casualties
- Best practice of today - R mass casualties
- Contribution of modern bio-technology
- Contribution of modern Information and Communication Technology
- Foresight into needs, possibilities and knowledge gaps in the future

DANISH HAZMAT TEAMS

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Danish Emergency Management Agency (DEMA) has developed a new national hazmat response concept to bridge the gap between the existing specialised technical remediating response provided by the DEMA regional response centres, and the scientifically based consulting and chemical analyses provided by the DEMA Chemical Division.

The new hazmat teams each consist of five team members. The team leader with a rank of captain is in command of two specially trained hazmat technicians from the regional centres, a chemical scientist from the central laboratory under DEMA Chemical Division, and a communication specialist.

The tasks of the hazmat team are on-site sampling and identification of unknown substances. Previously no specialised sampling capabilities have been present, and chemical analyses were performed only in the lab. By joining the technical and scientific staff in small units complete with sampling and analytical capabilities the over all time consumption from the time of the hazmat incident until all compounds have been preliminarily identified and sampled for evidence, is minimised.

The team is equipped with all levels of personal protection equipment including gastight level A. Combined SCBA-PAPR is used for breathing protection. The analytical techniques range from Raman/IR spectroscopy and specific sensors to GC/MS. For larger scale chemical releases wide range IR gas imaging is utilised to follow the cloud.

All personnel and equipment are transported in a Sprinter-style vehicle complete with office facilities and full communication including streaming video link from the team in hot zone out to the chemical scientist in the vehicle.

In addition to the tasks related to the work of the emergency services the hazmat teams also assist the Police in case of terrorist attacks involving hazardous materials.

POLICE PROTECTION – GOOD USER REQUIREMENTS LEAD TO GREAT KIT

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The UK Police Service recently updated their personal protective equipment to an ensemble which maintained existing protection levels but could be donned more quickly in response to a CBRN event. The evaluation of this equipment concluded in 2009, orders were placed and the new PPE has now been issued to police officers ready for deployment if required.

The user requirement for this project drew on five years of consultation with users from different specialist groups and from police forces around the UK. These views, some complementary but others contradictory, were combined and used to compile a coherent operational requirement from which a technical specification was drawn up and published to the market.

Industry responded extremely well and was very supportive of the project, and four separate manufacturers submitted a total of ten separate ensembles for evaluation.

These ensembles were submitted to a commercial assessment and then to a user and technical evaluation through an extensive programme. This included a large-scale user trial, physiological testing and assessment of a safe undressing procedure. Protection testing involved a variety of challenges including live agents and simulants and the methodologies used included swatch testing, respirator testing and full system testing using both a mannequin in live agent and a Man in Simulant Test.

The Home Office Scientific Development Branch worked closely with operational police officers and with technical testing providers to produce a well defined user requirement, a rigorous evaluation process and an effective testing programme.

This has meant that the operational requirement led directly to the new CBRN protective ensemble that is fit for purpose and has been issued to UK police CBRN responders.

DIFFICULTIES IN THE TREATMENT OF POISONING OF TOXIC CARBAMATES AS THREAT AGENTS

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Carbamates belong to a group of compounds having a broad spectrum of toxicity - from relatively non toxic to highly toxic compounds comparable with nerve agents. The current treatment of poisoning by organophosphates consists of the combination of cholinolytics like atropine and some oximes. The efficiency of oximes is not, however, satisfactory in the case of carbamates poisoning because oximes are not able to reactivate carbamylated acetylcholinesterase (AChE). It is considered that for treatment of carbamate poisoning administration of different cholinolytics only is effective. In this paper a number of oximes (toxogonine, pralidoxime, dipiroxime, HI-6, HS-3, HS-6, HGG-12), muscarinic (atropine, scopolamine, amysile) and nicotinic (arpenale, pentaphene) cholinolytics have been tested to protect mice against the poisoning by carbamates. The effectivity of few anticonvulsants (diazepam, phenazepam, clonazepam) was investigated also. The following carbamates were used: insecticides (carbaryl, aldicarb), drugs (physostigmine, aminostigmine, neostigmine, pyridostigmine) and a few pyridile and quinoline carbamates with almost irreversible action on AChE. Oximes alone or with mixture of cholinolytics and anticonvulsants were administered 15 min prior or 1 min after the intoxication. In the experiments *in vitro* the possibility of oximes to reactivate the carbamylated purified human erythrocyte AChE was studied. By use of different groups of oximes quite different effects were observed. All tested oximes reduced the toxicity of aminostigmine, aldicarb and neostigmine whereas no effect could be determined on the toxicity of physostigmine and pyridostigmine. The toxicity of carbaryl and irreversible carbamates was significantly increased by toxogonine, dipiroxime and pralidoxime. Oxime therapy reduced the protective effect of cholinolytics against poisoning of these types of carbamates. The high efficiency of the mixture of atropine, arpenale and phenazepam for treatment and prophylaxis of carbamate poisoning was shown. As demonstrated by experiments *in vitro* oximes did not reactivate carbamylated AChE. Thus, these data indicate that the efficiency of oximes against carbamate poisoning is, at best, very limited and unsatisfactory. Therefore, there has been an active search for a broad spectrum antidotes against poisoning by carbamates. Potential threat of terrorist usage of carbamates is connected with the high toxicity of compounds and the absence of universal antidotes.

FRENCH RESPONSE TO CBRN CHALLENGES : ‘INTEGRATED CBRN DEFENSE SYSTEM’

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Face to a high intensity and well-known threat (cold war), CBRN defence has been built until the nineties on a technological logic (research of individual top level requirements - isolated definition of the equipments). With few interfaces, the restricted number of equipments required to meet the operational capability justified this logic.

Considering the broadened scope of threats and of military operations involving CBRN defence, pursue of this logic would lead to a dramatic increase of technical complexity and costs associated to CBRN defence (equipments and interfaces, management - operational, support - ...). Thus, since 2003, the French MoD has been engaged in a deep evolution of its CBRN defence capability development and acquisition policy, moving from a technological to a systemic logic. It aims to the shift of the current CBRN defence equipments to an “integrated CBRN defence system”, progressively entering in service from 2015 to 2020. This global system will fill current capabilities lacks while optimizing efficiency/cost ratio and facilitating continuous adaptation to evolving threats.

Preparatory actions needed to develop the aforementioned systemic logic are currently organised under three major axis :

- definition of the system architecture :
 - o development of a performance assessment methodology, allowing to compare various system architectures ;
 - o study on the balance and the interfaces between functions (detection vs. medical countermeasures, individual vs. collective protection, ...) ;
 - o detailed definition of equipments, interfaces and communication system ;
 Noteworthy, this work relies on capabilities, engineering and risk assessment softwares to evaluate and optimize global system architecture.
- mastering technologies : achieving the full capability of the CBRN defence system need prior development and/or maturation of several technologies up to adequate performance levels, under sustainability (both from a technical (robustness) and cost (through-life possession cost) constraints.
Major R&T programs primarily focused on CBRN medical counter-measures, detection, protection and decontamination are currently in progress. Leading to the delivery of technological demonstrators from 2013, their evaluation will permit to precisely define the system architecture.
- risk assessment

The paper presents the current progress, especially the first vision of the integrated system architecture, the development of capabilities and engineering softwares and the on-going R&T programs.

THE CHEMICAL WEAPONS CONVENTION COALITION

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At a meeting on 2-3 December 2009 in The Hague, The Netherlands, parallel to the 14th Session of the Conference of the States Parties to the Chemical Weapons Convention, a group of 30 non-governmental organisations (NGOs) from Africa, Asia, Western/Eastern Europe, North America and the Middle East decided to establish the Chemical Weapons Convention Coalition (CWCC).

The CWCC is an independent, international body whose mission is to support the aims of the Chemical Weapons Convention (CWC) and to supplement the efforts of the member states of the Organization for the Prohibition of Chemical Weapons (OPCW) with focused civil society action aimed at achieving full membership of the CWC, the safe and timely elimination of all chemical weapons, preventing the misuse of chemicals for hostile purposes, and promoting their peaceful use.

Specifically the CWCC will:

1. Involve and engage a greater number of NGOs, academia, experts, chemical industries, chemical associations, professional organizations, and other interested stakeholders, especially from developing countries and non-States Parties to the CWC, and representing all regions of the world. This will allow NGOs to play a greater role and exert greater impact in advancing chemical weapons-related issues locally, nationally, regionally, and internationally with a view to facilitating CWC implementation and accession by the remaining non-States Parties.
2. Develop a common strategy and mobilize efforts to achieve full membership of the Chemical Weapons Convention, and monitor and promote full compliance with the CWC including the safe and timely elimination of chemical weapons, comprehensive national implementation, and peaceful uses of chemicals.
3. Promote the effectiveness of the OPCW's inspection and verification regime and identify weaknesses and challenges related to, for example, the destruction of declared chemical weapons stockpiles, the destruction or conversion of former production facilities, and for improved transparency of other chemical production facilities (OCPFs).
4. Undertake education, outreach and capacity-building activities at the local, national, regional, and international levels in accordance with the mission statement.
5. Promote synergies among NGOs in advocacy for weapons of mass destruction (WMD) demilitarization, disarmament, non-proliferation, and peaceful uses, through cooperation and interaction among stakeholders in the nuclear, chemical and biological regimes.
6. Advocate for improved transparency in the implementation of the CWC, including civil society access to OPCW documentation and activities (e.g., conferences and events).

THE CHEMICAL WEAPONS CONVENTION AND DESTRUCTION OF CHEMICAL
WEAPONS

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Abstract About 30 percent of Chemical Weapons has been destructed since the Chemical Weapons Convention enforced in 1997. However such development cannot keep up with the time table of CWC. Regulation of Chemical Weapons destruction in the CWC, development of Chemical Weapons destruction and Chemical Weapons destruction technique are described in this paper.

Key words the Chemical Weapons Convention (CWC), Chemical Weapons, destruction

STUDY ON IMPLEMENTATION AND CHALLENGES OF THE CHEMICAL WEAPONS
CONVENTION

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Abstract The Chemical Weapons Convention has been enforced for more than 10 years. How the CWC had been implemented by all State Parties? What the Organization for the Prohibition of Chemical Weapons (the OPCW) has accomplished in these years of treaty implementation? This paper will review the achievements of the Convention and study the key points of implementation of the Convention, the challenges that lie ahead and the future developments.

Key words the Chemical Weapons Convention (CWC), implementation, challenge

PREPARATION AND DECONTAMINATION CAPABILITY STUDY OF FUNCTIONAL MESOPOROUS SILICA

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Abstract: Ordered mesoporous siliceous materials such SBA-15 have recently received growing attention due to their appealing textural properties, high surface area, appreciable thermal and hydrothermal stability, but the inherent lack of adequate acid and basic sites limits their application in catalysis and adsorption-separation processes. To attain bifunctionalization of SBA-15 with acid and basic sites, we prepared functional mesoporous materials(AM/SBA-15) adding the magnesium and aluminium precursors by In-situ coating, physical grinding and wet impregnation, denoted as SBA-15-1, SBA-15-2 and SBA-15-3. The total weight percent of magnesia and alumina in samples was 32.7% and the Mg/Al mol ratio was kept to 2. The textural characterization of the resulting composites has been preformed by XRD and N₂ thermal adsorption. The addition of magnesium and aluminium salts did not obstruct the two-dimensional hexagonal mesoporous structure of SBA-15. The surface area decreased from 747 m²·g⁻¹ for SBA-15 to about 360 m²·g⁻¹ for AM/SBA-15, and the pore volume also decreased significantly. To assess the bifunctionalization of the resulting samples microcalorimetric adsorption of NH₃ and CO₂ at 150°C was employed. The initial heats and coverages of NH₃ and CO₂ indicated that the addition of magnesium and aluminium significantly enhanced acid and basic sites, and SBA-15-2 had the strongest acid and basic sites among AM/SBA-15. The adsorption and degradation of SBA-15, AM/SBA-15 and A-clay to mustard (HD) was investigated. The results indicated that the ordered mesoporous structure is beneficial to the adsorption to HD, and the elimination ratio all attained 99.9%. The mesoporous materials with higher acid and basic sites (SBA-15-2) possessed better catalytic degradation activity to HD, whose degradation ratio reached 90.1% in a week. And the degradation ratio of A-clay to HD was about 80.1% in a week.

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CERPE : A NEW EQUIPEMENT FOR MASS DECONTAMINATION IN THE FRENCH ARMIES

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CERPE (*CEntre de Reconditionnement des Personnels*) assures the decontamination of healthy personnel, protected or not against the CBRN threat, submitted to a nuclear, radiological, biological or chemical attack. These persons must be undecontaminated and unwounded – wounded contaminated personnel being oriented to a specific decontamination line managed by the Health Service.

The CERPE system allows the decontamination at a pace of 20 persons / h to 30 persons / h depending of their level of initial protection.

CERPE is divided into several zones, by modular principle, from reception to way out : reception, neutralization, undressing, shower treatment, decontamination control, dressing, and outside zones. These zones are built with tents and shelter on an area higher than 60 m². Decontamination is applied simultaneously to a population of the two sexes without possibility of visual interferences between the two groups.

Shower treatment is used with water or water / soap.

Control of cleaning is obtained thanks to detection devices : AP2C / AP4C (flame photometry) for CWA, Miniwarn / PAC III (electrochemical detection) for TIC's, and DOM DOR 309 LLR for radiological material.

A network of computers allows to follow the way of each person through CERPE keeping the personal data.

Environmental protection is taken in account thanks to treatment of effluents and storage tanks. Specific interests of CERPE also include ease of transportation and deployment.

This system results from a global contract awarded to a consortium led by NBC-Sys and UTILIS, which will finally lead to the delivery of 34 systems up to 2012.

Our presentation will give a detailed insight in the architecture and functions of the system, and report on operational experience collected in 2008 and 2009.

DECONTAMINATION EFFICACY OF POLYSTYRENE SUPPORTED N-CHLORO REAGENTS AGAINST CHEMICAL WARFARE AGENTS

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Chemical warfare agents are the chemical substances employed as weapons due to their direct toxic effects on animal, plants and any living organisms. Sulfur mustard (SM) is most potential chemical warfare agents for military point of view due to its multiple toxicological properties. It is highly toxic blistering agents and persistent chemical with highest incapacitating potential. It is a contact and inhalation hazard; therefore its removal or decontamination from environment is necessary. Decontamination includes removal of agents by physical means or rendering them harmless using chemical reactions or by biological methods. Decontamination of chemical warfare agents is important as it removes or reduces the toxic effects of chemical warfare agents. Out of these, chemical methods of decontamination are more effective and reliable as it involves transformation of the chemical structure of CW agents to nontoxic or less toxic products. Methods like hydrolysis and oxidation, for the chemical decontamination of sulfur mustard have been reported in the literature. Among these methods, oxidation using organic N-chloroamines is most preferred method. However separation, isolation of the consumed products and their purification is very difficult in these decontamination methods using N-chloramines. Therefore N-chloro containing polymer supported reagents have been attempted due to their insolubility in water resulting into ease of separation and reusability of reagents for decontamination of sulphur mustard. Polystyrene supported reagents have been considered for this purpose due to presence of aromatic moiety and its functionalization.

In continuation of our work, we have already reported synthesis, characterization and functionalization of polystyrene. Polystyrene supported N-chloro reagents: N-chloro amide/sulfonamide /Cyanamide poly (styrene -DVB) have been synthesized using amino methyl polystyrene containing 2% divinyl benzene as starting material. These polymeric reagents have been characterized by FTIR, Raman spectra, functional group loading and active chlorine contents. Efficacy of these reagents as decontamination agents against sulfur mustard, a chemical warfare agent and its simulants: 2-chloro ethyl phenyl sulfide (CEPS) and 2-chloroethyl ethyl (CEES) have been evaluated. Decontamination reaction was monitored by gas chromatography (GC). Nontoxic products obtained as a result of decontamination were identified by FTIR, GC-Mass and NMR spectra. Among the polystyrene supported N-chloro reagents studied, N-chloro-4 nitro benzamide poly (styrene -DVB) was found to be most effective decontaminating agent for sulfur mustard and its simulants. We are therefore reporting synthesis, characterization and decontaminating efficacy of polystyrene supported N-chloro reagents against sulfur mustard and its simulants in the present communication.

MODIFIED STATIC DIFFUSION CELLS FOR DECONTAMINATION
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A modified static diffusion cell model incorporating a showering component was developed to examine the comparative effects of various parameters such as shower temperature, duration, flow-rate, detergent or physical washing aid use upon percutaneous absorption of toxic skin contaminants *e.g.* sulphur mustard and nerve agents. Control of flow and duration is enabled via use of a peristaltic pump which supplies individual shower fluid lines to each diffusion cell. Control of shower temperature is achieved through heat conduction, by wrapping shower lines around a heated copper pipe a short distance from the shower inlets. This model was tested using four ¹⁴C-labelled compounds: the CW vesicant, sulphur mustard, and its widely used simulant, methyl salicylate, and organophosphate nerve agents, GD (Soman) and VX. The chemicals were applied (10 µl) to dermatomed (~500 µm) porcine skin with the aim of examining the comparative effect of shower duration on each respective chemical's penetration profile (measured by liquid scintillation counting of receptor fluid samples collected at regular intervals over 24 hours). Decontamination showering (at 0.6 ml cm⁻¹ min⁻¹) occurred at 1 hour post exposure reflecting an estimated timeframe taken for deployment of showering equipment by emergency services following an incident.

The data obtained suggest that shower duration can have an influencing role in effective CW decontamination. A short shower does appear to decrease the absorption of chemical contaminants through porcine skin of sulphur mustard, methyl salicylate and GD and, if extrapolated to man, would thus be expected to mitigate the potential risk from percutaneous chemical exposure. Paradoxically, the benefits from showering appear to be reduced if shower duration is extended for some agents perhaps reflective of differing physicochemical properties, a factor worthy of further examination in larger scale (clinical, simulant-based) trials.

This research was conducted as part of the EU co-funded ORCHIDS project (www.orchidsproject.eu).

OPTIMISATION OF MASS CASUALTY DECONTAMINATION PROCEDURES IN
VITRO

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Effective decontamination is an integral response to a large-scale attack involving the release of toxic materials (Okumura *et al.*, 1998). The purpose of this study was to assess percutaneous absorption of the simulant methylsalicylate (MS), and chemical warfare (CW) agents sulphur mustard (HD) soman (GD) and VX through skin subject to shower water containing different detergents.

Full thickness pig skin was excised post mortem from the dorsal flank of female animals (weight range 20-30kg). After dermatoming to a depth of 500µm, skin samples were placed into Franz-type static glass diffusion cells (area 1.76 cm²) maintained at a temperature of 32°C (±1°C). The receptor fluid (bathing the underside of the skin) comprised 50% aqueous ethanol. Treatment groups included controls (untreated) and those treated with either water or detergent solutions. A droplet (10µl) of ¹⁴C radiolabelled CW agent or simulant (nominal activity 0.5µCi µl⁻¹) was applied to the skin surface of each diffusion cell with decontamination being performed (where applicable) after 1 hour by showering the skin samples with warm (35°C) water (± detergent). Samples (250µl) of receptor fluid were taken at regular intervals up to 24-hours post exposure for analysis by liquid scintillation counting to quantify the percutaneous absorption of each contaminant.

The relative efficacy of each detergent varied according to which chemical contaminant was used. Whilst no single detergent could be identified as being consistently the most effective, these data demonstrate that generic effectiveness against a range of chemicals may be attained for decontamination of casualties in the event of a chemical incident.

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SKIN DECONTAMINATION OF VX: IN VITRO EVALUATION OF
ADSORBENT POWDERS.

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In a scenario of military or civilian exposure to chemical warfare agents (CWA), the emergency procedures include the immediate body surface decontamination. The immediate skin decontamination efficacy of VX and sulphur mustard by RSDL[®] or Fuller's Earth (FE) has been demonstrated. However, in a civilian urban context, these might not be quickly available to the first rescuers. Consequently, since the critical element is time and early physical removal of the contaminant is the most important, it is commonly agreed to perform immediate decontamination with any adsorbent systems available on site. However, there is a lack of scientific evidence for the decontamination efficacy of these systems. The objective of our work was to evaluate the skin decontamination efficacy of VX by flour and talc relative to FE. Split-thickness skin samples from pig ears were used as *in vitro* skin models. They were placed in diffusion cells then exposed for 5, 15 or 30 min to VX (5 mg/cm²). At the end of the exposure time, 70 mg of powder was spread on the skin surface then 15 sec later, was removed by using a cotton glove. Six hours post-exposure, the amount of VX present on the skin surface, in the skin (SF) and that penetrated through the skin (PF) was quantified. The decontamination efficacy was determined from the ratio $E (\%) = ((SF+PF)_{controls} - (SF+PF)_{decon}) / (SF+PF)_{controls}$. Our results showed that for a given decontaminant, E significantly decreased when the exposure duration to VX increased from 5 or 15 min to 30 min. FE was the most effective decontaminant. With up to 15 min skin exposure duration to VX, our results indicated that FE could be substituted with flour, but not with talc, without greatly affecting the decontamination efficacy.

**ENZYME STABILIZATION IN NANOSTRUCTURED MATERIALS FOR
USE IN BIOLOGICAL AND CHEMICAL WARFARE AGENTS
DECONTAMINATION**

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Enzymes used for decontamination of biological and chemical warfare agents are required to operate under harsh conditions such as extreme pH and temperature, organic solvents, adverse physiological disposition factors, etc., that often lead to enzyme deactivation. The stability of enzymes generally is a critical issue in biotechnology. Both storage and operational stabilities affect the usefulness of enzyme-based products. An effective way to overcome limitations caused by insufficient stability to some extent is enzyme immobilization, i.e. its physical attachment to a solid support. Moreover, immobilization might improve enzyme properties because substrate specificity might be enhanced and the effect of inhibitors might be reduced. It further provides a convenient means to separate and reuse the biocatalyst to improve process economics. The immobilization or encapsulation of enzymes has attracted continuous attention in the fields of fine chemistry, biomedicine and biosensor and has also been proposed and successfully tested for enzyme-based methods of environmental decontamination and personal detoxification. The effectivity of immobilization, including the performance of immobilized enzymes, strongly depends on the properties of supports, which are usually referred to particularly as material type, composition and structure. Substantial R&D efforts have been conducted to optimize the structure of the carrier materials in order to obtain better catalytic stability and efficiency. In this regard, nanotechnology provides opportunities to formulate desirable features in balancing key factors that determine the efficiency of biocatalysts, including specific surface area, mass transfer resistance, and effective enzyme loading. The incorporation of enzymes in polymer nanostructures provides diverse opportunities for chemically re-engineering enzymes for a wide range of applications. Nanostructured supports are believed to be able to retain the catalytic activity as well as ensure the immobilization efficiency of enzymes to a high extent. Hitherto different nanostructured materials have been used as supports, such as mesoporous silica, nanotubes, nanoparticles, nanofibres and crosslinked enzyme crystals or aggregates. Compared to other supports their surface area-to-volume ratios are very high, which can provide large specific surface areas for enzyme immobilization as well as stabilization. The other essential advantage offered by their chemical structures are the immense possibilities to establish a suitable microenvironment for a chosen enzyme. In terms of controlled porosity and flexibility, tailored balance between hydrophobicity and hydrophilicity, and the high degree of structural availability and functionality, respective nanostructures are particularly attractive for fabricating effective enzyme supports.

SYNTHESIS AND EVALUATION OF NOVEL DETERGENTS AS POTENTIAL
DECONTAMINATION AND DESINFECTION MEANS

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Decontamination of chemical warfare agents together with several biological pathogens are of high interest nowadays. At our institute, we are focused on preparation of novel detergents having in their structure quaternary nitrogen. Such detergents could be used as part of the decontamination means (thanks to their ability to form micelles which can increase velocity of nerve agent decontamination) and disinfection means (thanks to the cell membrane disruption). Recently, we have prepared several series of novel quaternary detergents derived from benzalkonium and pyridinium salts. These compounds are currently tested for their decontamination and disinfection potency.

**Carbon-supported polyoxomolybdates catalytic materials perparation,
chataacterization, and catalytic oxidation of Dipropylsulfide as Mustard(HD)
analogue**

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Abstract

A prototype material for sustained catalytic decontamination of mustard analogue used in self-decontamination suits was investigated. $H_5[PV_2Mo_{10}O_{40}](HPA)$ with Keggin structure was prepared as orange crystalline solids and was characterized with FT-IR, TG-DSC and Potentionmetic titration. HPA can be incorporated in activated carbon / charcoal (HPA/C). Evaluation of the adsorptive capacity of Carbon /charcoal for HPA indicates that Carbon L is highly effective in retaining the immobilized HPA even under catalytic conditions (oxidation of Dipropyl sulfide, DSP, by tetrabutyl hydroperoxide, TBHP). DSP can be effectively catalytically oxidated to sulfone and sulfoxide. The catalytic activity of the HPA/C system can be restored and reusability.

Keywords

polyoxomolybdates, catalytic oxidation, Dipropylsulfide, Mustard

SURFACE DECONTAMINATION OF CHEMICAL AGENTS USING ATMOSPHERIC
PRESSURE PLASMA JET WITH AIR FLOW DISCHARGE

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The plasma jet generated by gas discharge at atmospheric pressure has been the focus of research in the surface decontamination of chemical agents. Avoiding using chemicals and water, the plasma jet decontamination technology is dry and nondestructive to sensitive equipment and materials, and especially it can work under freezing environment. In this study, an atmospheric pressure dielectric barrier discharge (DBD) plasma jet generator using air flow as feedstock gas was developed and applied to decontaminate the chemical agents (sulfur mustard, soman and VX) on the material of aluminum, stainless steel, iron plate painted with alkyd, PVC, etc. The decontamination experimental results showed that the residual chemical agents on the material were lower than the security permissible values. The corrosion tests, including etiolation index, chromatic difference, gloss reduction and microcosmic appearance etc, for the material of plexiglass, neoprene, PVC, PE, phenolic resin, iron plate painted with alkyd, stainless steel, aluminum etc showed that the plasma jet was corrosive tinily for part of the material, but their performances were not affected. The portable calculator, computer display, mainboard, circuit board of radiogram and hygrometer could work normally after being treated by plasma jet.

Keywords: surface decontamination; chemical agents; atmospheric pressure plasma jet; dielectric barrier discharge (DBD); air flow discharge

CORROSION AND PROTECTION OF EQUIPMENT FOR DECONTAMINATION OF CHEMICAL WARFARE AGENT

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Chemical warfare agent (CWA) is the use of the toxic properties of some chemical substances to kill, injure or incapacitate an enemy in warfare and associated military operations. At present, the existing large quantities of chemical weapons need to be destroyed, the chemical industry and chemical agents unexpected incidents of terrorist incidents happened frequently. Decontamination, aimed at eliminating the hazard of CWAs, is required on the battlefield as well as in laboratories, pilot plants, and chemical agent production, storage, and destruction sites. The hypochlorite ion, OCl^- , is widely used for disinfection. The dibasic tricalcium hypochlorite, whose formulation is $3\text{Ca}(\text{OCl})_2 \cdot 2\text{Ca}(\text{OH})_2$, is the very common industrial sources of the hypochlorite ion. They can destroy the chemical agents such as isopropyl methyl phosphonofluoridate (Sarin), bis(2-chloroethyl)sulfide (Sulphur mustard) and other poison very effectively. But it should be noticed that when the effective chemical disinfectant used, the phenomenon of severe corrosion will appear on the surface of the decontamination equipments because of the nature performance of the dibasic tricalcium hypochlorite.

In this paper, the corrosion research methods, which consisted of metallographic microscope, scanning electron microscope and electrochemical methods, were used to character the corrosion resistance of the carbon steel covered with Ni-P alloy using electroless plating method in $\text{Ca}(\text{ClO})_2/\text{Ca}(\text{OH})_2$ alkaline solution. From the results, It was shown that the serious corrosion appeared on the surface of the carbon steel when dipped in the $\text{Ca}(\text{ClO})_2/\text{Ca}(\text{OH})_2$ alkaline solution after 1 h. Some parts were peeled off from the substrate when dipping in the alkaline solution after 24 hour. When coated with Ni-P alloy, the corrosion current of the sample decreased in the $\text{Ca}(\text{ClO})_2/\text{Ca}(\text{OH})_2$ solution. The corrosion resistance of the sample increased with the increasing of thickness of Ni-P coating. The carbon steel coated with Ni-P alloy has a well corrosion resistance performance in $\text{Ca}(\text{ClO})_2/\text{Ca}(\text{OH})_2$ alkaline solution.

CZECH / CZECHOSLOVAK UNIVERSAL SOLUTIONS FOR DECONTAMINATING MILITARY EQUIPMENT

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Results of the Czech / Czechoslovak R&D (proceeding for over four decades), production and experience with use of means for decontamination (detoxification, deradiation and disinfection) of equipment are presented, starting in order to substitute new means for *the first generation of decontamination system* produced according to the Soviet design since the early 1950s, based on two solutions: **OR-1** (designated for vesicants and like: dichloroamine in dichloroethane), and **OR-2** (designated for nerve G-agents: sodium hydroxide and monoethanolamine in water). Main aim of the R&D commenced in the late 1950s was to develop universal, efficient, less toxic and cost-effective solution.

The second generation (introduced in the early 1960s) for all three tactical levels, i.e. (1) in the sets for decontaminating personal arms and utensils (UOS-1 – in use after 2 upgradings till nowadays), (2) in the sets for decontaminating cars, combat vehicles and medium arms by the crews (AOS-1, AOS-2) as well as (3) for decontamination of heavy equipment items by special chemical units in the mass decontamination systems (ARS-12M, Linka 82, ST-T-815, ACHR-90) is represented by the **Universal Decontamination Solution UOR** (based on calcium hypochlorite with high content of active chlorine with addition of special detergent Alfa and small amount of diesel fuel). The same solution (without addition of diesel fuel) is used in the sets UOS-1M (it was used also in the sets AOS-1, AOS-2) with additive for spontaneous warming. Necessary warming (in order to achieve high efficiency in case of decontaminating mustard under ambient temperatures below + 10° C) is assured also in the above mentioned mass decontamination systems by other means.

The novel generation (introduced since the 1980s) is mainly represented by the new type of **Universal Decontamination Solution OR-3** (containing sodium hydroxide, ethyl alcohol, monoethanolamine and cyclohexylamine) designated for the medium tactical level decontamination sets UOS-3 (standard accessory of all cars, trucks and combat vehicles). Its efficiency is higher as compared with the widely used US decontamination solution DS-2. This generation encompasses also decontaminants for specific use at wet processes, i.e. **OR-4** (detergent Linka-1 with addition of sodium hydroxide in water), **OR-5** (detergent Linka-2 in water), **Deactivation mixture** (detergent Alfa in water), and **Laundry mixture** (detergent ODD-1 in water) designated for the field laundry PMP-79.

Parallel development of means based on “dry technique” i.e. utilisation of exhaust gases of jet engines applied in the universal decontamination systems TZ-74 (Czech-made jet engine M-701), and ST-T-815 (two Czech-made light jet engines SAPHIR) is also presented.

All mentioned solutions and technical means as well as experience with testing and use (mainly with their efficiency based on comparing used standard values for initial and residual contamination with GB, GD, HD, VX, and like) are described in detail, current efforts in further development are discussed.

INDIVIDUAL PROTECTION EQUIPMENT FOR SOLDIERS EMPLOYED IN
PEACEKEEPING OPERATIONS AGAINST EXPOSURE TO AIRBORNE TOXIC
SUBSTANCES

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Conflict areas where military units are deployed for peacekeeping operations are often characterized by a poor air quality, with presence of pollutants from industrial and other anthropogenic sources, which can reach dangerous levels, due a living environment degraded by the conflict, poor environmental legislation and lack of law enforcement. The Individual Protective Equipment designed for protection against CBRN agents is often unsuitable to protect personnel against toxic chemicals different than chemical warfare agents: therefore it is necessary to decide when and how to use a more specific equipment against toxic industrial chemicals.

This work suggests a strategy to address the problem of individual protection of soldiers against exposure to toxic substances with the aim to of proposing reasonable guidelines to commanders. According to the level of the conflict, from low intensity conflicts up to war situations, it is possible to decide on the level and the type of individual protection to adopt, in order to ensure protection of soldiers against possible health risks from exposure to airborne toxic substances, while accomplishing the military mission.

THE ACUTE TOXICITY OF NERVE AGENTS AND EFFICACY OF SELECTED DECONTAMINATION MEANS – *IN VIVO* STUDY

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The acute toxicity (i.m., i.v., i.p., p.o., s.c.) of selected nerve agents (VX, soman, tabun, sarin, RVX) and model pesticide (paraoxon, DFP) were assessed in mice, rats and guinea pigs. The results were evaluated 2 and 24 h after application of the toxic agent onto shaved dorsal skin of the animal. Abnormal behaviour as convulsions, lacrimation, mastication and salivation appeared within few minutes after application of the agent. VX as one of the most toxic agents was chosen for evaluation of decontamination efficacy. Four types of detergents means (Argos[®], Dermogel[®], FloraFree[®] and Neodekont[®]; in 5% water solutions) were used as decontaminants. The process of decontamination was performed 2 min after application of the nerve agent by wiping of the skin with cotton swab wetted in the detergent solution. The animals survived longer after decontamination; detergent Argos[®] was the most effective mean used in our study (ID_{VX} > 58.8).

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IN VITRO PENETRATION OF PARAOXON THROUGH THE PIG SKIN AND ITS
DECONTAMINATION USING DIFFERENT DECONTAMINATION MEANS

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Paraoxon percutaneous penetration study was performed in Franz-type of glass diffusion cells. An amount of toxic agent penetrating the skin was determined spectrophotometrically using Ellman's method.

Afterwards, influence of chosen decontamination means, showering duration and water temperature on the decontamination process was tested. According to the obtained results, the most important factor influencing the decontamination seems to be the time of decontamination mean application. The decontamination activity is increasing with the shorter time between skin contamination and decontamination mean application. Argos seems to be the most effective decontamination mean used in our study.

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SMDS : NEW CONCEPTS FOR AUTOMATIC DECONTAMINATION OF SENSITIVE EQUIPMENTS

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Decontamination processes operated by French armies on the battle fields (ground, air bases, ships ...) are effective. For decontamination of personnel and outfits (vehicles, aircrafts), efficiency is achieved via automatic or semi-automatic processes implemented in systems such as CERPE for mass decontamination, VLRA, SDA, SYMODA for vehicles and aircrafts. However, these systems do not allow decontamination of sensitive equipments (small weapons, respirators, helmets, electronic or optronic hardware, computers...). Therefore, current procedures for decontamination of such equipment are mainly manual.

Having in view the delivery of a partially or totally automated system allowing high-throughput decontamination of sensitive equipment, SDMS (*Système de Décontamination des Matériels Sensibles*) demonstrator was developed in close cooperation with French Army and German armed forces, aiming at the assessment of different technologies oriented to automatic decontamination. Selected technologies were :

- BDM (Batch Decon Module) : decontamination based on "washing" (based on liquid decontaminant) techniques, suitable for equipment such as respirators, helmets... ;
- VDM (Vacuum Decon Module) : decontamination by heat and vacuum, to equipments which are not compatible with liquid-based decontamination ;
- SHUTTLE : injection-aspiration system for decontamination of sensitive environments (e.g inside vehicles) needing that "decontamination system goes to the equipment" rather than "equipment goes to the decontamination system".

Performances of SDMS were measured face to C (HD, VX), RN (¹⁴⁰La) and B (*Bacillus Atrophaeus* spores) agents. Operational capability of SDMS was also assessed by the Army on field. Influence of different parameters (pressure, flow-rate, temperature, time-cycle ...) was evaluated with the aim to point exactly the perimeter of optimized functioning conditions, including safety.

Main results show the importance of positioning of equipments in a process of automatic decontamination, illustrated by the risk of a negative bulky effect (too close to other equipments) which prevents an homogeneous decontamination (and could help contamination by transfer), and by temperature heterogeneity inside BDM or VDM when filled up.

All these results will be used to develop a new system which will be adopted by the French Forces in the next years. Further development leading to an operational system will therefore involve optimisation of the configuration of decontaminant distribution systems (e.g. nozzles in BDM and VDM). Other parameters that require fine optimisation include duration of decontamination process, energy and consumable (e.g. decontaminant) consumption.

PEROXIDE IN NANOPARTICLES IS AN EFFECTIVE METHOD TO DISINFECT AREAS CONTAMINATED WITH B-AGENTS PRESERVING THE FORENSIC EVIDENCE.

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INTRODUCTION

Traditional disinfection techniques using wet sprays and wipes do not eliminate the main cause of contamination by bacteria, spores, fungi or even viruses. The permanent exchange between the surface and air, the so-called recurrent cycle, is an important source of (re)contamination. Especially in bioterror attack with airborne particles. More extensive decontamination with ClO_2 destroys all materials on the spot and all forensic evidence.

STUDY DESIGN

In Dutch hospitals studies were done to disinfect MRSA (multi-resistant *Staphylococcus aureus*) infected areas, like operation theatres, with the IC-4™, an innovative H_2O_2 ultra mist generator.

This unit sprays liquid with an array of 1.7 MHz activated ceramic discs controlled by an ultrasonic generator a container with detergent. As the fluid detergent cannot follow the frequency of the ultrasonic plates, the cavitation phenomenon takes place in the dispersion. A hydrogen peroxide spray with ultra small hovering particles is created.

RESULTS AND DISCUSSION

These nanoparticles show an excellent absorption in the air. No condensate nor droplets are formed, so it does not show not the so-called "umbrella" phenomena. As a consequence the nanoparticles disinfect behind barriers as well. Tests with a variety of bacteria-, spores- and virus-contaminated areas have shown reduction rates above log 5 in those 'hidden' areas. The micro-particles are attractive for micro-organisms, which absorb them resulting in lethal oxidation of the internal cell membranes. The DNA of the microorganisms can still be analysed for forensics.

The nanoparticle scaffold shields the active peroxide towards surfaces, which prevents corrosion of those surfaces.

CONCLUSION

The nanoparticles are not only very effective in the disinfection of MRSA infected hospitals, but also for disinfection after bioterror attack. This system will kill pathogens without damaging the DNA for forensic investigations and will be commercialized soon.

KEY WORDS

Disinfection, hydrogen peroxide, umbrella effect, MRSA, bioterrorism threat, forensic.

**STUDIES ON THE DECONTAMINATION OF CHEMICAL WARFARE
AGENTS HD, GB AND VX BY SODIUM DICHLOROISOCYANURATE IN
WATER**

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Kinetic evaluations were conducted of the efficacy of decontamination of chemical warfare agents: Sulfur mustard (HD), Sarin (GB) and O-ethyl S-(2-diisopropylaminoethyl) methylphosphonothioate (VX) by sodium dichloroisocyanurate in water. GC-MS and GC-FPD approaches were applied to detect and identify the decontamination products of HD, GB and VX by sodium dichloroisocyanurate solution. It was experimentally demonstrated that the efficiency of decontamination would be improved when the pH value of the decontamination solution was adjusted from 5 to 8~9 by sodium carbonate for all the three agents. The initial agents' concentration was 60 mg/L, 30 mg/L and 30 mg/L for HD, GB and VX respectively. The concentration of sodium dichloroisocyanurate was approximately 0.06 mg/L, 0.06 mg/L and 1 g/L for HD, GB and VX to decontaminate them to the safe concentration 1.5 mg/L, 0.07 mg/L and 0.01 mg/L according to the sanitary standard of drinking water for army in wartime. Mustard sulfoxide, di-isopropyl phosphonate (DIMP) and N,N-diisopropylformamide were the main products for HD, GB and VX respectively decontaminated by sodium dichloroisocyanurate solution.

EVALUATION OF THE EFFECTS ON FILTER PERFORMANCE CAUSED BY LONG-TERM STORAGE OF THE RESPIRATOR FILTER FILTER 90

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FOI CBRN Defence and Security has on behalf of the Swedish Defence Material Administration (FMV) evaluated the effects on filter performance caused by long-term storage of the respirator filter model Filter 90. The evaluation was performed to investigate the status of the filters and assess their life-time expectance

Half of the filters have been stored in room temperature and the other half in a non heated storage. The weight increases of the filters were measured after six months, one year and after two years. A more thorough evaluation was performed after 13 years and included the following tests:

The following tests were carried out:

Visual inspection

Weight

Pressure drop

Resistance times:

Sulfur dioxide

Ammonia

Chlorine

Hydrogen cyanide

Cyanogen chloride

Chloropicrin after different treatments

Aerosol penetration

The results from the tests were compared with results from similar tests performed when the filters were purchased. An assessment of the remaining life time of the filters was then made.

Adsorption kinetics of Benzene Vapor by a Two-layer Bed

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Abstract

A sintered microfiber sorbent (MFS) entrapped activated carbon powder using nickel fibers were prepared and a two-layer bed was composed by the MFS and conventional packed bed (PB) of granular activated carbon (GAC). The adsorption behavior of fixed bed to benzene vapor was carried out at various inlet concentrations. The Shilov equation was used to calculate the unused layer depth and adsorption capacity of the bed. Yoon-Nelson equation and the modified equation were used to simulate the breakthrough behavior of GAC bed and two-layer bed respectively. The results showed that, for both GAC bed and two-layer bed, the unused layer depth and adsorption capacity decreased with a decrease in the challenge concentration. The theoretical curves agreed with the experimental data very well for Yoon-Nelson equation to GAC bed and the modified equation to two-layer bed respectively.

Keywords

sintered microfiber sorbent, benzene, breakthrough, two-layer bed

**Revolution of Antichemical Technique in CBRN Collective Protection
-The antichemical abilities move forward from limited to infinite**

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Abstract: Impregnated carbon bed filters have been the crucial equipment of chemical, biological, radiative and nuclear (CBRN) collective protection for long time. The antichemical abilities of the filters are limited, which depend on the quality and the quantity of impregnated carbon. The regenerative adsorption techniques such as pressure swing adsorption (PSA), temperature swing adsorption (TSA), etc., which can provide lasting antichemical abilities in CBRN collective protection, were once recognized as the most promising techniques. However there are major drawbacks with the current PSA and TSA such as violent swing of pressure or temperature, high energy consumption, and complicated auxiliary facilities, which hamper their application. Three-dimensional cycle adsorption technique (TDCA), with all advantages of TSA and PSA and operating under nearly room temperature and pressure, is the best choice of antichemical techniques. TDCA will lead a revolution in CBRN collective protection, and make the chemical defense ability move forward from limited to unlimited.

Keywords: collective protection, antichemical ability, regenerative adsorption, three-dimensional cycle adsorption

EXPERIMENTS ON TREATING CONTAMINATION WASTEWATER WITH
COAGULATION-SETTLEMENT METHOD

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Abstract: A kind of contamination wastewater containing high concentration linear sodium alkylbenzene sulfonate (LAS) was treated with coagulation-settlement method. In the experiments, PAC was used as coagulant, PAM was used as aid coagulant, and CaO was used to adjust the pH value. The influences of PAC concentration, PAM concentration and pH value on the effects of wastewater treatment were studied. The results indicated that the removal rates of COD_{Cr}, LAS and turbidity increased nearly proportional to the PAC concentration and pH values when PAC concentration ranged in 1000 ~ 6000 mg/L and pH values ranged in 7.6 ~ 13. PAM was useful in accelerating the coagulation and settlement, but it has slight effect on the removal of COD_{Cr}, LAS and turbidity. After treated with coagulation-settlement, the removal rates of COD_{Cr}, LAS and turbidity could be higher than 60%, 70% and 90% respectively, indicating that the coagulation-settlement method was suitable for treating the contamination wastewater.

Keywords: contamination wastewater; coagulation-settlement; LAS; PAC; PAM

DECONTAMINATION OF HD AND VX IN PEROXIDE COMBINED WITH BLEACH ACTIVATOR SYSTEM

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Abstract: Decontamination is one of the supporting techniques for the nuclear, biological and chemical defense as well as the countering terrorism. In recent years, the decontaminant formula of peroxide combined with bleach activator has been paid attention to because of their virtues of excellent decontamination performance, non-corrosive and environmental friendly. In this paper, the universal decontamination formula of peroxide combined with bleach activator was optimized for degradation of chemical warfare agents. Based on the sodium peroxycarbonate (SPC), the activity of a series of bleach activators including ester compounds such as propylene glycol diacetate (PGDA), pentaacetylglucose (PAG) and Aspirin etc., acylamide compounds such as tetracetylglucure (TAGU), tetracetylenediamine (TAED) etc. were compared for decontaminating HD and VX. It indicated that TAED was the most excellent one to enhance the reactivity of SPC among the investigated activators. In SPC-TAED based decontamination solution, the optimization pH ranges to destruct HD and VX were respectively 7.5-9.0, 8.5-9.5, and the lowest molar ratios of active oxygen and HD, VX were separately about 2, 10 at the pH value of 8.5. Besides, 1-acetylguanidine (ACG) and acetamide as novel activators had been studied. The obtained results suggested that the SPC-ACG based formula was not superior to SPC-TAED on destroying HD, but it had taken on a better performance for detoxifying VX. The formula and the decontamination condition of the SPC-ACG system were also optimized. Based on the decontamination products of HD and VX in the SPC-TAED and SPC-ACG formula detected and identified by GC/MS, LC/MS and IC spectrum methods, the degradation pathways were deduced. It seemed that the degradation pathways of HD contained oxidation of sulfur and carbon, elimination of HCl, while oxidation of sulfur, nitrogen and α -carbon would be the main degradation pathways of VX.

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TOXICITY OF TNT CONTAMINATED NATURAL WATER ON SALMON ALEVINS

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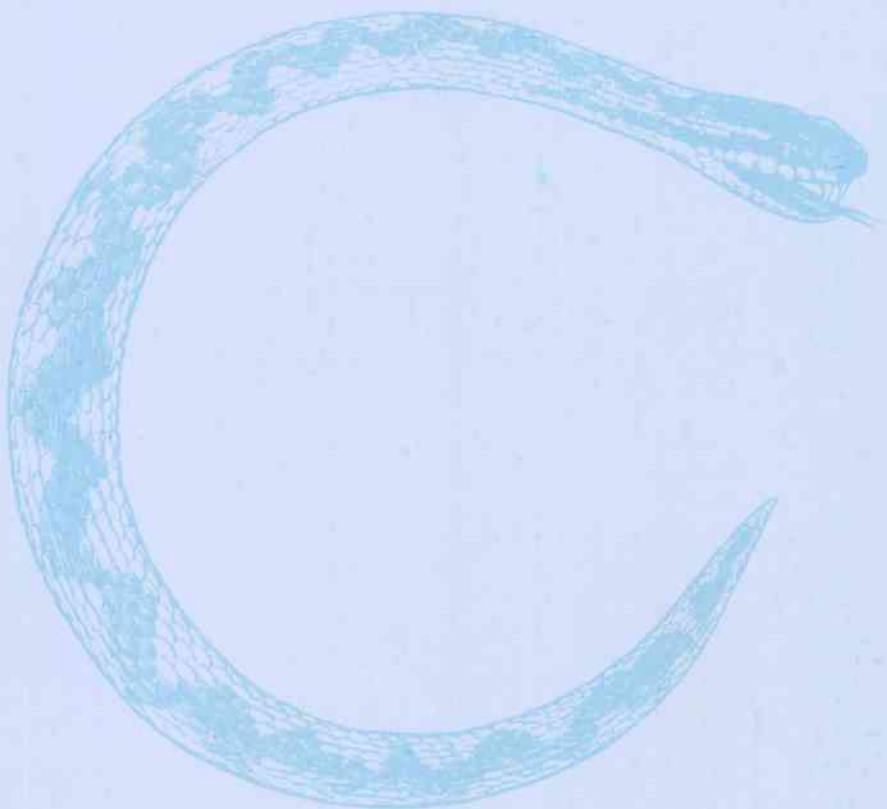
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A pond within an industrial area in Sweden was chosen as a model system to study effects on salmon alevins from a trinitrotoluene (TNT) contaminated water. Chemical screening revealed heavy contamination from TNT and its degradation products 2-amino,4,6-DNT (2aDNT) and 4-amino,2,6-DNT (4aDNT) ranging from 0,05g/kg to 230g/kg sediment (dry weight) within the water system. Pond sediment contained 8 g/kg TNT and pond water 3 mg/L of TNT.

In a dilution series pond water was mixed with tap water, which revealed increased death frequency in alevins occurred at 5 times dilution (≈ 0.4 mg TNT/L) of pond water. Uptake was clearly concentration related reaching 7, 9, 22 $\mu\text{g/g}$ tissue for TNT, 2aDNT, and 4aDNT at the highest TNT concentration. A time dependent uptake of TNT and its metabolites was documented at the water concentration 0.08 mg TNT/L. Metabolites showed a more efficient uptake compared to TNT, and the accumulation of 4aDNT was more pronounced during the late phase of the 40 day exposure study.

Disturbed physiological condition and delayed development in alevins were not studied, leaving questions on e.g. salmon reproduction without answers. Such toxic effects could thus not be excluded even at 125 times diluted pond water (0.016 mg TNT/L). The dietary intake of TNT and its metabolites are poorly studied. The study was sponsored by the Swedish Armed Forces.



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