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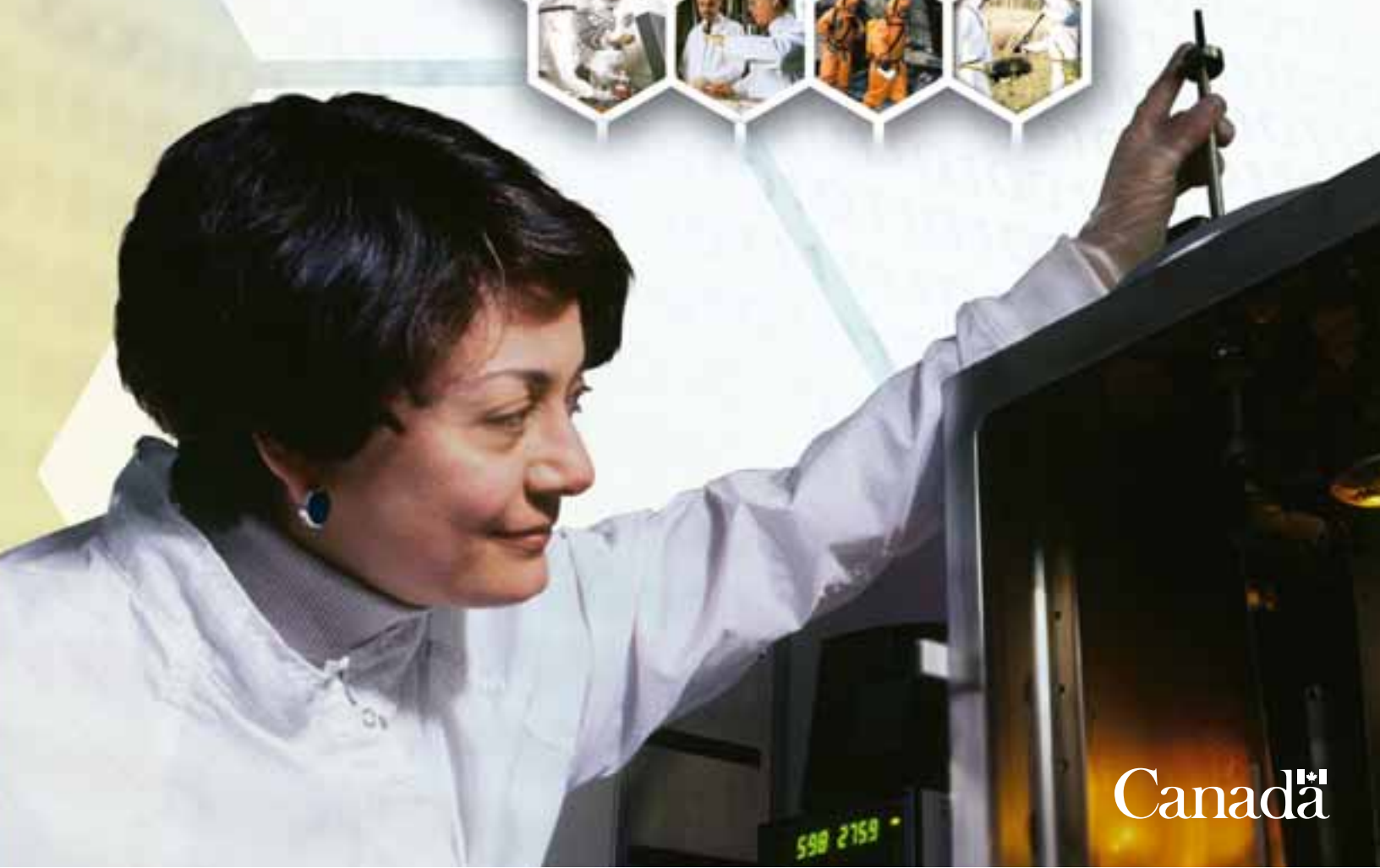
CRTI-IRTC

Proceedings of the

2004 CRTI Summer Symposium

15-16 June 2004

Gatineau Quebec



Canada

Forward

Proceedings of the 2004 CRTI Summer Symposium 15-16 June 2004 Gatineau Quebec

The Chemical Biological Radiological Nuclear (CBRN) Research and Technology Initiative (CRTI) was launched in May 2002 as a result of the Government of Canada's Public Security and Anti-Terrorism budget in December 2001. The CRTI is mandated to improve Canada's ability to respond to CBRN incidents through investments in science and technology.

In its first two years of operation, the Initiative has funded 53 Research and Technology, Technology Acceleration, and Technology Demonstration projects. It has further funded 59 Technology Acquisition projects to enhance the capacity of Federal Government science laboratories. Many of the projects have been very successful and are already demonstrating results.

The 2nd Annual CRTI Summer Symposium at the Chateau Cartier Resort in Gatineau, Québec provides an opportunity for the CRTI and the broader CBRN communities to learn about the progress of the projects from the first two rounds of funding. The goal of the Symposium is to facilitate an occasion to share and exchange the knowledge created by the CRTI partners and to learn about related allied work in CBRN. This interchange of ideas should further contribute to building Canadian capability and capacity in CBRN preparedness and response.

The following abstracts include all CRTI projects funded in 2002 and 2003 for either oral or poster presentation. CRTI is also pleased to include additional abstracts from researchers from allied and national CBRN communities. They are all notable by their breadth, quality, and the contributions they are making to national and international security.

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CRTI-0004TA

Biological Point Detection: MEMS Sensor Platform for Bio-agent Detection and Identification

PROJECT LEAD:
MEMS Precision Technology Inc.

FEDERAL PARTNERS:
DRDC Suffield

AUTHORS:
Dr John Dunfield and
Mr Brian Norling,
MEMS Precision Technologies,
3810 Fearn Way, Ladysmith, BC,
tel: (250) 245 0259,
fax: (250) 245 0259;

Dr William E. Lee,
Defence Research Development Canada Suffield, PO Box 4000,
Medicine Hat, Alberta, T1A 8K6,
tel: (403) 544-4706,
fax: (403) 544-3388.

Objectives

The scope of this project is to demonstrate a proof of principle for a MEMS chemical and biosensor for detection of potential toxic or infectious materials. To this end, we intend to construct a micro- fabricated resonator element to demonstrate the feasibility of our unique approach. Detection and signal transduction of the MEMS sensor is based on the relationship of frequency to mass in a resonating device (Hooke's Law). The resonator sensors will contain molecular recognition elements such as antibodies, nucleic acid probes or imprinted polymers. These will capture specific analyte materials on the resonator surface. The mass changes associated with specific capture will result in a change in frequency of the resonating elements.

This technology approach for detection is reagentless. The sensor, combined with recognition elements, is sufficient to provide signal when specific target molecules are presented. No labels (fluorescent, colored, radioactive etc.) are required for signal transduction. Another advantage of this technology over other reagentless methods is the low production costs of the sensors and the ancillary systems required to run it. Since the signal transduction process has differential frequencies of the resonator, miniature low cost differential amplifiers will be used. Expensive hardware such as lasers, light sources, optical detectors and lenses are not required. Nor are cumbersome features such as optical alignment or high voltage connections found in many current detection platforms.

Recent Progress

The core product development effort is to create an improved threat agent detection system that can be deployed in the field for military or civilian protection. The proposed system is simple to operate and provides real-time threat data necessary for rapid medical countermeasures. The heart of the system is a MEMS-based sensing element cartridge with the capability to accurately detect small numbers of bacteria, viruses, toxins or chemicals. The progress of the project rested upon resolving MEMS fabrication and bonding issues of the resonator devices to the enabling electronics. Several fabrication runs were undertaken, issues were resolved and the devices were successfully produced. The project team successfully bonded the connections.

Future Outlook

During the next several months, work in the project will focus on testing and evaluation of the resonator sensors. These devices will be tested for stability and for mass sensitivity. These tests will conclude the work for the CRTI project. The strengths of this technology include a high detection sensitivity, overall low cost, low power, small size and general robustness not yet demonstrated elsewhere. Microfabrication of the sensor elements will permit large-scale parallel bio-analysis.

CRTI 0006RD

RD Induction of Innate Immunity

PROJECT LEAD:
VIDO

FEDERAL PARTNERS:
Health Canada

INDUSTRY PARTNERS:
McMaster University, Hamilton;
NCFAD, CFIA, Winnipeg.

AUTHORS:
Markus Czub, Health Canada,
Winnipeg, (204) 789-6037,
email: Markus_Czub@hc-sc.gc.ca;

Lorne Babiuk, VIDO, Saskatoon,
tel: (306) 966-7475,
email: lorne.babiuk@usask.ca;

Jack Gauldie,
McMaster University, Hamilton,
tel: (905) 521-2100x76331,
email: gauldie@mcmaster.ca;

Steven Jones, Health Canada,
Winnipeg,
tel: (204) 789-5065,
email: steven_jones@hc-sc.gc.ca;

Stefanie Czub, NCFAD, CFIA,
Winnipeg,
tel: (204) 789-2021,
email: czubs@inspection.gc.ca.

Objectives

Recent events have demonstrated the threat of bioterrorism to Canadians and the food chain. Many highly infectious agents such as *Yersinia pestis* can infect both humans and animals, while Smallpox and Foot-and-Mouth Virus target only humans and animals, respectively. All these agents can be dispersed through both airborne and waterborne pathways. In the case of a bioterrorism attack, rapid diagnosis and therapy will be of immediate need, while in the long-term, pre-exposure prophylaxes will be required. The goal of our proposal is to develop products and procedures to provide immediate short-term protection to the airways and the intestines against various organisms, while at the same time delivering vaccines that can provide long-term immunity.

Recent Progress

The Vaccine and Infectious Disease Organization (VIDO) will screen a wide array of specific oligonucleotide (CpG) sequences to identify those which are most effective and suitable in a particular animal species to stimulate innate immunity. It will be necessary to determine optimal doses and delivery routes for CpG. The focus will be on inducingimmunity at mucosal surfaces of the respiratory and digestive tracts. Researchers will also establish screening methods for measuring changes in the immune response, such as cytokine expression profiles.

Several CpGs have been analyzed for their ability to induce specific cellular genes important for innate immunity, such as Il-6, IL12p40, IFN-γ, and B7-1. In addition, CpG response genes of bovine, ovine, porcine and equine origin have been cloned and expressed in transfected cell lines.

At McMaster University, a small animal model for poxviruses will be established. Poxviruses, especially Variola major causing smallpox, are among the most contagious and virulent infectious agents. At present, it is not clear whether infectious virus stocks of Variola major are still confined to federal high containment laboratories in the USA and Russia or whether the virus is already in the hands of terrorists. As this virus poses one of the most serious

threats worldwide, it will be of particular interest to study the rapid induction of innate immunity in an animal model using a closely related poxvirus.

Based on experimental data from VIDO, researchers from McMaster University have completed a number of *in vivo* and *in vitro* tests with CpG showing the impact of delivery of this compound to the mucosal surface of mice. It was shown that transmucosal, but not systemic, delivery of CpG oligodeoxynucleotides (ODN) to genital mucosa protected female mice against mucosal intravaginal (IVAG) challenge against herpes simplex virus type 2 (HSV-2). This protection was due to the ability of CpG to induce local innate immune responses at the epithelial surface in the vaginal tract since protection could be induced in mice lacking an adaptive immune system (RAG-2-/- and RAG-2-/- γc-/- mice). Local delivery of CpG ODN rapidly induced proliferation and thickening of the genital epithelium and caused significant recruitment of inflammatory cells to the submucosa. Local delivey of CpG to the vaginal mucosa resulted in inhibition of viral replication but not entry of virus into genital epithelial cells. Thus, mucosal delivery of CpG ODN induced an anti-viral state in mucosal epithelial cells. The ability of CpG to induce a local antiviral state was not due to interferon-γ (IFN-γ). Since CpG acts by signaling through Toll-like receptor 9 (TLR9), human HEK-293 cells transfected with murine TLR9 and a murine macrophage cell line that naturally expresses TLR9 were used and it was demonstrated that this anti-viral state was dependent on and mediated through TLR9. Researchers went on to study the parameters of CpG-induced protection against IVAG HSV-2 challenge. Protection *in vivo* occurs if CpG is delivered 48 hours before or up to 6 hours after mucosal infection.

Within the biosafety level 3 and 4 containment laboratories at the Canadian Science Centre for Human and Animal Health (CSCHAH), experimental animal work using Ebola virus and *Yersinia pestis* will be established and carried out. Both agents are extremely contagious and associated with a high fatality rate in humans. In its aerosolized, i.e. weaponized form, *Yersinia pestis* is almost invariably fatal and non-treatable. We will investigate the immediate effects of CpG molecules on animals exposed to either one of these deadly pathogens. Initial animal experiments using Ebola virus have been carried out in high containment labs in Winnipeg and were used to generate biological samples for the establishment of the screening procedures.

All the studies on animals will be evaluated employing clinical, molecular, and microbiological parameters. In addition, the Pathology Section of the National Centre for Foreign Animal Diseases (NCFAD) of the Canadian Food Inspection Agency (CFIA) at the CSCHAH will conduct histopathological and immunohistochemical analyses on tissues relevant to our studies.

Future Outlook

Genomic studies using DNA microarrays are being conducted to determine which genes may be involved in a CpG-induced antiviral state. To date, researchers have analyzed RNA isolated from cells treated with CpG ODN, control ODN or left untreated. In future studies, cells as well as tissues obtained from animals infected with various infectious agents, such as Ebola virus, Poxvirus or *Yersinia pestis* will be used for further analyses.

In addition, a semi-quantitative RT-PCR for both murine and human TLRs has been developed. This assay detects mRNA for all 9 murine and all 10 human TLRs. A comparison of the expression of TLRs in various mucosal tissues in mice and humans is currently underway. This relies both on the use of mRNA from whole mucosal tissues and using Laser Capture Microscopy (LCM). Further, the profile of expression in primary cultures of both human and mouse mucosal epithelial cells is being examined.

CRTI-0011TA

Hand Held Real Time Biological Detector

PROJECT LEAD:
General Dynamics Canada Ltd.

FEDERAL PARTNERS:
Defence R&D Canada – Suffield

AUTHORS:
Eric Newman PEng PMP
email: eric.newman@gdcanada.com
and

Ray Kacelenga PhD
email: ray.kacelenga@gdcanda.com
– Integrated Sensor Systems,
General Dynamics Canada Ltd.

Objectives

The main objective of this CRTI project was to develop a low-cost, hand-held, real time biological aerosol detector based on a laser induced fluorescence particle detector called the Biological Agent Real Time Sensor (BARTS). The key factor to success was the development of a smaller, less expensive optical cell based on a lower cost UV LED light source (or a less costly continuous wave UV diode laser) rather than the more expensive UV pulsed laser used in the existing BARTS. Successful integration of this new optical cell dramatically reduced the cost and increased the environmental durability without impacting detection performance. The use of the new continuous wave light source also allowed additional changes to the capture electronics and data analysis subsystem that further reduced the cost, size, weight, and power requirements of the sensor.

The scope of the project was limited to research and development of a hand-held biological agent detector, demonstration of the performance of such a detector and the construction of three prototype units. Full productionization of the design was not attempted as part of this project although GD Canada fully intends to proceed to production with the new sensor.

The project consisted of nine main tasks and a number of subtasks that are described in detail below. They were completed over a period of twelve months from 01 April 2003 to 31 March 2004. A milestone (go/no-go) point was placed after the development of the new light source (the highest risk task) to limit liability and offer an appropriate place to abandon the project should the potential for success be limited. At that decision point, the team assessed the likelihood of success on the rest of the project and made a decision on whether to continue with the project. The Project Manager then implemented the "go" decision in consultation with PWGSC and DRDC Suffield.

Project Tasks:

1. Explore LED and Diode Laser Light Sources
2. Redesign the Optical Cell
3. Modify Sensor Control and Data Capture Electronics
4. Design Battery Power Subsystem
5. Design Interface for Air Concentrator
6. Acquire Material for System Integration and Build
7. System Integration and Build
8. Document Final Design
9. Development of in-house test capability

Recent Progress

The recent progress in this abstract covers the one-year period from 01 April 2003 to 31st March 2004 and includes all work conducted against the CRTI contract requirements. The work conducted during this reporting period may be divided into the following major categories:

Light Source –
Early in the project, a significant amount of effort was dedicated to the investigation of the viability of using a UV LED light source in place of the traditional laser light sources. UV LED light sources offer advantages in the areas of size, cost and overall life cycle requirements. Engineering sample LEDs were procured and a variety of methods of harnessing the power of several lower output LEDs and concentrating their outputs were investigated. In July of 2003, a decision with respect to which light source would be used was made based on performance of UV LED based prototypes against the UV laser diode prototype. It was determined that although UV LEDs in the correct wavelength were available, their power output and angle of divergence were such that a UV LED based optical cell was not practical. As a result, the optical cell development proceeded with the continuous wave UV diode laser, while maintaining insight into the fact that future industry UV LED development would eventually yield a UV LED with suitable characteristics to generate an NaDH fluorescence response. Subsequently, an 85 mW, 380 nm engineering LED sample was procured from Nichia Corporation. The divergence angle of this LED source turned out to be too wide at +/- 55 degrees making it difficult to capture much of the energy. Nichia Corporation has gone back to the drawing board in an attempt to reduce the divergence angle to +/- 25 degrees. The new engineering samples are expected anytime between March and June 2004. In the meantime, INO have redesigned the capture optics and increased the fluorescence aperture in order to capture an additional 120 % fluorescence signal. The scatter optics have also been redesigned to capture an additional 30 % scatter signal. The building of the new optics will occur subsequent to the project completion date. Although these cell enhancements were done in anticipation of a viable LED source, all changes will improve sensor performance regardless of the light source.

Optical Cell Enhancements –
The project has yielded several optical cell enhancements during the past year. The most significant of these enhancements was the relocation of the scatter PMT optics to the incident light axis in order to take advantage of the dominant scatter phenomenon in the cell. This change has improved both the particle counting and sizing efficiency. Other enhancements include the redesign of the air nozzle, the introduction of a mirror to increase the amount of fluorescence detected, introduction of optical filter packs at the light source and scatter and fluorescence PMTs, and introduction of mechanical optical alignment features to simplify manual alignment of the laser light source.

Battery Power/Chassis Design –
A custom removable and rechargeable battery pack was designed to permit use of the hand-held biological agent detector for a typical first responder mission profile of one hour. The chassis design includes ergonomic and human factor considerations revealed through meetings with the Calgary Fire Department Hazardous Materials Team and later validated through marketing contact with various US based Homeland Security agencies.

Aerosol Test Path (ATP) –
The ability to quickly validate design changes and sensor performance under customized test conditions is critical to any efficient development program. Although external testing was conducted as part of this project, an internal aerosol test facility was developed. The ATP is a trailer- mounted, stainless steel ducting that incorporates intake and exhaust filtering and variable aerosol control. The airflow rates across the equipment under test are also variable. Other features include: accommodation of slit samplers for referee data, on board incubator and power distribution,

suite of control sensors, viewing ports and access doors, as well as a full complement of safety equipment. The ATP has permitted real time verification of design concepts, calibration of optical cells, and preparation of equipment prior to testing at external facilities.

Future Outlook

The future plans for this project include:

(a) Complete the assembly of prototype units # 2 and # 3;

(b) Integrate, when received, the 365 nm, 100 mW LED into the illumination source sub-assembly;

(c) Continue with activity to automate an Aerosol Test Chamber at GD Canada;

(d) Compile the final CRTI report and project close-out.

CRTI-0019TA

Real-Time Confirmatory Bio-Detection and Identification: Rapid Validation and Fieldable Device Prototyping

PROJECT LEAD:
IatroQuest Corporation

FEDERAL PARTNERS:
DRDC Suffield

INDUSTRY PARTNERS:
Dycor Technologies Ltd.,
Fluorosense Inc.

AUTHORS:
Guy Rodomista and Dr Denis Godin,
IatroQuest Corporation,
1000 Chemin du Golf, Verdun, QC
H3E 1H4,
tel: (514) 362-1091,
http://www.IatroQuest.com;

Dr William E. Lee, DRDC- Suffield,
PO Box 4000, Medicine Hat, Alberta,
T1A 8K6,
tel: (403) 544-4706;

Tim Friesen, Dycor Technologies Ltd,
17944 106-A Ave, Edmonton,
Alberta, T5S 1V3,
tel: (780) 486-0091,
http://www.dycor.com;

Bill Sinclair and Mike McDonnell,
Fluorosense Inc, 1948 Merivale Rd.,
Suite 101, Nepean, Ontario,
K2G 1E9,
tel: (613) 224-1192,
http://www.fluorosense.com.

Objectives

This project proposes the development of a limited number of operational fieldable prototypes of a breakthrough in biosensing technology based on the use of nano-technological 'smart' materials termed Bio-Alloy™. These materials are based upon the discovery (global patents allowed / pending) and development of photoluminescent (PL) nanostructured (2-3 nm features) semiconductor (e.g. doped silicon) materials to which bioengineered Recognition Elements (REs; current focus on antibodies; nucleic acids, enzymes, and chemical ligands also possible) are chemically immobilized. Validation will be done using BW agent simulants and possibly 'live' agents in conjunction with DRDC Suffield. Studies could also be conducted in association with the Canadian Food Inspection Agency on ultra-rapid detection and identification of food-borne biological agents.

Recent Progress

To date, the evaluation of the first breadboard platform (BBD1) has been completed, and the evaluation of the second breadboard platform (BBD2) has been initiated. At this time, all elements (fluidics, optics, firmware & software) are showing positive performance. The verification process is progressing using validated "in-house" test platforms to confirm presence or absence of target. BBD2 is capable of independently addressing three Bio-Alloy™ chips simultaneously, through 3 independent opto-electronics channels. Different fluidics scenarios are being investigated: variable flow path thickness (0.1, 0.3 and 1mm) and sample introduction by capillarity, wicking and active pumping. To date, the results have been encouraging using the sample introduction by capillarity. The other options will be evaluated in order to optimize the measurement cycle.

Future Outlook

Ultimately, the scope of the project is to provide proof-of-concept and functionality for the development of a fieldable biosensing device that utilizes the underlying Bio-Alloy™ smart material technology. At this stage, the focus is on optimizing the Bio-Alloy™ chips, biosensing cartridge and opto-electronics in order to deliver a robust and reproducible test platform. The final prototype will have capability to determine the presence of biological analytes in environmental and food samples. Recent international conferences related to counterterrorism technology requirements continue to highlight the urgent need for solutions in cost-effective, widely distributed, bio-detection and identification systems.

CRTI-0027RD

Biological Dosimetry and Markers of Nuclear and Radiological Exposures

PROJECT LEAD:
Health Canada,
Radiation Protection Bureau

FEDERAL PARTNERS:
DRDC Ottawa

INDUSTRY PARTNERS:
Atomic Energy of Canada Limited,
McMaster University,
Credit Valley Hospital

AUTHORS:
Slavica Vlahovich

Objectives

The objective of this project is to establish a National Biological Dosimetry Response Plan (NBDRP) and develop rapid methods of radiation exposure assessment to increase throughput in large-scale events. Biological dosimetry assesses radiation exposure when physical dosimetry is not available. It can be a means of screening the general population for radiation exposure and identifying first responders who must be restricted from further exposure. It can also be a means of assessing long-term risks following radiation exposure. In the event of a radiation emergency, timely assessment of radiation exposure and response will help guide the actions of emergency officials, first responders and health care personnel.

Recent Progress

The first component of this project is the development of a National Biological Dosimetry Response Plan (NBDRP). This is an emergency response plan for the coordinated delivery of dosimetry services by a network of laboratories across the country. This dosimetry service network will be able to respond to national and regional needs in a nuclear or radiological event. The initial phase of NBDRP development is complete. The plan builds upon a collaborative agreement among three laboratories to assess radiation exposure using the dicentric chromosome assay (DCA). The existing network now consists of four laboratories.

The DCA measures dicentric and ring chromosomes that are caused by radiation. These laboratories are working towards the ISO 90238 standard for biological dosimetry. This will require (1) standard operating procedures, (2) standard training documents, and (3) intercomparison of results from standard slides evaluated by trained staff. To date, staff have been trained, standard operating procedures have been drafted and calibration curves for predicting radiation exposure are being developed.

In order to extend the network, clinical laboratories across the country are now being recruited for performing the DCA. These laboratories will form the Canadian Cytogenetic Emergency Network. A workshop planned for May 2004 has been organized to provide participants with theoretical and practical knowledge about the current state of biological dosimetry in Canada and to create a forum for discussion about network operations. Once established, the NBDRP will be maintained in a state of readiness for emergency response by continuing a program of intra- and inter-laboratory comparisons and participating in emergency exercises.

The second component of this project is the development and implementation of improved assays for estimating individual radiation exposure and response following a radiological incident. With a goal of achieving faster and automated methods to screen large numbers of samples, a flow cytometric version of the DCA (FDCA) is being developed. Progress includes producing chromosomes in suspension, successful staining of centromeres, first on slides, then in solution, and detecting centromeres in solution using the flow cytometer. Further steps are being taken to optimize the staining.

A method of premature chromosome condensation fluorescence *in situ* hybridization (PCC-FISH) is being investigated to determine if it can be used for the early detection of personal exposure within 4-12 hours post-irradiation. A literature search was completed and experiments are in progress to optimize the technique. Dose response curves for different radiation qualities are being developed in collaboration with a U.S. laboratory.

Other techniques being examined include a modified FDCA procedure using fluorescence *in situ* hybridization (F-FISH), evaluation of apoptosis, and spectral karyotyping (SKY) in lymphocytes. Work has begun on evaluation of apoptosis and FISH of chromosomes in suspension, a prerequisite for the application of FISH to the flow cytometric assay. SKY may provide an estimate of the level of damage, and subsequently the magnitude of the dose, within 24-48 hours following exposure. SKY can also be used in follow-up investigations to monitor future health risk. Dose response curves for low-LET radiation are about 50% complete. SKY analysis performed so far on human peripheral blood lymphocytes indicates a high detection rate of chromosome rearrangements using this technique.

Future Outlook

Techniques are being developed to determine the exposed individual's absorbed dose directly using electron spin resonance (ESR) in tooth enamel within 24-72 hours post-irradiation. However, since collection of tooth enamel from exposed individuals could be problematic, the ESR tooth enamel assay is being developed in animals that could also be involved in the exposure (e.g. mice, cats, and dogs). All experiments for low-LET radiation tooth enamel dose response curves using ESR in human and canine teeth have been completed and manuscripts published. Work is in progress to generate a neutron calibration curve. Once proven in the laboratory, any or all of these methods will be expanded to laboratories across the country to improve the response time to radiological or nuclear events.

In addition to these cytogenetic assays, state-of-the-art genomics and proteomics technologies are being used to identify specific biological markers of radiation exposure. Biological markers can be used as indicators of an individual's response to damage caused by radiation. This is more biologically relevant than a measure of exposure and may be useful in assessing the long-term risks following radiation exposure. It is expected that data on individual responsiveness will revise the traditional means of risk assessment and triage that is based on population studies and does not take into account individual variability. To date, a list of radiation responsive markers has been identified and variation in the endogenous levels of these markers is under investigation. Human Research Ethics approval for analysis of human blood irradiated *in vivo* is pending. Where applicable, a prototype for a field deployable assay will be developed and tested. This could be used for surveillance purposes or rapid identification of potentially exposed individuals. Moreover, individual plasma profiling has the potential of providing information about exposure to other stressors such as biological or chemical agents.

CRTI-0027RD (a)

Design and Implementation of the National Cytogenetic Emergency Network (CEN) for Biological Dosimetry following Radiological/Nuclear Accidents

PROJECT LEAD:
Health Canada,
Radiation Protection Bureau

FEDERAL PARTNERS:
DRDC Ottawa

INDUSTRY PARTNERS:
Atomic Energy of Canada Limited,
McMaster University,
Credit Valley Hospital

AUTHORS:
Susan M. Miller and
Catherine L Ferrarotto,
Consumer and Clinical Radiation Protection Bureau, Health Canada, Ottawa ON;

Diana Wilkinson, DRDC Ottawa, Department of National Defence, Ottawa, ON;

Donald P. Morrison, Radiation Biology and Health Physics Branch, Atomic Energy of Canada Limited, Chalk River, ON;

Douglas R. Boreham, McMaster Institute of Applied Radiation Sciences, McMaster University, Hamilton, ON;

Jo-Anna Dolling, McMaster Institute of Applied Radiation Sciences, McMaster University, Hamilton, ON and Genetics Department, Credit Valley Hospital, Mississauga, ON.

The project team is developing a network of laboratories across Canada to provide the capacity for rapid biological dose estimates using the dicentric chromosome assay (DCA) in emergency situations. The DCA measures dicentric and ring chromosomes, which are induced by radiation, in cells blocked in metaphase. This method has been used internationally for over 30 years and has been standardized by ISO (ISO 19238). The team is currently working towards this standard in all four laboratories to form the core of the national network.

In cases where only a small number of dose estimates are required, up to 1000 metaphases per blood sample are analyzed, allowing detection of exposures as low as 0.15 Gy. However, in dealing with a large number of samples from potentially exposed individuals, where turnaround time is critical, the detection threshold can be raised to 1 Gy, thus reducing the number of metaphases to be analyzed.

In a major emergency situation, even with the combined capacity of the four core labs, only a limited biological dosimetry service would be available due to limitations in both equipment and expertise. Therefore, to increase Canada's response capacity, the project is expanding the network to include cytogenetic laboratories across the country. As part of this initiative, a workshop on biological dosimetry was held in May 2004 in Toronto as part of the Great Lakes Chromosome Conference, which was attended by directors of interested cytogenetic laboratories.

Blinded slides, prepared for DCA analysis following *in vitro* irradiation of blood from a healthy volunteer donor to a range of γ-ray doses, were distributed to the workshop participants and to the four core laboratories. Fifty metaphases will be analyzed per slide, to mimic triage scoring, and dose estimates will be calculated. Results from all laboratories will be collated and analyzed. Following this initial scoring exercise, laboratories will be sent slides on an ongoing basis to maintain their expertise in the DCA and to ensure their readiness to respond to local and national radiological/nuclear emergencies.

CRTI-0027RD (b)

Markers of Radiation Exposures in Support of Biological Dosimetry

PROJECT LEAD:
Health Canada,
Radiation Protection Bureau

FEDERAL PARTNERS:
DRDC Ottawa

INDUSTRY PARTNERS:
Atomic Energy of Canada Limited,
McMaster University,
Credit Valley Hospital

AUTHORS:
Diana Wilkinson

Recent international developments have raised issues of security that have led to the advancement of public health measures intended to guide the actions of emergency officials, first responders and health-care personnel. Of primary concern for the emergency health care personnel is a rapid and accurate identification of the incident and its associated health risks. Triage methodologies are strongly dependent on this identification being correct. In the event of radiological exposure, a responding physician must have evidence confirming a biological effect prior to initiation of triage. Physical monitoring provides guidance but may be misleading and thus inappropriate for initiation of certain treatment strategies. Traditional biological methods use the rate of change in blood cell counts from peripheral blood samples as an indicator of radiation exposure and a predictor of radiation dose. It is the intention of this project that the methodologies being developed will provide supportive biological information to the physicians so that conclusive results may lead to more rapid triage.

The project will use genomic and proteomic strategies to identify biological markers of radiation exposure. Through clinical studies of cancer patients receiving radiation therapy, some of these markers have already been identified. The project will determine the range of basal level expression for some of these markers and compare these background values in non-irradiated individuals to those in irradiated patient populations. It is hoped that this research will answer a number of questions. First, can the radiation-induced biomarkers be used as specific indicators for radiation exposure or are they more likely to be representative of a generalized stress response caused by physical stress, biological, chemical or radiological agents? Can biological markers be used as indicators of an individual's response to damage thus providing a more biologically relevant measure of exposure that may be useful in assessing the long-term risks following radiation exposure? Will this information on individual responsiveness be in agreement with the traditional risk assessment values extrapolated from cytogenetic damage assays that are based on population studies and do not take into account individual variability? Can identification of biomarkers of response assist medical professionals in customizing triage methodologies by providing them with individual-specific medical information? And finally, can this knowledge of biomarkers of response facilitate the development of a field deployable assay for surveillance purposes or rapid identification of potentially exposed individuals?

A list of radiation responsive markers has been identified and the variation in the endogenous levels of these markers is under investigation. The team will present preliminary information on the endogenous levels of these markers in a non-irradiated control population. It will also present experimental strategies designed to answer the earlier identified questions and how this information can support the presently existing program for biological dosimetry.

CRTI-0027RD (c)

Development of a High-Throughput Biological Dosimeter for Radiation Exposure

PROJECT LEAD:
Health Canada,
Radiation Protection Bureau

FEDERAL PARTNERS:
DRDC Ottawa

INDUSTRY PARTNERS:
Atomic Energy of Canada Limited,
McMaster University,
Credit Valley Hospital

AUTHORS:
R.C. Wilkins, S.M. Miller,
C.L. Ferrarotto, B.C. Kutzner,
P.V. Bellier and J.P. McNamee,
Consumer and Clinical Radiation Protection Bureau,
775 Brookfield Road,
Health Canada,
Ottawa, ON, K1A 1C1.

Biological dosimetry assesses radiation exposure when physical dosimetry is not available. It can be a means of screening the general population for radiation exposure and identifying first responders who must be restricted from further exposure. It can also be used to assess long-term risks following radiation exposure. In the event of a radiation emergency, timely assessment of radiation exposure will help guide the actions of emergency officials, first responders and health care personnel.

Traditionally, biological dosimetry is performed using the dicentric chromosome assay (DCA), which measures dicentric and ring chromosomes that are caused by radiation. This CRTI project is establishing a National Cytogenetic Emergency Network (CEN) using the DCA. However, this is a microscope-based assay which requires specialized training and is labour intensive. Adapting dicentric analysis to flow cytometry would allow for higher throughput and greater accessibility.

A new centromere-specific antibody is now available which allows uniform staining of centromeres in all chromosomes. This is a key feature required for successful dicentric detection by flow cytometry. Microscopy data showed that 99% of dicentrics identified by DAPI had both centromeres labelled with this antibody. Samples from the same experiment were analyzed using the DCA and the same number of dicentrics per cell was found with both techniques.

For dicentric detection by flow cytometry, a method for fluorescently labelling the centromeres of chromosomes in suspension has been developed. The project team is currently optimizing the conditions that would allow identification of single and double labelling of chromosomes. Once this assay is established, it will be valuable as a screening tool following a radiological or nuclear emergency.

CRTI-0027RD (d)

Biological Dosimetry and Markers of Nuclear and Radiological Exposures

PROJECT LEAD:
Health Canada,
Radiation Protection Bureau

FEDERAL PARTNERS:
DRDC Ottawa

INDUSTRY PARTNERS:
Atomic Energy of Canada Limited,
McMaster University,
Credit Valley Hospital

AUTHORS:
D.R. Boreham, J. Lavoie,
N. McFarlane, M-E. Bahen, L. Ryan,
K. Schnarr, R. Wilkins, J. McMamee,
and J.A. Dolling, McMaster Institute
of Applied Radiation Sciences,
McMaster University,
1280 Main St. West, Hamilton,
Ontario,
tel: (905) 525-9140, ext. 27538,
email: boreham@mcmaster.ca

Objectives

The overall objective of this project is to establish a National Biological Dosimetry Response Plan (NBDRP) that utilizes classical and novel approaches in biological dosimetry to rapidly determine the magnitude of a radiation exposure to a population. Biological dosimetry assesses radiation exposure when physical dosimetry is not available. McMaster University is actively involved in research that focuses on development of new molecular tools to assess radiation exposure in biological systems.

The specific aim of this research is to expand and optimize the application of two biological processes that occur after radiation exposure. The levels of radiation-induced programmed cell death (apoptosis) and chromosome aberrations (damage) in white blood cells (lymphocytes) have been shown to correlate with radiation exposure. The advantage of measuring apoptosis and chromosome aberrations in lymphocytes is that these endpoints can be measured by taking a small blood sample. This presentation will outline the progress made regarding the most suitable assays to measure apoptosis and chromosome aberrations for emergency biological dosimetry.

Recent Progress

When human white blood cells are irradiated, they can die through a process of programmed cell death called apoptosis. There are a number of ordered biochemical processes that take place once a cell has committed to undergo apoptosis. It has been previously shown that the level of apoptosis measured in irradiated lymphocytes is proportional to dose. The team has completed tests that compared a series of techniques and assays and determined that an automated flow cytometry approach in conjunction with labelling a cell surface marker Annexin V is most likely the simplest and fastest method for emergency biological dosimetry purposes. The project team has shown that the technique is sensitive enough to measure doses at least as low as 0.25 Gy. An unexpected result showed that low energy neutrons had a similar relative biological effectiveness, per unit dose, as gamma radiation for inducing apoptosis. The process of radiation-induced apoptosis in human lymphocytes will be described in the following presentation. The current detection techniques tested by the project team to measure apoptosis will be reviewed and the sensitivity and speed for automated scoring using flow cytometry will be discussed in the context of different radiation qualities.

Chromosome aberrations have been used for more than 40 years to measure radiation damage in human lymphocytes. The team's efforts have focussed on measuring chromosome aberrations using a powerful new technique in molecular cytogenetics called spectral karyotyping (SKY). An objective of this project is to develop these techniques for both immediate and long-term radiation risk assessment. Molecular cytogenetics is a technical term used to describe biochemical techniques that allow identification of chromosome damage caused by a DNA damaging agent like radiation. Humans have 23 pairs of chromosomes (karyotype) and SKY uses a process called fluorescence in situ hybridization (FISH) that "paints" each chromosome pair a unique colour. When chromosome damage occurs after radiation exposure, rearrangements between chromosomes is common and visualization of specific colour junctions can identify aberrations. It has been shown that gamma radiation causes the formation of relatively simple chromosome rearrangements that are proportional to dose. The shape of the aberration curve is very similar to the curvilinear dose response seen for classical aberration measurement using the simple dicentric assay. The advantage of SKY, however, is that even simple appearing dicentrics can be characterized as originating from complex rearrangements between three or more chromosomes. This is important because the level of rearrangement complexity is believed to be directly proportional to the degree of cellular harm (risk). Interestingly, SKY results have shown that the complexity of chromosome aberrations associated with exposure to low-energy neutrons is large compared to gamma radiation. At neutron doses around 1 Gy, almost every cell has at least one chromosome that is aberrant. The presentation will describe molecular cytogenetics and the techniques used to measure chromosome aberrations caused by radiation of different qualities. The sensitivity of SKY will be compared to other more classical techniques and the advantages of SKY for emergency biological dosimetry will be discussed.

Future Outlook

Future experiments will test the usefulness of apoptosis and chromosome aberrations using SKY to assess the risks of very low-dose exposures in humans. The project has ethical approval to obtain blood samples from human patients who have been exposed to whole body low doses of diagnostic radiation. The team will continue to optimize the sensitivity of its techniques and analyze blood samples from these individuals in an attempt to define the lower limit of detection for these biological endpoints. The team also intends on investigating the newest application of SKY, known as m-banding, which involves multiple colour banding of individual chromosomes. This technique could provide even higher resolution for emergency biological dosimetry because it can detect intra-chromosomal rearrangements indicative of exposure from high linear energy transfer (LET) radiation, such as alpha radiation, that might be associated with a dirty bomb detonation.

CRTI 0029RD

Protecting the First Responder against CB Threats

PROJECT LEAD:
Royal Military College of Canada (RMC), DND

FEDERAL PARTNERS:
RCMP, DRDC Suffield, Director NBC Defence (DNBCD), Health Canada

INDUSTRY PARTNERS:
3M Canada, DuPont Canada, Research Development and Engineering Command (RDECOM, US Army Edgewood)

AUTHORS:
Eva Gudgin Dickson and Paul Bodurtha, Dept. of Chemistry and Chemical Engineering,
PO Box 17000, Station Forces,
Royal Military College, Kingston,
Ontario, K7K 7B4.
email: Dickson-e@rmc.ca,
Paul.Bodurtha@rmc.ca.

Objectives

Over the last few years, the first responder community, such as firefighters, police, and emergency medical teams, has been faced with the challenge of examining their capability to respond to a new class of potential disasters, terrorism using various particularly toxic materials such as chemical, biological, or radiological agents. While the community is in the process of equipping and training its members with appropriate personal protective equipment (PPE) as time and budgets will allow, there are still uncertainties as to the best equipment and operational procedures to be used. The first responder community needs guidance in how to appropriately select and use existing off-the-shelf equipment in order to meet their immediate needs as well as, ultimately, equipment designed against appropriate standards.

To assist first responders in obtaining the best possible equipment, the project will: a) provide guidance in the use and selection of protective equipment in order to enhance preparation for response to a CB incident; and b) drive the development (for Canada) of protective equipment guidelines and standards for response to a CB event.

Recent Progress

Protective equipment performance evaluations
In order to develop the best possible guidance for first responders, the project will determine actual protective equipment performance under the most realistic exposure conditions possible.

Respiratory protection
Man in simulant (MIST) methods are under development for evaluation of respirator protection under operationally relevant conditions, while modeling is being used to supplement this information. A more reliable procedure has been developed for measuring the respiratory protection against aerosols (protection factor) for first responders wearing a negative pressure respiratory mask. A mobile operational protection factor instrument has been acquired that will be used for these evaluations. In addition, a model to predict protective performance of air-purifying respirator filters against a wide variety of toxic industrial chemicals and military gases is being developed and validated.

Body protection: vapour-liquid
MIST assessments of protection provided by first responder equipment are also being performed to study the percutaneous exposure to, and to model effects from, vapour and liquid contact. Methods for evaluation and risk assessment for vapour exposure to toxic agents such as mustard and VX have previously been developed, using man-in-simulant test (MIST) methods, in conjunction with a number of other national programs. These have been used for prediction of effects of chemical agent vapour exposure to firefighters wearing standard turnout gear. In these evaluations, individuals wearing the protective gear are exposed to a chemical agent vapour simulant while performing a series of operationally relevant activities, and vapour penetration at a variety of body locations is monitored. These methods are used for evaluation of equipment for the police line officer, with the particular participation of the RCMP in selection and evaluation of equipment. In addition, in collaboration with a variety of first responder organizations, these methods have been extended to operational assessment of first responder equipment in the case of exposure to liquid agents. Based on the distribution of observed dosages measured at the skin after liquid contamination of the equipment at different locations, predictions can be made of the mean and worst-case likely effects that would be seen for rescuers performing the types of specific activities. Preliminary evaluations of firefighter and tactical police equipment using simple liquid exposure patterns have been completed.

In collaboration with project CRTI-0161TA (CBRN Blast Protective Helmet Development), the vapour and liquid explosive dispersal protection provided by the prototype helmet, along with the bomb disposal overgarment system used by the RCMP, has been evaluated. These results have been used in the design of the next generation prototype to be produced under that project.

Body protection: Bio-aerosol
A procedure has been refined to determine how well protective equipment can protect the first responder from skin/respiratory tract contamination while working in an area contaminated by a bacterial spore aerosol such as anthrax. In this procedure, first responders performing a series of operationally relevant activities are exposed to a non-toxic aerosol of *Bacillus globigii* spores (anthrax simulant). Samples are collected from the exterior of the protective wear and, after removal of the protective wear, from the same positions on the skin of the volunteer, and also from within the respirator. The samples are processed to show how many Colony Forming Units (CFU) were present on both the outer and inner sample points for each position, and are used to determine a protection factor.

Skin permeation
In vitro skin absorption test systems have been established at both Health Canada and DRDC Suffield to examine dermal exposure of toxic chemicals, such as pesticides and chemical warfare agents. The research at Suffield will involve the absorption of the nerve agent VX, and the pesticide parathion, on viable skin tissue samples from a pig (swine). Two potential exposure scenarios have been evaluated at Health Canada for studying the absorption of the chemical agent simulant, methyl salicylate, and the pesticides parathion and malathion, by viable human skin. The first scenario involves the collection of skin sections and receptor fluid right after exposure (3 or 30 minutes), and the second scenario has receptor fluids collected hourly for six hours following a 30-minute exposure. Based on these studies, the amount of toxic material that would permeate through skin over these durations of exposure can be predicted.

Standards development
A standards team has been organized amongst various key partners as well as outside organizations representing first responders and standards developing bodies. A preliminary position paper, titled *A Canadian standard for respiratory and*

skin protection for official first responders to a Chemical or Biological Event – Criteria, Selection and Use Guidelines, is currently being prepared. The standards development and recommendation involves two components: 1) personal protective equipment (PPE) testing / performance criteria, and (2) selection and use guidelines for the end user (including training and education). The team has also compiled a variety of Canadian and international standards relevant to the area.

Future Outlook

A few key activities that will be performed within the next year include operational protection factor determination for first responder respiratory protection system; determination of bioaerosol protection factors for a variety of first responder equipment including the CBRN blast protective helmet; and determination of realistic contamination patterns provided by liquid explosive and spray dissemination, and exposure levels that would result in first responders in such a contaminated environment. Preliminary guidance to first responders on selection of respiratory and body protection will also be provided. Final recommendations for guidance and standards will occur in 2006 at the closeout of the project.

CRTI-0052TA

Rapid Carbon-14 Analysis by Accelerator Mass Spectrometry

PROJECT LEAD:
IsoTrace Laboratory,
University of Toronto

FEDERAL PARTNERS:
Health Canada,
Fisheries and Oceans

INDUSTRY PARTNERS:
High Voltage Engineering
Europa B.V.

AUTHORS:
Dr. Jack Cornett, Health Canada - Radiation Protection Bureau,
tel: (613) 952-9071,
email: Jack_Cornett@hc-sc.gc.ca;

Dr. W. E. Kieser, IsoTrace Laboratory, University of Toronto,
tel: (416) 978-2241,
email: Liam.Kieser@utoronto.ca.

Objectives

To provide equipment and to develop and test procedures for the rapid, sensitive and high throughput analysis of organic samples (especially those related to human health and the environment, e.g. in the food chain), so that the level of carbon-14 contamination which might result from a variety of CBRN events can be accurately determined. This will provide a capability for both assessing the extent of ^{14}C contamination in a particular area and for certifying the efficacy of remediation work. This project includes:

- The purchase of a high capacity CO_2 gas-fed ion source and its integration into the IsoTrace Accelerator Mass Spectrometry (AMS) system;
- The purchase and modification of an elemental analyzer to produce CO_2 from the environmental samples;
- The construction of a gas transfer line to provide purified CO_2 at the appropriate rate to the ion source; and
- The integration of the software controls of all components to facilitate automated analysis.

Recent Progress

The CO_2 gas-fed ion source was ordered on March 19 2003 and delivery is expected by April 30 2004. During a visit in November 2003 to Oxford University where the prototype for this source is installed, it was in routine operation for AMS ^{14}C analysis, having undergone several engineering improvements, which are included in the one being shipped to the project team. At IsoTrace, ion optical matching calculations have been completed, vacuum components to connect the source to the rotatable electric analyzer in the injection line are currently in production in the machine shop, and electrical and other components required to install the source have been delivered.

After careful examination of elemental analyzers from 5 manufacturers, visits to a number of sites where they are being used and telephone discussions with other users, it was decided that the Elementar vario ELIII was the most suitable for adaptation to the requirements of producing CO_2 for an AMS source. This unit and its ancillary equipment have been ordered and delivery is expected by April 23 2004. Space is being prepared for its installation in a sample preparation laboratory for the initial testing phase.

Hiring procedures for the chemical technologist who will be in charge of sample preparation, introduction and analysis and for the electronics technologist, who will provide the support for automating the locally built components and integrating them and the existing control systems, are approaching completion: a short list of candidates have been interviewed and the successful ones will begin work by April 12 2004.

When the ion source arrives and is connected to the AMS system, acceptance tests will be carried out, according to a protocol agreed on by High Voltage Engineering and IsoTrace. Concurrently, training sessions for the operation of the elemental analyzer will occur, the design for the gas transfer system, based on a similar system in operation at Oxford, will be finalized and components will be purchased or produced in local shops.

Following acceptance of the ion source, work will begin on the assembly and test of the gas transfer system and on the integration of the software controls for the three new systems with the current AMS control systems. Concurrently, Health Canada and Fisheries and Oceans will provide typical samples for testing and to check for any limitations in the elemental analyzer combustion system.

Future Outlook

Following connection of the elemental analyzer to the ion source and the control software integration, analysis protocols for the various types of samples will be developed and draft versions of these will be circulated to all project partners. After final revision and acceptance of these protocols, information will then be provided through HC-RPB and DFO-AERU to first responders and to those who will be involved in wide-area decontamination and remediation by seminars, workshops and specialized training sessions.

CRTI 0060TA

Rapid Triage Management Workbench (RTMW)

PROJECT LEAD:
WorldReach Corporation

FEDERAL PARTNERS:
National Research Council of Canada

AUTHOR:
Laura Brown

The Rapid Triage Management Workbench (RTMW) is a system designed to manage the communication of medical information during a chemical, biological, radiological or nuclear (CBRN) event. RTMW is designed to be particularly useful in mass casualty events. The system is portable and capable of being deployed in the field, in rural and urban settings, with a minimum of training. A medical data capture module provides first responders and medical caregivers in the field with the means to record casualties' medical information quickly and accurately. These data are then entered into a central database that allows other response team members to access it. The RTMW system can be used at any location that has an Internet connection or stand-alone capabilities in the event of an Internet failure.

Objectives

RTMW will enable the response team to function efficiently and effectively by making relevant medical information immediately available to all response team members. In particular, it will provide all members of the team with accurate and up-to-date information on casualties' current status. RTMW will be designed with an appropriate level of access security, to ensure the security and privacy of patient information stored in the database.

RTMW will provide multiple benefits:

- Rapid triage with up-to-date medical data will increase efficiency in patient care and transportation.
- Caregivers will provide effective and efficient care because the right people and equipment will have been triaged to where they can do the most good. This will minimize provider fatigue and unnecessarily prolonged exposure to potentially hazardous environments.
- Health care facilities will receive relevant patient data prior to admission.
- The safety of response team members will be improved as information on potential medical hazards is quickly communicated to all cluster members.
- The response team or relief agencies will be able to provide patients' families with accurate, up-to-date information.
- Public health organizations will have accurate information and will therefore be able to provide advice and directions to the public based on current data.
- RTMW will use the current triage marking system used by Emergency Medical Services; all first responders will be familiar with the RTMW triage system; therefore training is minimized.

- CBRN preparedness staff will use RTMW as a teaching tool in the art of triage. RTMW could also be part of first responder curriculum on a national level.
- By facilitating medical information exchange in a well-organized response, RTMW will maximize lead times for decision-making that can minimize the overall impact of the event.

Recent Progress

RTMW makes use of the Internet as well as the latest database technology. The hardware and software components have been selected so that standard PC / Windows equipment can be used, eliminating the need to procure and maintain special equipment for this system. The system has two major components: (1) a portable component used in the field, and (2) a stationary database accessible through the Internet.

RTMW is bilingual and includes an on-line help facility and supporting documentation. Further, RTMW is designed using user-centric design methods. The Human Oriented Technology (HOT) Lab at Carleton University applied proven techniques to identify requirements and design the interface.

The following have been completed:

- All project plans
- Critical Design Review
- Software Requirements Specifications
- Privacy Impact Assessment
- User Interface Design

To date, all milestones have been met.

Future Outlook

- **RTMW will be completed in the summer of 2004**
- **It will be used in a full-scale exercise in Ottawa in the fall of 2004**
- **A marketing team is currently developing a marketing strategy to integrate RTMW within the WorldReach Crisis Management product suite.**
- **Proposal in place for further funding to add software modules so as to expand the suite of CBRN Emergency Medical Information Management software tools.**

CRTI-0064RD

New Technologies for Surveillance of Biowarfare Agents and Identification of Engineered Virulence Genes

PROJECT LEAD:
The University of British Columbia

FEDERAL PARTNERS:
Health Canada - National Microbiology Laboratory and DRDC-Suffield

AUTHORS:

Julia Rathmann, Chad Malloff and Wan Lam, BC Cancer Research Centre, 601 West 10th Avenue, Vancouver, BC, V5Z 4E6,
tel: 604-877-6149,
email: wanlam@bccrc.ca.

Steve Pleasance and Rachel Fernandez, Microbiology & Immunology, University of British Columbia, #300-6174 University Blvd, Vancouver, BC, V6T 1Z3,
tel: 604-822-6824,
email: rachelf@interchange.ubc.ca.

Louis Bryden, Shannon Hiebert and Michael Mulvey, Canadian Science Centre for Human and Animal Health, 1015 Arlington St., Winnipeg, MB, R3E 3R2,
tel: 204-789-2133,
email: Michael_mulvey@hc-sc.gc.ca

Yimin Shei and Barry Ford, DRDC Suffield, PO Box 4000 Station Main, Medicine Hat, Alberta, T1A 8K6,
email: barry.ford@drdc-rddc.gc.ca.

Objectives

An innocuous bacterium becomes a lethal weapon by the introduction of a virulence gene. The technology for gene transfer in organisms such as *Bacillus anthracis* and *Yersinia pestis* has existed for over a decade, and therefore there is an urgent need to develop the capability to identify introduced virulence genes in engineered biowarfare strains. Current methods lack resolving power to find unknown insertions. Whole-genome sequencing of all suspected biowarfare agents is impractical, and microarray-based approaches, while powerful, are limited to genes present in the reference strains. The project team will adapt its novel DNA scanning technology, which couples the resolving power of two-dimensional DNA electrophoresis with comparative genomic hybridization, to rapidly identify engineered genes. This technology is called Bacterial Comparative Genomic Hybridization or BCGH. To identify an unknown virulence gene using BCGH, the engineered biowarfare strain harbouring the novel gene is compared against a related lab reference strain. DNA fragments from the two strains are combined, displayed in 2-dimensions, blotted onto a membrane, and sequentially probed (hybridized) with DNA from each individual strain. The engineered gene is identified as a novel spot(s) that can be excised from a parallel gel, cloned and sequenced to reveal its identity. This information can be used to tailor therapy and develop surveillance strategies.

- The project team will profile *Bacillus anthracis* (anthrax), *Yersinia pestis* (plague), *Francisella tularensis* (tularemia), *Burkholderia pseudomallei* (melioidosis), and *E. coli* O157, *Salmonella typhi, Shigella flexneri* and *Yersinia enterocolitica* (the food-borne pathogens). Restricted pathogens and BioSafety Level 3 organisms will be cultured by NML and DRDC. For these, DNA only will be provided to the UBC labs.
- Display parameters (fragmentation conditions, gel composition, temperature, time, etc.) will be determined empirically for each of the organisms. The sensitivity, quality assurance and quality control of BCGH will be assessed using a panel of spiked genes representing a spectrum of sequence composition.
- Technology transfer will be jointly executed between the University and Federal project participants.
- Standardization and refinement at Federal sites will be carried out by NML and DRDC.

- State of the art software (BioNumerics from Applied Maths) will be used to analyze and archive the 2D-DNA profiles, as well as to communicate between the partner laboratories.
- Expected project completion date is December 2006.

Recent Progress

- Personnel at DRDC and NML have completed Biosafety Level 3 training.
- NML and DRDC labs have isolated *Y. pestis* and *B. anthracis* DNA for UBC labs.
- A technical workshop was held at UBC in October 2003.
- NML and DRDC labs have established functional 2D DNA facilities, and personnel have been trained.
- In-house software developed at UBC is being used and adapted to predict optimum DNA display parameters *in silico* based on sequenced genomes of biowarfare strains.
- Display parameters have been developed for DNA of lower G+C content at UBC.
- DNA displays have been generated for *Y. pestis, B. anthracis* and *Y. enterocolitica*.
- Non-radioactive imaging protocols are being developed at the NML and UBC sites.
- Analysis and archiving of 2D DNA displays has been initiated and will be on-going.
- BioNumerics software has been evaluated at NML and found to be useful.
- Progress and results were presented at:
  - the American Society for Microbiology Northwest Branch Meeting in Vancouver, BC in August 2003 ("Profiling Bacterial Genomes to Identify Acquired Genes")
  - the American Society for Microbiology Biodefense Meeting in Baltimore, MD in March 2004 ("Two-Dimensional Bacterial Genome Display for the Identification of Engineered Genes").

2004-2005:

- **DNA will be isolated from *S. typhi, S. flexneri* and *E. coli* O157.**
- **DNA displays will be generated for *S. typhi, S. flexneri* and *E. coli* O157.**
- **BCGH conditions will be tested using a panel of spiked genes and constructs.**
- **Clinical isolates will be compared using BCGH for validation purposes.**
- **Standardization and quality control of 2D DNA display and BCGH will be conducted.**
- **Analysis and archiving of 2D DNA displays will be on-going.**

Future Outlook

Expected final outcomes:

- **Completed 2D display and BCGH analysis of *Y. pestis, B. anthracis, F. tularensis, B. pseudomallei, S. typhi, S. flexneri, E. coli* O157, and *Y. enterocolitica*.**
- **Completed protocol standardization and technology transfer to NML and DRDC including streamlining of protocols for routine use in diagnostic and forensic laboratories.**

In a bioterrorism event, the identification of engineered genes will facilitate diagnosis, surveillance, vaccination, and therapeutic measures that can be targeted at the virulence gene or gene product to control disease outbreaks.

CRTI 0072RD

Nanodosimeters Based on Optically Stimulated Luminescence

PROJECT LEAD:
DRDC Ottawa

FEDERAL PARTNERS:
Health Canada

UNIVERSITY PARTNERS:
University of Toronto

INDUSTRY PARTNERS:
Bubble Technology Industries Inc.

AUTHOR:
Lorne Erhardt, DRDC Ottawa,
3701 Carling Ave. Ottawa ON,
tel: (613) 991-5900,
email: Lorne.Erhardt@drdc-rddc.gc.ca

Objectives

In the event of a radiological incident, tracking the spread of radioactive material will be of utmost importance. Information on the distribution of contamination will be required both to guide evacuation and to plan responses while minimizing the risk to all involved. The aim of this project is to create small, low-cost dosimeters that could be quickly and easily deployed by the thousands over a contaminated area, providing a detailed map of the contamination pattern.

Early work in this area pointed to the use of Optically Stimulated Luminescence (OSL) as the most promising technology for such a dosimeter. The dosimeter design consists of a single crystal of OSL material (which acts as the detector's sensitive volume), a laser diode to stimulate emission from the crystal and an avalanche photodiode to detect the emission. This design lends itself well to miniaturization and system-on-chip architecture. It is felt that the dosimeter's sensitive volume can be miniaturized to nanoscale proportions and integrated with control, read-out and communication electronics. With these detectors, multiple probes could be spread over a contaminated area and could track the contamination in real time. Simulations have shown that this approach would produce extremely accurate contamination mapping and would be very robust: the set of detectors would survive even if there were a large number of individual failures.

The project is designed to follow two separate but related tracks: Bubble Technology Industries Inc. (BTI) is designing the dosimeter and associated electronics and the University of Toronto Electronic-Photonic Materials Group (EPMG) is taking the BTI design and adapting it to a chip-level design. This project will result in two prototype dosimeters. The first, fabricated by BTI, will be a completed and tested prototype minidosimeter with integrated control, read-out, communications and GPS electronics. The second will be a prototype nanodosimeter (produced by EPMG) based on the BTI design and tested with the minidosimeter electronics.

Recent Progress

As it stands, the project is slightly ahead of schedule and a great deal of progress has been made on the prototype dosimeters, both at BTI and at the University of Toronto. Efforts at BTI were focused on the production of the minidosimeter prototype, while EPMG has been designing a custom avalanche photodiode for use with the nanodosimeter.

A prototype minidosimeter was designed and constructed at BTI, and tested for its response to ionizing radiation. After this characterization phase, the efforts at BTI turned to the integration of all the required electronics with the sensitive portions of the dosimeter. The vast majority of the effort in the past year has been in deciding on the communications architecture and integrating the appropriate electronics into the minidosimeter system. Communications hardware was purchased and custom software was written to allow data from the minidosimeter to be transferred on a wireless network. In addition to the communications, a single-board 18 parallel-channel GPS receiver module with small footprint was integrated into the minidosimeter circuit board, which allowed minidosimeter position data to be transferred on the wireless network along with the dose information. A plug-and-play, self-organizing, self-healing network of minidosimeters has been created. Multiple communications channels (such as telephone, cell phone, PDA, satellites, wireless modem, airborne platform) from the access point can be utilized to ensure deployment in any and all environments (urban, rural and remote). The network management and traffic can be accessed locally or remotely. Together, these achievements result in a prototype minidosimeter with all the associated electronics that has been demonstrated to operate as required by the project team. This was a key milestone in the project and has been successfully met.

Research at the University of Toronto over the past year has focused exclusively on the development and fabrication of an avalanche photodiode (APD), which can be operated in the Geiger mode, with spectral response tailored to the output of the OSL material. This required a thorough review of the current scientific literature to determine the status of Geiger mode avalanche photodiodes in the visible wavelength range. Particular attention was paid to the identification of suitable materials, structures and techniques to fabricate such a photodiode. A model was developed to simulate the separate absorption and multiplication regions of the APD design; the associated process flow was also developed. A series of structures were prepared by molecular beam epitaxy (MBE) in order to reveal key design variables influencing optical and electronic properties of structures. Measurements of the compositional, structural, electrical and optical properties of these structures were made to establish feedback to growth and processing steps. A prototype avalanche photodiode structure was constructed and characterized, and its response was determined to be in the desired wavelength range. This was viewed as a critical step for the eventual production of a nanodosimeter, and with its completion a key milestone has been successfully achieved.

Future Outlook

The next steps for this project involve the refinement of both the prototype minidosimeter and the avalanche photodiode. BTI will be incorporating the minidosimeter's sensitive volume and all the electronics into a small package to allow it to be used autonomously. This milestone in on-track to be completed, ahead of schedule, by December 2004. The prototype minidosimeter will then be tested extensively by BTI, DRDC Ottawa and Health Canada to determine its utility. At the University of Toronto, the next phase will be focused on a complete assessment of the performance of the prototype APD device and its refinement. Following this will be the development of an appropriate light emitter for the nanodosimeter read-out, integration of the APD and light source with the OSL material, and finally integration of the BTI designed electronics with the aforementioned devices. This will result in a prototype nanodosimeter and should be completed, on schedule, by July 2005.

CRTI 0080TA

ARGOS Decision Support System for Radiological-Nuclear Emergency Management

PROJECT LEAD:
Health Canada (Radiation Protection Bureau / Nuclear Emergency Preparedness and Response Division)

FEDERAL PARTNERS:
Environment Canada (Canadian Meteorological Centre / Environmental Emergency Response Division), Natural Resources Canada (Radiation Geophysics), Health Canada (Radiation Protection Bureau / Environmental Radiation Hazards Division)

AUTHOR:
Brian Ahier, Health Canada (Nuclear Emergency Preparedness and Response Division),
tel: (613) 954-6674,
email: brian_ahier@hc-sc.gc.ca

Objectives

National and international benchmarks, reports, and exercises have shown that in a serious radiological or nuclear (RN) event, coordinated information and actions are critical to achieving effective emergency response. Under Health Canada's lead, the Federal Nuclear Emergency Plan (FNEP) provides the national preparedness and response framework for RN emergencies affecting Canadians, supports the provincial response, and provides the radiological consequence management framework in support of Canada's National Counter-Terrorism Plan. FNEP emergencies involve 20+ federal organizations, many of which are responsible for key consequence assessment data. Robust data management tools are required to integrate and assess this data, and support decisions on response measures.

In this project, Health Canada's Nuclear Emergency Preparedness and Response Division is collaborating with Environment Canada - Canadian Meteorological Centre (CMC) and other key federal and international partners to implement the ARGOS RN Decision Support System in Canada as an operational FNEP response capability. ARGOS is made available through partnership with the Danish Emergency Management Agency and Prolog Development Center A/S (PDC). Canadian implementation of ARGOS involves core software enhancements in order to interface with Canadian meteorological and radiological surveillance, monitoring, modelling and forecasting data sources and capabilities. CMC is accelerating development and distribution of local and regional meteorological modelling capabilities supporting RN emergency response. Health Canada is also working with other FNEP partners, including Natural Resources Canada - Radiation Geophysics and Health Canada - Environmental Radiation Hazards to integrate aerial and fixed point surveillance and laboratory sample analysis data.

This project will result in an operational decision support system that will facilitate a fast, coordinated response to an RN incident, improved emergency data management and effective decision making supporting first responders, the operational community, and the public. To date, it has resulted in a new level of data integration for RN emergency response, new methods of data delivery, and advances in atmospheric emergency modelling capabilities that are being leveraged for other emergency groups.

Recent Progress

Progress on this project began with completion of the Project Charter in December 2002, the first within the CRTI framework. Following membership of Health Canada in the ARGOS Consortium of member countries and the negotiation of contracts with the industry partner, design meetings were held to review Canadian data sources and specifications, and determine the design specifications for the project phases. Phase 1 covered the development of ARGOS data import facilities and modules for Canadian data. Phase 2 focused on the interface with CMC modelling capabilities, including atmospheric plume trajectories and dispersion modelling. Phases 3 and 4 address the interface with Health Canada's fixed point gamma surveillance network and Laboratory Information database, and CMC's advanced short-range atmospheric dispersion models.

To date, PDC and CMC have collaborated on interfacing Canadian meteorological resources with the ARGOS core software. PDC and Health Canada have worked on the enhancement of system functionality. CMC has worked in parallel on the delivery of key meteorological data to the ARGOS system, and on enhancements to their atmospheric modelling capabilities, which are used directly within ARGOS. Health Canada has continued work on the operational implementation of the ARGOS system and associated computer infrastructure within its offices, model verification, and the interface with web-enabled GIS capabilities for rapid data exchange between FNEP partners.

ARGOS is now installed and operational on Health Canada's dedicated infrastructure, with the following core capabilities:

- Automatic synchronization with a CMC weather server for import of current and forecast meteorological data
- On-demand CMC atmospheric model requests launched from within ARGOS, and linked to user-supplied source term information
- Short-range dispersion and dose model linked to CMC meteorological files
- Dispersion model outputs linked to internal dose pathways models
- Import of NRCan aerial surveillance data
- Link to Health Canada's fixed point gamma surveillance network
- Visualization of results (by isotope, time step and output type); and
- Local data replication allowing core operations in case of communications failure

Future Outlook

The goal of this project is to implement ARGOS as an operational federal emergency response tool within Health Canada, and to make it available to the FNEP response organization, by the end of 2004. The next steps are to complete development and interface with the short-range particle model, fixed point surveillance and laboratory results, and integrate into FNEP emergency procedures. CMC is also working, through other CRTI projects, on urban dispersion and improved deposition models. When these are completed, they will be accessible to ARGOS through a single consistent interface, thereby extending the system capability well beyond the original project scope. Finally, access to the ARGOS outputs will be made available to FNEP partners through the FNEP Emergency Communications Website and the EMAP-Nuclear GIS web-distribution system, developed and implemented in partnership with Environment Canada – Atlantic Region.

CRTI 0085TA

Evaluation of GM-CSF for Acute Radiation Syndrome

PROJECT LEAD:
Cangene Corporation

FEDERAL PARTNERS:
Health Canada

AUTHORS:
Darin Lee, Vadim Tsvetnitsky, Donald IH Stewart
email: dstewart@cangene.com,
3403 American Drive,
Mississauga, Ontario,
L4V 1T4, Canada.
tel: (905) 405-2930.

Objectives

Radiation overexposure is considered a potential threat to both civilian and military personnel in various circumstances. It may also be envisaged that deliberate exposure in a military or terrorist situation is of reasonably high probability due to proliferation of global nuclear capacity and traffic in spent nuclear fuels.

The primary goal of this project is to demonstrate the utility of Cangene's pipeline GM-CSF product LEUCOTROPIN™ for haematopoietic reconstitution following radiation-induced bone marrow aplasia in rhesus macaques exposed to uniform total body x-irradiation. The primary application for the drug in radiation exposure would be for the early treatment of patients exposed to low to medium dose radiation. However, the drug may also be useful for protection of individuals likely to be exposed to radiation such as rescue workers and others. The study utilizes cynomolgus monkeys as a model animal and results would suggest optimal treatment regimes for affected humans.

An important part of this project involves development and evaluation of a longer acting form of GM-CSF, produced by modifying the protein with polyethylene glycol (PEG), which may offer less frequent and therefore more convenient dosing regime for the patients. PEGylated proteins may have a number of advantages over their unmodified counterparts including increased half-life, reduced antigenicity and improved solubility.

Recent Progress

To achieve these goals, Cangene has produced LEUCOTROPIN™ according to its established cGMP process at 2,100 L scale in the company's manufacturing facility. Three lots of freeze-dried and liquid formulations were prepared and made available for evaluation in the animal studies, which were initiated on April 01, 2004 at the University of Maryland and are scheduled to run for 90 days. Two treatment protocols are being assessed where GM-CSF is administered either early on (within 20 hours) or with a delay (by 3 days) after x-irradiation, allowing evaluation of two likely real-life scenarios.

We have prepared laboratory-scale quantities of GM-CSF conjugated to 20 kDa monomethoxypoly (ethylene glycol) (mPEG) and assessed the effect of PEGylation on *in vitro* cell proliferation using the TF-1 myeloid progenitor cell line. Somewhat contrary to expectations, attachment of a single mPEG molecule per protein (monoPEGylated GM-CSF) did not lead to any loss of *in vitro* activity when compared to the nonPEGylated form, while as anticipated both di- and tri-PEGylated GM-CSFs forms exhibited three- to ten-fold reductions in activity respectively.

We have completed a larger scale (100 mg) synthesis, purification and characterization of monoPEGylated 20 kDa mPEG-GM-CSF. These materials will be used to perform additional investigations of *in vitro* liquid formulation stability and *in vivo* serum stability. It is anticipated that a successful demonstration of enhanced serum half-life in the *in vivo* studies will lead to future studies of mPEG-GM-CSF in the treatment of acute radiation syndrome.

Future Outlook

Upon the completion of the radiation protection study scheduled for the end of July 2004, Cangene plans to review generated data in consultation with the Radiation Protection Bureau, Health Canada, and prepare supplemental New Drug Submission (NDS) should the results be judged positive. This should be completed as planned by the end of October 2004.

CRTI-0087RD

Therapeutic Antibodies to Ebola and Marburg Viruses

PROJECT LEAD:
Health Canada

FEDERAL PARTNERS:
Canadian Food Inspection Agency

INDUSTRY PARTNERS:
Cangene Corp., University of Alberta

AUTHORS:
Vadim Tsvetnitsky, Eric Wiersma, Donald IH Stewart, Heinz Feldmann, Mavanur Suresh and Steven M. Jones
email: Steven_jones@hc-sc.gc.ca,
1015 Arlington Street, Winnipeg, R3E 3R2, Canada.
tel: (204) 789-5065

Objectives

The filoviruses Ebola and Marburg are Category A Biological Agents as defined by Centres for Disease Control (CDC). These viruses cause an acute hemorrhagic fever in man with a mortality rate ranging from 23-90% and are prone to human-to-human transmission. The Marburg virus was weaponized in the former USSR and a Japanese sect recently attempted to obtain the Ebola virus for use in a bio terrorist attack. As neither therapeutic nor prophylactic treatments are available for these filoviruses, they present a great risk to both military and civilian populations.

Therapeutic antibodies have been suggested by many in the field to be the most promising strategy at present (Bray and Paragas, 2002), as this approach provides a short-term strategy, something which is urgently required to address the CRTI priority of therapeutic measures for immediate reaction and near term consequence management capability. Passive protection against a lethal Ebola virus challenge has been demonstrated in small animals (Wilson, *et al.*, 2000; Takada *et al.*, 2002) and post-exposure treatment with convalescent plasma seemed to have a protective effect in humans (Mupapa, *et al.*, 1999). A challenge in developing effective neutralizing antibodies has been the limited knowledge on viral proteins targeted by the host immune response. However, recent studies have developed a better understanding in this area and determined that the transmembrane glycoproteins (GP) are the key targets.

In this project, different forms of the viral transmembrane glycoproteins will be used as immunogens for the development of therapeutic monoclonal and polyclonal antibodies. In a first step, caprine/ovine polyclonal antibodies will be raised and tested for efficacy and safety in Ebola and Marburg protection models (mice, guinea pigs). The caprine/ovine polyclonal antibody approach will provide a supply of therapeutic drug for short-term delivery. To provide a long-term solution, recombinant monoclonal antibodies will be developed for both Ebola and Marburg viruses. While the lead time to generation of the recombinant antibodies will be longer than for the caprine/ovine antibodies, the recombinant products will be of better-defined specificity and will be available for indefinite supply.

Recent Progress

To achieve these goals, the project team has successfully rescued, using a technique called reverse genetics, recombinant vesicular stomatitis virus (VSV) vectors containing the glycoproteins of Ebola (VSV-EBOVGP) or Marburg (VSV-MARVGP). These viruses will be used to immunize goats for the production of polyclonal neutralizing serum and mice for the production of neutralizing monoclonal antibodies. In a separate study not funded by CRTI, mice were immunized with the VSV-EBOVGP vaccine and were completely protected from challenge with 1 million lethal doses of Ebola virus. Splenic RNA from mice surviving challenge was sent to the University of Alberta and has been used to develop immune mouse phage display of FAb. Two independent complete phage library constructions were demonstrated. The First Ebola-biased Fab antibody phagemid library was generated with an estimated complexity of $7x10^7$ clones. The second independent Ebola-biased Fab antibody phage library has been generated with an estimated complexity of $6x10^7$ clones. These libraries will be the basis of selecting potentially useful anti-Ebola GP 1,2 antibodies. The phages are currently being panned against VLPs and mock membrane fraction prepared by NML Winnipeg. The Fab-expressing phagemid library was used for two rounds of panning against these targets and enriched antigen-specific Fab phage particles were isolated. Phage particles were expanded and prepared for binding studies, which are still in progress. Cangene has produced a naïve human Fab phage library with an estimated complexity of $1x10^{10}$ clones and is currently panning this library against both a transmembrane region deleted mutant of Ebola GP and the secreted form of Ebola GP (sGP).

Future Outlook

Once the goat antiserum has been produced, Cangene will use proprietary technology to produce a hyperimmune serum, of clinical grade, for testing in *in vivo* models of Ebola and Marburg hemorrhagic fever. The panning of the phage libraries will be followed by production of recombinant antibodies, human and murine, which will be tested *in vitro* and *in vivo*. The aim of the project is to develop a panel of monoclonal antibodies, which can be used in combination to treat humans infected with either Ebola or Marburg. The next step will be to bring these products into clinical use.

CRTI-0091RD

The Development of Recombinant Monoclonal Antibodies for the Treatment and Detection of Bio-Terrorism (BT) Agents

PROJECT LEAD:
National Microbiology Laboratory, Health Canada

FEDERAL PARTNERS:
DRDC Suffield, National Centre for Foreign Animal Diseases (NSFAD) – Canadian Food Inspection Agency.

AUTHORS:
Dr. Amin Kabani

Objectives

This project is focused on the development of protective and diagnostic monoclonal antibodies for the detection, prophylaxis and post-exposure treatment of bacterial and viral agents. The project is initially limited to antibody development for Alphaviruses, Foot-and-mouth Disease (FMD) Virus, and Anthrax. However, the knowledge gained in this project will advance vaccine design for other potential agents of bio-terrorism and infectious pathogens in general of both humans and animals.

In case of a terrorist event, the public health system must deal with thousands, or even hundreds of thousands, of potentially exposed persons. Effective post-exposure treatment is required. At present, antibiotics are available only for some bacterial agents and must be administered within hours after exposure to be truly effective. Supportive treatment is all that can be offered for many of the viral agents. A cocktail of recombinant monoclonal antibodies can provide immediate protection to both bacterial and viral agents. However, to use antibodies as post-exposure treatment, rapid methods must be in place to correctly identify the microbial agent involved, since the right choice of antibodies must be used in order to be effective. Therefore, this project addresses the development of monoclonal antibodies for specific detection and identification of BT agents as well as for treatment purposes. Besides the human pathogens of anthrax and Alpha viruses, this project also addresses the need to develop rapid diagnostic reagents for the identification of an important animal pathogen, FMD Virus (Foot-and-Mouth Disease), which can cause an enormous economic impact in the Canadian livestock industry. An outbreak of FMD in Canadian livestock will require many tests to be performed before the re-establishment of international livestock trade. Validated tests with monoclonal antibodies will help to speed up the recovery of such an outbreak.

This project aims to:

- Develop monoclonal antibody-based treatments for the BT agents of anthrax and Alpha viruses (Venezuelan Equine Encephalitus [VEE], Western Equine Encephalitus [WEE], and Eastern Equine Encephalitus [EEE]).
- Develop monoclonal antibody-based rapid diagnostic reagents for BT agents of anthrax, Food-and-Mouth Disease virus and Alpha viruses.
- Identify candidate microbe components for vaccine development against BT agents (anthrax, Alpha viruses and Food-and-Mouth Disease virus).

Recent Progress

Work is progressing as planned in all six phases of the project:

Phase 1. Immunogen design: Initial design completed and vaccines prepared for immunization of animals to produce antisera and antibodies. Work to refine the design of better immunogen is expected to be carried out throughout the project as new information is gained from the research.

Phase 2. Antigen production: Recombinant anthrax toxins are being produced at the University of Toronto via a contract awarded to Dr. Jeremy Mogridge. FMD virus antigens have been produced and tested at CFIA-NCFAD. The protective domain of the anthrax PA toxin component has been successfully expressed by a new recombinant DNA method at the DRDC-Suffield laboratory and the product is being tested for various practical applications for diagnosis and vaccine development.

Phase 3. Hybridoma monoclonal antibody production: Monoclonal antibodies to different anthrax toxin components, FMD virus, and synthetic peptides that represent parts of the anthrax toxin and FMD virus have been produced and are being characterized. In some cases, antibodies produced to synthetic peptides have been found to recognize either the natural anthrax toxin or the FMD virus.

Phase 4. Recombinant antibody production/construction and testing: Antibody fragments to different Alpha viruses have been produced by genetic engineering methods at the DRDC-Suffield laboratory and such reagents are being tested for potential application in diagnostic tests. Methodologies for production of "humanized" antibodies by genetic engineering approach is being studied at the CFIA-NCFAD laboratory.

Phase 5. *In-vitro* assay development: Various enzyme immunoassays for detection and identification of anthrax toxin, FMD and alpha viruses are being developed in all three participating federal laboratories.

Phase 6. Vaccine development: Serological response of human volunteers immunized with the licensed anthrax AVA vaccine is being studied. The serum antibody response of cows and monkeys immunized with the live attenuated spore anthrax vaccine is being evaluated at CSCHAH. Mapping of antibodies to anthrax toxin and FMD virus to identify what they recognize is being done by immunologists at the CSCHAH. Previously unknown sites on the anthrax toxin were identified as potential vaccine candidates for the development of next generation of subunit synthetic peptide vaccine.

CRTI-0100TA

Systems Level Simulant Test Chamber for CB Personal Protective Ensembles and Equipment, with an Articulated Mannequin Capability

PROJECT LEAD:
Director Science and Technology Human Performance, Defence R&D Canada

FEDERAL PARTNERS:
Director Nuclear Biological Chemical Defence and DRDC Suffield, Royal Military College, Department of National Defence

INDUSTRY PARTNERS:
Amtech Aeronautical Ltd.

AUTHORS:
Dr. Scott Duncan, Head, Physical Protection Group, CBDS, DRDC Suffield;

Dr. Alex Markov, Amtech Aeronautical Limited,

Mike Greenley, Greenley & Associates;

Ted Timmons, Greenley & Associates;

Dr. Eva Dickson, Chemistry Department, Royal Military College;

Maj Pierre Caron, Directorate Nuclear Biological Chemical Defence, DND;

Ken Torrance,
Amtech Aeronautical Limited,
tel: (403) 529-2350,
email: ken.torrance@amtech-group.com

Julie Tremblay-Lutter,
DRDC/DSTHP 4,
tel: (613) 995-7627,
email: Julie.Tremblay@drdc-rddc.gc.ca;

Dr. Ken Johnson, DRDC/DSTHP,
tel: (613) 992-2877.

Objectives

This CRTI Technology Acceleration project will establish a world-class CB test and evaluation facility, known as the CB^{plus} Chamber, at DRDC Suffield. The Chamber will enable the first responder and military communities to research, develop and evaluate complete ensembles of CB Personal Protective Equipment (PPE) against liquid, vapour and aerosol CB hazards utilizing non-toxic organisms and chemical compounds as simulants. In addition, the Chamber will be used by government and industry to develop new PPE concepts and materials.

Systems under test will be worn by an articulated mannequin, representing human anthropometric body shape that will mimic human movements. A separate headform that simulates human facial movements is also included for testing integrated CB headwear systems.

The CB^{plus} Chamber establishes a number of Canadian firsts in systems level CB PPE research, development and evaluation, including:

a. The capability for testing over a broad range of controlled temperature (+5 to +50°C), humidity (10 to 90% relative humidity) and air flow (wind speed) (up to 25 km/hr).

b. The capability for testing with chemical simulants in vapour, aerosol and liquid form.

c. The capability for testing with chemical and biological simulants in the same chamber.

d. A state-of-the-art mannequin that simulates body movements such as walking, running and bending at the waist.

e. A state-of-the-art headform.

Automated Chamber operations will permit precise, repeatable control over simulant release and environmental conditions while the mannequin and headform will provide reproducible body and facial movements. This precision and repeatability will enhance existing test capabilities and also enable the certification of PPE for the first responder and military communities. Of particular interest will be the ability to conduct studies of longer duration (6-12 hours) that are required for certification, yet not possible with human volunteers.

The Chamber will also allow government PPE procurement teams to practice simulation-based acquisition, using the Chamber to confirm PPE requirements, evaluate potential PPE suppliers and conduct final acceptance testing of PPE. Similarly, industrial PPE providers will be able to access the Chamber in support of their internal R&D programs and to achieve product certification.

The CB^{plus} Chamber is an integral component of collaborative efforts with two current CRTI projects - 00161TA CBRN Blast Protective Helmet (Med-Eng Systems Inc., Ottawa, ON) and 0029RD New Standards for Broad Spectrum PPE for First Responders (RMC, Kingston, ON).

Recent Progress

Progress since the last CTRI Symposium, in June 2003, has been substantial and includes the successful accomplishment of a number of key tasks and milestones, including the following:

- Chamber design/build contract award
- Mannequin requirements complete
- Chamber specification approved by DRDC
- Environmental Assessment (EA) complete and approved by DRDC-S Environmental Officer
- Mannequin contract award
- Chamber design complete and approved by DRDC
- Subsystem construction started
- Building construction started

Recent technical achievements have included:

- A three-dimensional computational fluid dynamics analysis of the air velocity in the test section demonstrating that a uniform turbulent air velocity profile can be obtained within the required tight tolerances in a ductwork system that is substantially less than ideal.
- Materials tests identifying several previously untested materials that are resistant to and do not absorb the chemical simulant. Such materials are necessary to provide an ultra low background level of simulant, especially during application and removal of the simulant dosimeters worn under the PPE.

Future Outlook

The project is progressing as planned with only small changes to the schedule arising to date. The deliverables for this project include the following equipment, documentation and services:

- **Commissioned, fully operational CB^{plus} Chamber**
- **Chamber Operator's Manual**
- **As-built construction drawings**
- **Final Report documenting the work performed under the contract**
- **Technical support for the two collaborative CRTI projects**

The CB^{plus} Chamber will be a leading Canadian capability in the research, testing, evaluation and certification of CB PPE for both first responders and the military. The CB^{plus} Chamber will ultimately provide higher performance CB protection to first responders and the military, reducing the casualty rate in the event of a CB terrorism incident.

CRTI-0105TA

Real Time Threat Imaging with CBRN Sensor Networks

PROJECT LEAD:
McFadden Technologies

FEDERAL PARTNERS:
Health Canada, RCMP

AUTHOR:
Dr. Robert McFadden

Objectives

The early and sensitive detection of terrorist agents offers significant opportunities for enhancing the security of the responders community through attack interdiction, consequence mitigation and incident management. Practical realization of these opportunities requires mature technologies for gathering the large volumes of terrorist agent sensor data in real time and for making these data available to operations managers and decision makers as usable information.

Recent Progress

The operational technologies of CRTI 0105TA, the Mobile Real Time Radiation Surveillance Network, provide open architecture support for a network of mobile radiation (gamma and neutron) sensors with bidirectional real time communication to a central server over both wide area and local wireless networks as well as Ethernet. The network also provides for static Vital Point or chokepoint sensors as well as chemical and biological sensors. Sensor data is associated with GPS data and time stamp. Sensors and the supporting electronics are suitable for both vehicle and person-carried operations and make possible the covert surveillance of a public event and protection of the area. Reliable, consistent data reporting is managed by the system. Vehicle-based operations have no impact on routine patrol operations. Open software architecture supports a variety of database, query, GIS, mapping, aerial and satellite image software applications.

The large volume of network data is transformed into information congruent with RCMP Communications and Operations Centres procedures for incident identification and management. Innovative threat imaging software supports incident analysis and management and off line training and simulations. Novel signal detection technology characterizes the urban radiological environment and excursions from normal.

Future Outlook

The operational experience with the implementation of a mobile network of radiation sensors based on the RCMP patrol of the National Capitol Region and the extension to static and chemical sensors is demonstrated and discussed.

CRTI-0120RD

Development of a Novel Molecular Imprinting Methodology for Sensing Applications

PROJECT LEAD:
National Research Council of Canada

FEDERAL PARTNERS:
Department of National Defence, Directorate of NBC Defence;
Defence R&D Canada - Suffield

AUTHORS:
Karim Faid, Raluca Voicu, Abdi Farah, Christophe Py and Raluca Barjovanu, NRC- Institute for Microstructural Sciences;

Farid Bensebaa, Kidus Tufa and Zhao Li, NRC- Institute of Chemical Processing and Environmental Technology;

Jolanta Lagowski and Dumitru Pavel, Memorial University of Newfoundland;

Jean-François Legault, Department of National Defence, Directorate of NBC Defence;

Carmela Jackson Lepage, Department of National Defence, Defence R&D Canada - Suffield

Objectives

The principal objective of this project is to enhance the capabilities of first responders or military personnel to determine the presence of hazardous chemical compounds in the environment. This will lead to the development of portable and direct sensing devices capable of equipping first responders in their interventions and helping them in their training. The use of innovative imprinting techniques to deposit artificial recognition elements on selected surfaces will produce robust and affordable devices adaptable to a variety of detection and identification purposes. Arrays of chemically and spatially-resolved functional groups have been imprinted onto substrate surfaces, enabling the recognition of complementary molecules.

Molecular imprinting is an emerging technology based on the use of artificial recognition elements. These artificial recognition elements provide an alternative to the use of the somewhat fragile elements (such as enzymes, proteins or antibodies) used in traditional sensing devices, which lack storage and operational stability. Standard molecular imprinting is a process by which functional monomers are allowed to self-assemble around a template molecule and are subsequently crosslinked into place. The template is encapsulated in a stable three-dimensional polymer matrix. The template molecule can then be removed, leaving behind a cavity that will bind molecules identical to the template molecule. The imprint is like a lock that is only compatible with the correct key, similar to biological systems, such as enzymes and substrates, antibodies and antigens, and hormones and receptors.

Recognition between a molecular receptor (host) and a substrate (guest) in a matrix containing structurally related molecules requires discrimination and specific binding; this can happen only if the binding sites of the host and guest molecules complement each other in size, shape, and chemical functionality. When these arrays are coupled with sensors employing standard surface analytical or photonic techniques, targeted species will be detectable and identifiable in real time. Moreover, this technology, through the control of surface chemistries, may find application in various other sectors, such as in the pharmaceutical and biotechnology industries.

A research methodology has been developed to carry out this research project. The NRC-IMS and NRC-ICPET teams are jointly developing the polymer materials to be used as recognition templates. NRC-IMS is carrying out the chemical patterning and molecular recognition parts of the project. The fabrication and characterization facilities of both IMS and ICPET are being used. Computer simulation studies are carried out at MUN with inputs and feedbacks from both DND and NRC-IMS.

Recent Progress

The proof of concept of 2D Molecular Imprinting has been made.

The development and characterization of the model system, selected to perform the proof-of-concept, has been carried out. Multi-functional monomers and polymers have been designed, synthesized, characterized and used for the development of the surface synthetic recognition cavities. The selective rebinding of the molecular target has been performed and probed using standard surface characterization techniques, such as X-ray Photoelectron Spectroscopy (XPS), Grazing Angle Fourier Transform Infra-Red (GA-FTIR), Atomic Force Microscopy (AFM) and Fluorescence microscopy. The patenting of this methodology is being pursued and a first manuscript has been submitted for publication.

The main strength of this proposed methodology is the integration of the recognition and detection subsystems on a chip. The incorporation on a chip of the chemical and/or biological recognition elements along with the analytical element (such as micro-photonic analytical method) will allow the development of self-contained, compact, robust real-time sensing devices. Fine tuning of the various materials and processes will be continued, so that the specific and selective detection and recognition of untagged molecular target will be achieved. The design and development of an analytical system capable of detecting the rebinding of untagged molecules will be started. The development of a virtual library of host-guest interactions involving real agents will be carried out.

Future Outlook

The main outcome of this project is to provide enabling technologies for use in building adequate prevention, surveillance and warning capabilities. Moreover, the availability of such real-time sensing and screening devices may have a direct effect on public confidence through the given reassurances that potential threats can not only be handled efficiently but also prevented through the use of state-of-the-art detection technologies. The proof-of-concept phase of this project is to be completed by December 2005.

CRTI 0131TA

HI-6 Nerve Agent Antidote System

PROJECT LEAD:
Defence R & D Canada

FEDERAL PARTNERS:
Department of National Defence

INDUSTRY PARTNERS:
UGM Engineering Ltd.

AUTHORS:
Maj. Don Van Loon

Objectives

Since the early 1990s, defence personnel from several nations, including Canada, have relied upon an auto-injector containing the nerve agent antidote HI-6 to provide immediate treatment regimen for chemical agent exposure. HI-6 was chosen primarily because it provided superior effectiveness against a broader range of nerve agents. Deficiencies with the current system include: the lack of a commercial source of supply of GMP-grade HI-6, a cumbersome system of multiple auto-injectors, the absence of HI-6 for parenteral administration and an incomplete data package to support a regulatory submission.

The project seeks to develop a nerve agent antidote system comprised of a 3-in-1 auto-injector and HI-6 in a vial for parenteral administration. The auto-injector will contain an oxime (HI-6), anti-cholinergic (atropine) and an anti-convulsant (avizafone). Pre-clinical studies will support the preparation of Clinical Trial Applications to Health Canada. Federally, partnerships have been established with OCIPEP and the former Solicitor General, now subsumed within the new Public Safety and Emergency Preparedness portfolio, and Health Canada. In addition, the project seeks to collaborate with allied defence partners.

The project objectives include:

- Develop an optimized route of synthesis for HI-6 dimethanesulfonate (DMS) suitable for industrial scale-up;
- Produce a quantity of GMP HI-6 DMS accompanied by a Drug Master File;
- Identify or develop an optimized route of synthesis for avizafone suitable for industrialization;
- Produce a quantity of GMP avizafone accompanied by a Drug Master File;
- Select or develop an auto-injector capable of meeting the 3-in-1 auto-injector requirements to agreed-upon specifications;
- Conduct formulation activities to support each drug product;
- Complete the necessary pre-clinical studies; and
- Prepare a Clinical Trial Application in Common Technical Document format.

The following milestones and timelines are anticipated:

- HI-6 Drug Substance – new alternate synthesis route: Q3/2004
- HI-6 Small batch production: Q1/2005
- Avizafone Drug Substance: Q3/2005HI-6 Parenteral Drug Product: Q4/2005
- 3-in-1 Drug Product: Q4/2006
- Non-clinical Trials completed: Q4/2007
- Clinical Trials Application: Q2/2008

Recent Progress

Baseline Project Documentation

A Baseline Project Document has been completed. It identifies and supports all tasks and deliverables of the project, and includes a Gantt chart with planned start and finish dates. A cost estimate has been prepared for each task, supported by vendor quotations.

Drug Substances

- HI-6 Progress
  - The project has identified a method to convert previously available HI-6 2Cl to the required HI-6 DMS salt on a small-scale level. Work is ongoing to confirm that this process will be viable for converting large amounts of HI-6 2Cl, should this be required.
  - Coupled with the conversion process investigation is the identification of a new route of synthesis to produce HI-6 2Cl. The preliminary data is promising.
  - Since the desired drug substance is the HI-6 DMS salt, investigations are also underway to develop a new direct-to-DMS route of synthesis. Results for this process are expected in the next 3-6 months.
- Avizafone Progress
  - Due to lack of information on the production of avizafone, the project initiated proof of concept investigations into the production of this drug substance.
  - Stage one of the proof of concept is complete with the production of a small quantity of avizafone. Further clarifying work is required to document a full production method, and will take place in the next 6-9 months.

Auto-injector

Engineering design students were asked to research new designs for an auto-injector. The resulting information will be used to identify the requirements and specifications of the new auto-injector.

Future Outlook

The development of a novel synthesis route for HI-6 DMS continues. An optimized process will be selected by mid-2004, to be followed by small batch production to demonstrate industrial viability. The conversion of HI-6 2Cl to DMS will be extended to the 1kg level.

Efforts will continue to identify additional partners. If successful, HI-6 production will be scaled-up to industrial production, if demand warrants.

Anticipated deliverables include provisional patents for novel routes of synthesis for HI-6 DMS and the conversion of HI-6 2Cl. It is expected that 1 kg of HI-6 DMS will be converted from a new source of BCME-derived HI-6 2Cl. In addition, the final HI-6 alternate synthesis route will yield three consecutive lots of HI-6 DMS from the small batch production process.

In the longer term, product formulation for both the vial and 3-in-1 auto-injector will be initiated, and pre-clinical studies will commence. All products and data sets will be developed in a manner that will facilitate a final regulatory submission.

CRTI 0133RD

New Technologies for the Rapid Assessment of Radioactive Contamination

PROJECT LEAD:
Trent University

FEDERAL PARTNERS:
Health Canada,
National Research Council

INDUSTRY PARTNERS:
MDS Sciex

AUTHORS:
Dr. Vladimir Epov, Trent University,
tel: (705) 748-1011 x 7020,
email: vepov@trentu.ca;

Prof. Douglas Evans, Trent University,
tel: (705) 748-1010 x7364,
email: devans@trentu.ca;

Dr. Jack Cornett, Health Canada;

Dr. Patricia Grinberg, NRC;

Dr. Chunsheng Li, Health Canada;

Dr. Ralph Sturgeon, NRC;

Dr. Scott Tanner, MDS Sciex;

Dr. Vladimir Vais, Health Canada;

Dr. Scott Willie, NRC

Objectives

The aim of the project is to develop innovative technologies using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for the rapid analysis of radionuclides that pose a serious health threat after a radiological or nuclear terrorist attack.

Recent Progress

Rapid sample preparation
Sample preparation is an important step in the total analysis of a sample. Combustion was studied for the rapid decomposition of different types of samples. Temperature regime, gaseous atmosphere and combustion devices were compared and optimized. The influence of sample matrix and sample size on the speed and completeness of decomposition was investigated. The most efficient digestion was found for vegetation samples (i.e. leaves and grass) in a muffle furnace using compressed air. More than 92% of the sample matrix can be removed with a combustion temperature of 400-550˚C. The total time for combustion of 1-2 g of sample is 10-15 min, while 10-20 samples can be placed in the muffle furnace simultaneously.

Instrumentation
Optimization of instrumental conditions is necessary to obtain the lowest detection limit and the best analytical precision. Different ICP-MS instruments (i.e. ELAN-5000, ELAN-DRCII and ELEMENT) and different introduction systems (i.e. conventional nebulizer, ARIDUS desolvating unit and high sensitivity APEX system) were compared for the determination of radioisotopes by ICP-MS. Various combinations of ICP-MS instruments with introduction systems were tested with different matrices (i.e. clean matrix, in the presence of high uranium concentrations and using six types of biological samples). The lowest detection limits for the clean matrix were obtained using the APEX coupled with the ELEMENT-2. For the determination of actinides in samples containing high uranium concentrations and also for the determination of radioactive cesium in the presence of Ba, the ELAN-DRCII was found to be the best instrument. Direct analysis of six different types of biological samples indicated that separation of radioisotopes from the matrix is necessary. The choice of the introduction system for the determination of actinides depends on the level of uranium present in the sample.

Dynamic reaction cell (DRC)
With few exceptions, isobaric atomic interferences cannot be resolved spectrally in conventional ICP-MS even using a high-resolution double focusing analyzer. However, ion-molecule reactions that are specific to convert one of the isobars to a product ion of different (and thus not interfering) mass can be promoted with high efficiency within the mass spectrometer using a quadrupole reaction cell

with dynamic bandpass tuning to suppress other interfering molecules. Using stable isotopes, we observed that Ba^+ can be reactively reduced by 5 orders of magnitude through oxidation with N_2O while Cs^+ does not react. This should make possible the detection of $^{135}Cs^+$ and $^{137}Cs^+$ in the presence of Ba. Chemical resolution of the m/z 238 isotopes of U and Pu can be obtained using ethylene as a reaction gas. Unfortunately, this provides little improvement in the resolution of the m/z 239 isobars. However, the high efficiency for reaction of U^+ and UH^+ with CO_2, coupled with the non-reactivity of Pu^+, allows for the sub-ppt determination of the isotopes of Pu in the presence of 7 orders of magnitude excess U. The method provides the potential for analysis of the actinides with reduced sample matrix separation and improved throughput.

Ion chromatography (IC)
Ion chromatography is a very helpful approach to avoid matrix effects and some spectral interferences, and also to preconcentrate the analyte. Ammonium molybdophosphate was used for selective preconcentration and separation of Cs. Both the acidity of the sample and the sample loading flow rate were optimized. A 0.5% ammonium solution was found to be the best choice to extract Cs from the column. A cationic exchanger, AG50W X 8, was used to separate Cs from high concentrations of Mo, because during ICP-MS analysis, ArMo interferes with all three radioactive Cs isotopes. Different ion chromatographic resins were studied for the separation of actinides from the sample matrix and for their preconcentration. TRU resin from the Eichrom Company was found to be very efficient for separation of actinides from biological matrices. On-line separation is being developed.

Electrothermal vaporization (ETV)
Electrothermal vaporization was used as a sample introduction system to complement sample nebulization. This device is based on the difference in volatilization rates for Cs and Mo so that the analyte can be directly determined without the necessity of removing Mo prior to the measurement. The influence of instrument operating conditions and vaporization characteristics of Cs, in the presence of high concentrations of Mo, were investigated. It was verified that air oxidation, for 30 s after the pyrolysis step, is recommended for removal of most of the Mo present in the sample. Under these conditions, 2500 ppm of Mo resulted in a contribution equivalent to only 26 ppt for m/z 137. In contrast, without the use of air oxidation, the same contribution is achieved with only 500 ppm Mo.

Determination of Pu in biological samples
Pu was spiked into different biological samples and determined by ICP-MS, after rapid sample digestion, separation of Pu from the residual sample matrix using ion chromatography, and determination of Pu using the ELAN-DRCII with the APEX as the sample introduction system.

Future Outlook

The next steps of the project are: (1) optimization of the new device for rapid sample digestion; (2) development of analytical techniques for the determination of other actinides (i.e.,Am, Np, U, Th); (3) application of ETV as a sample introduction system for ICP-MS analysis of radioactive Cs and U; and (4) high temperature pyrolysis for the separation of highly volatile radioisotopes (i.e. I-129) prior to introduction to ICP-MS.

CRTI-0154RD

Rapid (<1h) DNA-Based Diagnostic Tests to Identify Two Bacterial Biothreat Agents

PROJECT LEAD:
Defence R&D Canada – Suffield

FEDERAL PARTNERS:
Health Canada – National Microbiology Laboratory

AUTHORS:
Eric Leblanc, Infectious Diseases Research Center, St. Foy, QC,
tel: (418) 654-2705,
email: eric.leblanc@crchul.ulaval.ca;

Doug Bader, Defence R&D Canada – Suffield, Medicine Hat, AB,
tel: (403) 544-4650,
email: doug.bader@drdc-rddc.gc.ca;

Louis Bryden, Health Canada - National Microbiology Laboratory, Winnipeg, MB,
tel: (204) 789-2000,
email: louis_bryden@hc-sc.gc.ca;

Michael Mulvey, Health Canada- National Microbiology Laboratory, Winnipeg, MB,
tel: (204) 789-2133,
email: michael_mulvey@hc-sc.gc.ca;

Jean-Pierre Gayral, Infectio Diagnostic (IDI) Inc., St. Foy, QC,
tel: (418) 681-4343,
email: jpgayral@infectio.com;

Michel G. Bergeron, Infectious Diseases Research Center, St. Foy, QC,
tel: (418) 654-2705,
email: michel.g.bergeron@crchul.ulaval.ca.

Objectives

Appropriate and timely identification of bacterial pathogens is critical in minimizing the impact of bioterrorism events. However, classical, culture-based identification methods are time-consuming and not easily adapted to field practice. Thus there is a need to create rapid diagnostic assays to augment and improve biodefensive response strategies among operational communities for both lab-based and field-based applications. In order to address this need, the Infectious Diseases Research Centre (IDRC) |of Université Laval, Defence R&D Canada - Suffield (DRDC Suffield), Health Canada - National Microbiology Lab (HC-NML), and Infectio Diagnostic (IDI.) Inc., will contribute to the design, development and testing of rapid (<1h) fluorescence-based PCR assays for the specific, ubiquitous, and sensitive detection and identification of *Yersinia pestis* and *Francisella tularensis*. Assays will be developed for the Smart Cycler™ platform by targeting unique sequences in conserved chromosomal genes and pathogen-associated virulence genes. Liquid and dried reagent formulations and a rapid sample processing procedure to prepare samples for analysis will be developed. Assays will be unique and innovative in their design, and performed directly from clinical and environmental specimens.

Recent Progress

Several evolutionary conserved chromosomal genes and virulence genes were selected as targets for multiplex PCR assay design. Relevant strains useful for ubiquity and specificity testing were identified and procured from partner collections and from outside sources based on geographic and phylogenetic diversity. Genomic DNA required to generate sequence information for probe and primer design was prepared from selected microbial strains using methods that provided sterile preparations and fulfilled the requirements for subsequent use (sequence analysis, molecular typing, PCR). Sequence information was generated for several conserved chromosomal gene targets (*tuf, atpd, recA, fus, hsp60*) from several strains of each pathogen, and also from closely related species. Sequence information for virulence genes associated with *Y. pestis* (*pla, ymt, caf1*) and *F. tularensis* (*fopA, tul4*) was also generated. This information, along with existing sequence information in IDRC's sequence database, was used to design PCR primers and probes for assay development.

In the case of *Y. pestis*, plasmid DNA sequence analysis of several *Y. pestis* strains revealed conserved gene targets on both pPCP1 and pMT-1 plasmids. Sequence analysis of chromosomal DNA fragments from several relevant *Yersinia* strains, including closely-related

Y. pseudotuberculosis, allowed the identification of a unique *Y. pestis* chromosomal polymorphism. From this work, a specific, sensitive and rapid multiplex PCR assay was designed to include plasmidic and chromosomal target sequences of *Y. pestis*. In the case of *F. tularensis*, a multiplex assay targeting conserved (*tuf*) and virulence (*fopA*) gene targets of *F. tularensis* was developed and tested. Multiplex assays were initially developed using standard PCR protocols coupled with agarose gel electrophoresis, where each amplicon was distinguished by its size. Agarose gel detection assays were subsequently adapted to fluorescence-based amplicon detection using SYBR Green I dye, where each amplicon was distinguished by analysis of the melting curves generated by the Smart Cycler™ instrument. An internal control was included in the assays to verify the efficiency of each PCR reaction.

Future Outlook

Over the next year, assays for both organisms will be evaluated using spiked clinical and environmental samples. The assays will also be developed for use with fluorescent probes (i.e. Taqman chemistry). During this time, rapid sample preparation methods will be investigated and specification/qualification assessment of critical assay components will be initiated by the project's industrial partner in preparation for developing and manufacturing dried reagents and assay protocols for live agent testing. Preparations to conduct live agent testing in the federal laboratories (planned for the final phase of this project) will be initiated. The final outcomes of the project will include rapid (<1h) DNA-based diagnostic assays for *Yersinia pestis* and *Francisella tularensis* validated to industrial standards; capability at two federal sites to detect and identify *Yersinia pestis* and *Francisella tularensis* in various clinical and environmental sample types using these assays; species-specific and strain-specific sequence data for future molecular research of these organisms.

CRTI 0161TA

CB Blast Protective Helmet

PROJECT LEAD:
Explosive Disposal and Technology Branch-RCMP

FEDERAL PARTNERS:
DRDC-Suffield, RMC, DRDC-SIHS

AUTHORS:
C. Kessler
email: ckessler@med-eng.com,
J-P Dionne and A. Makris,
Med-Eng Systems Inc.,
tel: (613) 739-9646;

John Bureaux, Explosive Disposal and Technology Branch – RCMP.

Objectives

When first responders such as EOD technicians face a situation which may or may not be CB-related, there is often some doubt as to what equipment to use. In the case of a potential explosively-driven agent, full CB equipment, including SCBA or gas mask and full-body protection is required; however, since many CB agents are volatile, the explosive charge is usually low, and the level of blast protection required is typically not as high as for traditional explosive devices. On the other hand, if the device is suspected to contain a large explosive charge, the CB protective equipment is unnecessary, and better blast protection is required. In the past, EOD units have had to own and maintain all of the equipment for both types of threats, and if the nature of the threat was determined to be different than originally thought, the technician had to change the entire protective ensemble in the field, losing precious time and requiring two different sets of equipment. Further, existing CB-protective EOD equipment does not have appropriate levels of fragmentation or blast loading protection. This was recognized as a serious gap that is now being addressed.

The new CB Blast Protective Helmet is a modular system in which a common helmet shell is used with two different, easily-changeable visors, one which can accommodate a CB face mask, the other with enhanced blast protection. This helmet also has several sizes of removable comfort liners, allowing for many different users (with varying head sizes) to use the same helmet shell, even while wearing a CB-protective balaclava.

MES is currently involved in the final stage of the development of this helmet under the auspices of the CRTI program, a $1.8 million project. For this program, MES has teamed up with end users with extensive experience in CB Blast applications from the RCMP, and worked in close collaboration with researchers from RMC and DRDC-Suffield to evaluate the performance of the helmet prototypes against various types of CB agents and explosive threats. User trial feedback has generated several design improvements to the functionality in the CB role. Extensive blast integrity testing has been carried out at DRDC-Suffield, along with explosive dispersal testing of chemical agent simulant. In addition, MIST testing (Man-in-Simulant Testing), consisting of exposing first responder volunteers from the RCMP to simulated CB agents has taken place at RMC, and similar tests will take place shortly at DRDC-Suffield with biological simulants.

Recent Progress

With the initial design complete and prototypes available, 2003-2004 was a period of major development testing for the CB Blast Protective Helmet.

Improved fragmentation protection over that of the previous system has, perhaps, been the most important gap addressed through this program. Fragmentation testing, for both the helmet shell and for the two visors, has taken place throughout the design process, as a result of user feedback and technical challenges. The fragmentation protection is determined in accordance with MIL-STD-662F, giving a V50 rating for each component. V50 is defined as the fragment velocity at which 50% of the impacts are stopped by the material. A 17-grain, chisel-shaped fragment simulator was used in these tests. Meeting the design goals for V50 ratings while maintaining an appropriate overall weight has been an ongoing challenge. The current specifications are as follows: shell, 610 m/s; EOD visor viewing area, 780 m/s, non-viewing area, 700 m/s; CB visor, 700 m/s. The previous CB visor available (SRS-5) only provided a V50 protection of 250 m/s for the visor and 425 m/s for the shell.

Full scale blast testing, using instrumented mannequins wearing full EOD equipment and facing representative charges, was performed in October 2003 at DRDC-Suffield. With a pressure transducer at the ear and a tri-axial cluster of accelerometers at the centre of the head of the mannequins, these tests show the reduction in both overpressure and head acceleration, which can cause eardrum injuries and concussive injuries, respectively. By performing both protected and unprotected tests, the reduction of ear overpressure and head acceleration due to the helmet can be quantified. When facing 0.567 kg C4 from a distance of 0.6 m, the average reduction in overpressure at the ear was 98%, and the average reduction in head acceleration was 91%.

Also in October 2003 at DRDC-Suffield, explosive dispersal testing was performed, in which a mannequin was subjected to an explosively-driven chemical agent simulant (methyl salicylate). The mannequin, with adhesive passive adsorbent dosimeters (PADs) and colour indicator paper placed in strategic locations all over the skin surface, wore a full chemical protective undergarment, EOD suit, and CB Blast Protective Helmet with CB visor over a series of SCBA and gas mask systems. Since the limited testing of this type in the past has taken place inside a chamber, with clean rooms for the doffing process, and the facilities available required an outdoor test, a new test protocol was devised for outdoor testing at Suffield. The indicators at the skin surface showed that no discernible amount of liquid or vapour contamination occurred.

In November 2003, Man-in-Simulant-Testing (MIST-vapour) took place at the RMC in Kingston. In this test, human subjects (RCMP volunteers) had PADs placed on the skin surface, were dressed in cooling undergarments, CPUs, full EOD suits, SCBAs, and the CB Blast Protective Helmet with CB visor. They were then asked to perform several physical activities while inside a chamber full of chemical simulant vapour. These activities (bending, lifting, climbing, walking) were intended to stretch and stress the seals of the entire personal protective equipment system, particularly any interference between the visor and the face mask of the SCBA or any movement that may cause penetration of agent to the skin dosimeters.

These MIST tests also provided important user feedback, which has been used, with other user trials and fit trials throughout the design process to make the CB Blast Protective Helmet both more comfortable and more useable, as well as safer through better fit.

Finally, drop tower impact testing, performed in accordance with the standards for riot helmets, was carried out in December 2003 and January 2004 in order to determine the best design and choice of material for the impact liner.

Future Outlook

There is still a significant amount of testing that remains to be done. Now that the design of the prototype is frozen, many of the tests performed during development must be repeated to determine final specifications for the helmet. Another series of blast testing is scheduled for May 2004 at DRDC-Suffield, as design changes since the previous testing may have improved the blast performance. MIST-aerosol testing, which uses a biological – rather than a chemical – simulant, is tentatively scheduled for September 2004. Moreover, a wide range of tests are scheduled to ensure the reliability of the CB Blast Helmet, including temperature shock, vibration, cyclical testing, electronics and electromagnetic verification, storage and transportation testing, and environmental testing. The CB Blast Helmet will have undergone more extensive testing than any existing blast helmet.

CRTI-0196RD

Development of Rapid Detection Field Tests and Training Programs for Veterinary First Responders to Address Agro-Terrorism with Animal Pathogens

PROJECT LEAD:
Canadian Food Inspection Agency

FEDERAL PARTNERS:
National Research Council, National Microbiology Laboratory of Health Canada

AUTHOR:
Shane Renwick DVM MSc, Director, Animal Health Laboratory Services, Laboratories Directorate, Canadian Food Inspection Agency (CFIA)

Objectives

Canada is currently free of major transmissible animal pathogens such as foot and mouth disease (FMD) and Hog Cholera (HC). This freedom has allowed for an internationally-recognized, superior health status for Canadian livestock; development of an efficient livestock production industry; and an export trade in live animals and animal products worth billions of dollars per year. Ironically, it is largely because of its excellent health status and a consequent lack of natural or acquired immunity, that Canadian livestock is particularly vulnerable to infection by exotic animal pathogens. It has also meant that veterinary first responders have not had opportunities to gain direct experience in managing such outbreaks within Canada.

An outbreak of an exotic animal disease caused by agro-terrorism would result in swift and severe economic damage to Canada. The extent of this damage is illustrated by the recent "natural" introductions into Canada of BSE in 2003 and highly pathogenic avian influenza in 2004. Outbreaks can result in the immediate closing of Canada's borders for exports of animals and animal products. Economic and social consequences could be as exteme as those experienced by the 2001 British FMD crisis in which damage to the economy exceeded $30B (CDN). If agro-terrorists were to introduce a zoonosis (ie. an animal disease capable of infecting humans) such as zoonotic Avian Influenza (AI) or Nipah virus (NV), human health could well be affected.

Agro-terrorism using an animal disease would likely present itself differently than a natural disease incursion. A multi-focal simultaneous outbreak of an exotic disease, or an emerging disease, could occur. A disease could present different clinical signs than usual due to an aerosol exposure to the disease agent instead of the normal animal-to-animal transmission route.

Early detection, warning to the agro-sector and rapid action are essential in containing and eliminating disease and mitigating negative consequences on health, economy and public confidence. This will depend on being well prepared to detect signs of disease in animals early and accurately, to differentiate quickly between diseases which have similar signs and to manage longer term consequences through containment and eradication. These efforts require highly trained veterinary first responders (VFR) in the field, well equipped with robust, rapid diagnostic screening tests, and with the ability to communicate with scientific experts in real time.

Recent Progress

Animal diseases may be diagnosed rapidly using new technologies that detect antigen (protein) or genome sequence (DNA) specific to a particular pathogen or, later in the disease process, antibodies in the blood (serum) of a recovering animal. For example Enzyme-Linked Immuno-Sorbent Assay (ELISA) technology can produce tests, which are read by a simple colour reaction and may be suitable for use as dipstick or penside tests for either antigens or antibodies. Polymerase chain reaction (PCR) technology can detect DNA sequences unique to certain pathogens and can be used in mobile field units. DNA or protein microarrays can be designed to detect and differentiate multiple antigens, antibodies or DNA.

The project is developing new, rapid, highly sensitive diagnostic tests based on the key platform technologies that have the greatest potential as field tests for VFR. These tests will be mobile and robust for use under field conditions. They will produce highly reliable, accurate results rapidly, support differential diagnosis, allow for automation for handling large numbers of samples, and allow for electronic collection and transmission of data. These tests will be applied to rapid diagnosis of the FMD, HC, AI and NV.

Technologies groups that are under development include Real Time Polymerase Chain Reaction (RT-PCR) for FMD, HC, and AI; DNA/Protein Microarrays for FMD, HC, AI; and Rapid Antigen/ Antibody Detection Systems.

The latter group will include sub-projects that will:

1. **Develop Fluorescence Polarization Assay (FPA) technology, dipstick ELISA or acoustic rupture event scanning for FMD, HC and AI;**
2. **Differentiate convalescent animals from those vaccinated against FMD by multiplex ELISA;**
3. **Develop Rapid Diagnostic Colloïdal-Gold Immuno-Blotting Methods for HC and AI;**
4. **Develop field tests for the detection of NV.**

Future Outlook

Each technology group is led by a Canadian Food Inspection Agency (CFIA) laboratory equipped with biocontainment facilities (Level 3 or 4) and having specialized expertise in select animal pathogens and key technologies. A Training and Communications group has been formed to study transmission of data from the field to the laboratory and to train first responders.

CRTI 0203RD

Standoff Detection of Radiation

PROJECT LEAD:
DRDC Ottawa

FEDERAL PARTNERS:
Health Canada, Atomic Energy of Canada Limited

INDUSTRIAL PARTNER:
Bubble Technology Industries Inc.

AUTHOR:
Dr. Dean S. Haslip, DRDC Ottawa,
3701 Carling Avenue, Ottawa, ON,
K1A 0Z4,
tel: (613) 998-3231,
email: Dean.Haslip@drdc-rddc.gc.ca

Objectives

This project will construct a fieldable prototype standoff radiation detector. Conventional radiation detectors work on the principle of "direct" detection, whereby the radiation must actually enter the detector to be counted. A significant drawback to this is that a radiation surveyor must enter a radiation field in order to detect it. This project will construct a detector based on "indirect" detection, allowing detection of a radiation field from a distance. This will allow detection of contaminated areas prior to entry, and characterization of such areas to identify those of high and low dose rate, to facilitate mission planning. The project is thus closely aligned with the CRTI Investment Priority "Immediate Reaction and Near-Term Consequence Management Capabilities".

Recent Progress

Standoff detection of radiation is a significant challenge. There are very few avenues by which "indirect" detection may be accomplished. The most promising of these is by detecting the faint light emitted by ionized molecules in the air surrounding a radioactive source. Fortunately, these emissions occur in colour bands of specific relative intensities, which make them easier to sense against high levels of background light from other sources. Furthermore, because the spectrum of emissions is unique, misidentification of extraneous light is significantly lessened.

Several techniques could be used to sense these emissions. Our system employs custom-fabricated mirrors and optical filters to simultaneously image a scene of interest in several wavelength bands. These images are then processed to look for the signature of radiation-induced photoluminescence. In field tests with a laboratory prototype system, the team has demonstrated the ability to detect alpha, beta, and gamma radiation from considerable distances, well beyond the limits of conventional direct detection.

The project began by designing the fieldable prototype detector, from both an optical and mechanical standpoint. The optical design of this system was based on hundreds of simulations of various optical

systems with an optical design code. The procurement phase of this project was substantial, not least because the components of this detector are highly specialized and must be fabricated within exacting standards. All of these parts were extensively tested by the project team as they were delivered by the various manufacturers. With the exception of a few minor components, this system is now complete, and the project team has begun system testing.

This project is scheduled for completion in March 2005. Activities in the last year of the project will involve the final elements of systems integration, and field-testing. A few minor components have yet to be manufactured, and this work will continue in the first half of this year. There will also be a great deal of testing with the goal of identifying problems with the compatibility and integration of sub-systems. Data acquisition software will undoubtedly undergo some small changes to improve the interface and expand functionality. Finally, it will be important to identify those challenges specific to operating this device in the field. For instance, the stability of the device in the field or during transport is an important property that must be established.

Future Outlook

Ultimately, perhaps the most important question that this project will answer is the sensitivity of the standoff detector, and standoff detection in general. This can only be answered conclusively through a program of field testing. One element of this testing will involve establishing the instrumental backgrounds under a variety of field conditions. This will help set detection limits, but will also enable the lowering of these limits by providing input to the data analysis routines. This field testing will also determine the sensitivity of the detector under a variety of conditions, and specify the factors that limit this sensitivity.

Bubble Detector Film

PROJECT LEAD:
DRDC Ottawa

FEDERAL PARTNERS:
Health Canada,
Atomic Energy of Canada Limited

INDUSTRIAL PARTNERS:
Bubble Technology Industries Inc.

AUTHORS:
Dr. Dean S. Haslip, DRDC Ottawa,
3701 Carling Avenue, Ottawa, ON,
K1A 0Z4,
tel: (613) 998-3231,
email: Dean.Haslip@drdc-rddc.gc.ca.

Objectives

This project will develop a sensitive, non-electronic, real-time indicator of radiation exposure suitable for detecting radioactive contamination, particularly alpha- or beta-emitters. This new technology will have many applications in radiation safety and emergency response. For instance, the Bubble Detector Film (BDF) could be made into a disposable strip with an adhesive backing that could be stuck to the pant leg or boot of a first responder. If the first responder walks into a contaminated area, the strip will become contaminated and produce a visible and timely warning. Another significant application involves making swipes from the BDF. Swipes are traditionally used to sample potentially contaminated surfaces, and must be analysed in a laboratory setting. Contaminated BDF swipes, however, would be instantly recognizable without sophisticated analysis. The BDF thus has clear applications to the CRTI Investment Priority "Immediate Reaction and Near-Term Consequence Management Capabilities".

Recent Progress

Bubble Detector Film is based on some fairly complicated chemistry, which must at least be appreciated in order to understand the progress that this project has made. Part of this process includes the reactions taking place in a photographic emulsion. A photographic emulsion consists of a silver halide in a gelatin matrix. Upon exposure to radiation, some of the silver ions in the emulsion are converted to metallic silver. In the presence of a photographic developer, these metallic silver grains catalyze the production of more metallic silver, permitting a chemical amplification of up to one billion. Some developers release hydrogen ions when they are oxidized. When these are used, the chemical amplification also produces an increase in hydrogen ion concentration, which is synonymous with an increase in acidity, or a decrease in pH. Radiation exposure is thus linked to a decrease in pH, which can be easily detected with electronic probes or through the use of pH-sensitive dyes. Unfortunately, this system is not sufficiently sensitive to be used for radiation dosimetry.

The other half of the BDF is the conventional Bubble Detector (BD). In a BD, superheated droplets are dispersed in a gel medium. Neutron interactions in this gel deposit sufficient energy in a sufficiently small volume to nucleate the droplets. The droplets then become visible as bubbles, and can be counted to

assess radiation dose. This technology is generally not used for radiation other than neutrons, because other forms of radiation do not produce sufficient energy densities to nucleate the droplets.

The Bubble Detector Film marries the technology of the photographic emulsion to that of the bubble detector. The concept is to impregnate the BD with a photographic emulsion, and to use a BD gel medium whose physical integrity is sensitive to pH. Thus, when radiation exposure occurs and the pH drops, the gel matrix is weakened and the superheated droplets are released. This combination should have the sensitivity required for contamination detection and monitoring.

Early progress on this project was focused on developing the components of the Bubble Detector Film. As such, the project has selected a suitable photographic emulsion for the BDF, and has identified a number of compatible developers. The project team has also selected and fabricated an acid-degradable cross-linker for the BDF. This has been done in collaboration with a group at the University of Ottawa.

Subsequent developments in the project have concentrated on integrating these components. To wit, the project team has demonstrated the dissolution of the bubble detector gel as a result of the action of the developer. In addition, the project has demonstrated that bubble nucleation is actually occurring in this gel (as opposed to a less localized dissolution), and the pressure pulse arising from this bubble formation has been observed and quantified. Work in the early part of 2004 has centred on the micro-encapsulation of dye droplets, which forms part of the visual interface for the BDF. The micro-encapsulation has been successfully carried out, and the resulting droplets have been characterized as a function of the production parameters.

Finally, in recent weeks, all of the BDF components have been assembled into a functional prototype device. This sets the stage for activities in the final year of the project, which is scheduled for completion in March 2005.

Future Outlook

The demonstration of a prototype device is a significant milestone in this project. Of note however, is that this prototype device responded to visible light, and not to ionizing radiation. Of course, the photographic emulsion should behave identically in an ionizing radiation field as it does to a visible light field, but this needs to be demonstrated. Furthermore, a major activity of the coming year will be characterizing this technology in terms of its dosimetric properties and its sensitivity to various forms of ionizing radiation.

The project has produced a prototype sensor, but this prototype has not been optimized. This optimization will be a significant activity in the coming year. In a system as complex as the BDF, there are many parameters that can be adjusted and that will have an impact (and possibly a substantial one) on the properties of the final product. As such, it is clear that many such parameters need to be adjusted in the BDF system to produce a device that is maximally sensitive and stable.

Of course, this project is building to a point at which larger-scale production of the BDF is feasible and desirable. As such, considerable thought needs to be put into the optimization of production techniques, so that Bubble Detector Film can be produced with high quality and minimal unit cost.

CRTI 02-0007TA

Medical Countermeasures against Ricin

PROJECT LEAD:
DRDC – Suffield

FEDERAL PARTNERS:
Twinstrand Therapeutics Inc. (Burnaby, B.C.),
Cangene Corporation (Mississauga, Ontario).

AUTHORS:
Dr. John W. Cherwonogrodzky, Department of National Defence, DRDC – Suffield, C/o Stores Bldg. 560, Canadian Forces Base – Suffield, Ralston, Alberta, Canada, T0J 2N0.
tel: (403) 544-4705,
email: John.Cherwonogrodzky@drdc-rddc.gc.ca;

Dr. Thor Borgford, President, Twinstrand Therapeutics Inc., 8081 Lougheed Highway, Burnaby, British Columbia, Canada, V5A 1W9.
tel: (604) 415-7180,
email: borgford@twinstrand.com;

Dr. Donald Stewart, Director, Research & Development, Cangene Corporation, 3403 American Drive, Mississauga, Ontario, Canada, L4V 1T4.
tel: (905) 405-2930,
email: don_stewart@cangene.com.

Objectives

a) Milestones and Timelines
The project is much like a "relay race" where 3 participants with different and exceptional expertise work together.

i) The "first runner" is Twinstrand Therapeutics Inc. They will develop harmless defective ricin toxoids by recombinant technology (October 2003) and assess the antigenicity (March 2004). The lead toxoid will be produced in bulk in yeast (July 2004), then characterized (November 2004). Completion of their role will be after assessment of the toxoid in tissue cultures (August 2005), in mice (October 2005) and after submission of a final report (November 2005).

ii) The "second runner" is Cangene Corporation. The toxoid will be used to produce antiserum in goats and synthetically in tissue cultures. For the first part, a facility has to be identified (disease-free animals for 5 years) (March 2004), the animals immunized/vaccinated (March 2005) and GLP quality antibodies produced (August 2005). For the second part, the clones must be created (June 2004) and the antibodies purified (December 2004). Completion will be the assessment of these 2 antibody sources (October 2005) and the submission of a final report (November 2005).

iii) The "third runner" is DRDC-Suffield. Animal Care Committee, Study Forms and Schedule 1 approval will be acquired (March 2004). Bulk amounts of ricin will be acquired (July 2004). Animal sensitivity and analytical assays for ricin will be developed (December 2004). Efficacy of antibodies for protection/therapy will be assessed (June 2005). Completion will be a comparison of antibody efficacy against the threat by different routes (e.g. aerosol), defining strengths and limitations, and the submission of a final report (November 2005).

b) Relevance
Ricin is a toxin found in castor beans, making up about 1-3% of the weight. Although only a few milligrams is a lethal dose for a human, production of castor beans is over a million tons a year. Given its toxicity and availability, ricin is viewed as a probable terrorist threat. Indeed, there have been recent incidences in the UK, France and the US (e.g. the letter with ricin sent to the Senate). There are no medical countermeasures against ricin and toxicity leads to death in a few days. The CRTI-supported project will produce antibodies for protection/therapy, similar to antiserum snake-venom kits used as therapies.

Recent Progress

This is a new CRTI project. Approval of the Charter was received in August 2003, the contract approval for Twinstrand Therapeutics Inc. was received in October 2003 and for Cangene Corporation, in February 2004. Despite the recent activation, this project is on time and on budget. Achievements have been:

- Twinstrand Therapeutics Inc. has developed the toxoid clones and has begun pilot plant testing of the lead toxoid in preparation for bulk production.
- Cangene Corporation has constructed a random protein bacteriophage library and has isolated a few expressing ricin-like groups.
- DRDC-Suffield has an Animal Care Committee protocol (JC-03-01) and a Study Approval Form (04-003) in place. A Use of Schedule 1 Agent form has been submitted and approval is expected soon.

Following the ricin letter incident at the US Senate, an unsolicited news article by MSNBC was issued. This article can be accessed at "http://www.msnbc.msn.com/id/4153753".

Monthly teleconferencing sessions have proven useful for keeping all parties informed, resolving decisions and for maintaining milestone timelines.

Future Outlook

a) Planned Activities and Future Milestones

The activities and milestones as noted in the previous section are on schedule. In brief, Twinstrand Therapeutics Inc. will produce bulk amounts of the ricin toxoid and will characterize it. Cangene Corporation will take this toxoid and produce antibodies, both in animals (polyclonal antisera) and in tissue culture (humanized mouse monoclonal antibody), with characterization to assess the quality. DRDC-Suffield will test –and evaluate these antibodies for protection/therapy against ricin poisoning (by different routes of challenge) in the mouse model. All participants will record their results in a final report.

b) Ultimate Products, Deliverables

The ultimate product will be similar to the accepted anti-snake venom antiserum therapy. Small bottles of antibodies will be produced to treat civilian or military targets or first responders exposed to lethal amounts of ricin. An insert package will describe the use, limitations and assessment.

The deliverables will be final reports by all the participants. The other deliverable will be the availability of the product, should the military or civilian agencies wish to acquire the anti-ricin antibodies as a preventative measure.

c) "Value Added" Benefits

The CRTI project has only recently been started and already there have been unexpected benefits.

1. Upon the attack on the US Senate, there was public concern on the threat. The news article by MSNBC was a confidence building measure. It reassured the public that they were being looked after, that measures were in the works, and that security was being enhanced.
2. There was a realization that just having a countermeasure on the shelf might benefit security. Evidence of a successful countermeasure may be if it is never used because it has deterred the terrorist.
3. First responders have indicated that entering a possibly contaminated area knowing there are no countermeasures available is a concern to them and distracts them from the work to be performed. Just having a possible treatment on the shelf has an enormous psychological benefit that is likely to improve response to an incident.

CRTI 02-0021RD

Direct Detection and Identification of Bioweapons Nucleic Acids Based on Cationic Polymers

PROJECT LEAD:
Industrial Materials Institute, National Research Council Canada

PROJECT PARTNERS:
Steacie Institute for Molecular Sciences, National Research Council Canada, Health Canada, Université Laval, Centre hospitalier universitaire de Québec - Centre de recherche en infectiologie, Infectio Diagnostic Inc.

PROJECT CHAMPION:
Dr. Michel Dumoulin, IMI-NRC
tel: 450 641-5181,
email:
Michel.Dumoulin@cnrc-nrc.gc.ca

PROJECT MANAGER:
Dr. Caroline Vachon, IMI-NRC
tel: 450 641-5185,
email:
Caroline.Vachon@cnrc-nrc.gc.ca

PROJECT TEAM:
Dr. Michel G. Bergeron, Centre de recherche en infectiologie;

Dr. Mario Leclerc;

Dr. Denis Boudreau, Université Laval;

Dr. Benoit Simard, SIMS-NRC;

Dr. Teodor Veres, IMI-NRC;

Dr. Louis Bryden;

Dr. Michael Mulvey, Health Canada;

Dr. Jean-Pierre Gayral, Infectio Diagnostic Inc.

PRESENTER:
To be determined

Objectives

This project will provide proof of concept for the development of novel nucleic acid biosensors that should allow rapid detection and identification of biological pathogens. The proposed technology features simple preparation, trapping, and preconcentration of samples combined with polymeric transducers. Certainly, all bacterial species as well as fungal species could be detected by this approach. However, for the purpose of this project, the initial target sequence will be from a virulence gene of *B. anthracis*. This technology will permit the detection of less than a thousand copies of genetic target. This represents a significant improvement over available technologies that require target amplification (PCR). Furthermore, we will take advantage of the polymer specificity to demonstrate the capacity of the technology to distinguish target material differing from other genetic material by only one nucleic acid. The detection step will be accomplished in less than one hour.

Recent Progress

Current detection technologies of biothreat agent nucleic acids rely on prior amplification, a time-consuming critical step sensitive to inhibitors present in the sample, and prone to false positive results, due to cross contamination of reagents or laboratory infrastructures. A revolution in the field will occur when low-cost portable devices capable of rapid detection and identification of nucleic acids without prior amplification become available, and this project aims at developing such a sensitive, rapid, and compact technology. The approach will combine minimal sample preparation, highly selective capture and preconcentration of the targets with real-time optical detection using water-soluble, cationic, polymeric transducers.

We have already realized important progress for establishing proof of concept that our polymeric transducer can be used to rapidly detect *B. anthracis*. So far, we were able to detect less than 1000 copies of DNA isolated from the *influenza* virus within 30 minutes. This detection step was directly done in solution without prior PCR amplification. Moreover, the discrimination between the perfect match and a DNA strand containing a single mismatch is excellent.

The next critical steps will involve the detection of DNA from *B. anthracis* with our polymeric transducer, which will require the isolation and deactivation of suitable fragments from this pathogen. We have tested several methods to purify and fragment *B. anthracis*, and obtained fragments of different lengths. We are currently working to analyze and select the fragments most suitable for detection. The final steps will involve concentration of the DNA and direct detection by optical measurements.

Future Outlook

This one-year project is scheduled for completion on September 30, 2004. We have made significant progress in reaching our final goal: providing proof of concept that our technology can rapidly detect and identify pathogens. Tasks and milestones will be completed on schedule. Once implemented in a portable device, this novel and simple technology could therefore enable on-site, rapid detection and identification of potential bioweapons for first responders and public health providers. It should also provide better capabilities for medical triage procedures and highly performing tools for detection and classification of events. Finally, such innovative developments will contribute to the efficient diagnosis of infectious diseases and genetic disorders.

CRTI 02-0024RD

Probabilistic Risk Assessment Tool for Radiological Dispersal Devices

PROJECT LEAD:
DRDC Ottawa

FEDERAL PARTNERS:
Canadian Nuclear Safety Commission, Public Safety and Emergency Preparedness Canada, Canadian Border Services Agency, Canadian Security Intelligence Service

UNIVERSITY PARTNER:
University of Ontario Institute of Technology

INDUSTRIAL PARTNER:
Science Applications International Corporation Canada

AUTHOR:
Dr. Dean S. Haslip, DRDC Ottawa, 3701 Carling Avenue, Ottawa, ON, K1A 0Z4,
tel: (613) 998-3231,
email: Dean.Haslip@drdc-rddc.gc.ca

Objectives

This project aims to create a comprehensive probabilistic risk assessment addressing all aspects of RDD construction and use, including source acquisition, construction risks, delivery mechanisms, consequences of use, and possible countermeasures. This risk assessment will be developed via the fault- and event-tree analysis used throughout the nuclear and software industries. Wherever possible, data on source security, border security, intelligence trends, health physics, and dissemination modalities will be accessed through project partners. In addition, the project will target critical knowledge gaps in construction feasibility and radiological dispersion through experimental investigation and modeling. The project clearly addresses the CRTI Investment Priority "S&T Dimensions of Risk Assessment".

Access to this risk assessment (specifically, the risk assessment database) will be expedited by the key deliverable of this project, a software tool that allows user interaction with the database. Functions of this software will include the ability to search for possible risks based on combinations of user inputs (such as specific sources or other RDD components), and to identify critical gaps in our defence against radiological terrorism. The tool will also provide the user with information on selected RDD modalities, including the nature and extent of a potential hazard, and possible countermeasures or steps to take in remediation.

Recent Progress

In this early phase of the project, work has focussed on data harvesting. Investigations have been made related to materials of concern, and to the transport of radioactive materials. Both of these have clear impacts on the availability of radioactive materials. Investigations have also been made of possible targets, including some examples of so-called critical infrastructure.

The project team has initiated collaboration with Sandia National Laboratories in the US, a major centre of expertise in the area of radionuclide dispersal. This collaboration will likely take place under the auspices of the Public Security Technical Panel. Discussion with Sandia has identified the key information gap in the area of radiological dispersal. The project team has already begun planning how to address this gap through experimental trials.

In the near term, activities on this project will be largely devoted to data harvesting. There are many potential areas of investigation that have not yet been adequately mined by the project. These activities will continue well into the current year.

The project team will also begin investigating software tools for performing Probabilistic Risk Assessment. A number of such tools exist, but a flexible tool will be needed because of the highly non-standard nature of this project. That is, the construction of a generic Radiological Dispersal Device is not a well-defined engineering system in the way that a nuclear reactor is. This means that the Probabilistic Risk Assessment methodology will need to be modified somewhat to accommodate this more complex situation.

Development of a Graphical User Interface for the risk assessment tool to be produced by this project is expected to begin shortly. This will help to further scope the project, thus identifying additional data harvesting that needs to be done. It will also give the user communities the opportunity to provide further guidance on their requirements from the project.

Future Outlook

The summer of 2004 will also see the beginning of the project's experimental program. Important components of this program will be the intercomparison of experimental data from this project with data taken at Sandia National Laboratories. When there is good agreement between these data sets, the project team can move into the previously uninvestigated areas that form the information gap mentioned above.

In a year's time, as the above activities wind down, the focus of the project team will shift to populating the risk assessment database and developing the software tool. These activities are scheduled to complete in March 2006.

Canadian Network for Public Health Intelligence

PROJECT LEAD:
Centre for Infectious Disease Prevention and Control, National Microbiology Laboratory, Health Canada

FEDERAL PARTNERS:
Defence R&D Canada), Health Canada

OTHER PARTNERS:
TDV Global Incorporated, Canadian Public Health Laboratory Network (CPHLN), TR Labs, University of Guelph, Canadian Council of Medical Officers of Health

AUTHOR:
Dr. Amin Kabani, National Microbiology Laboratory, Canadian Science Centre for Human and Animal Health, Health Canada, Room 4180, 1015 Arlington Street, Winnipeg, MB, R3E 3P6, tel: (204) 789-6090, fax: 204-787-4699.

Objectives

The Canadian Network for Public Health Intelligence (CNPHI) is targeted at improving the capacity of the Canadian public health system to reduce human illness associated with infectious disease events by supporting intelligence exchange, surveillance activities and outbreak investigations. This will be achieved by establishing a framework to collect and process surveillance data, disseminate strategic intelligence, and coordinate response to biological threats.

Integration of surveillance, epidemiology and laboratory information, maintained within an infrastructure that has the capacity to identify, communicate and respond is the foundation to bio-terrorism preparedness and effective Public Health. Many unique pockets of expertise relating to infectious diseases and data collection systems exist in Canada, but a national framework to allow the timely integration of these is lacking. CNPHI aims to facilitate the integration of relevant public health intelligence into a common national framework to support coordination among jurisdictions.

For the most part, the timely sharing of public health information in provinces and across Canada occurs within strict silos defined by: Jurisdiction (e.g. local vs. provincial vs. federal); Agency/department (e.g. First Nations and Inuit Health Branch vs. Population and Public Health Branch; Health Canada vs. CFIA; Public Health vs. RCMP and DND); and Discipline (e.g. laboratory vs. epidemiology; respiratory vs. enteric; medical community vs. law enforcement and public defence).

CNPHI is a model to integrate relevant public health intelligence (i.e. strategic or interpreted data) into a common national framework to support coordination between multi-level jurisdictions; coordination that must happen for effective use of data to identify risks, initiate response and build response capacity.

CNPHI Goals

- Enhance Canadian biological event detection, response and preparedness by facilitating national integrated real-time data sharing of laboratory and epidemiological data, and by supporting response capability and capacity.
- Maintain and respect the present jurisdictional boundaries while leveraging current Canadian resources and infrastructure in innovative new ways for the benefit of the broader stakeholder community.
- Develop an innovative IT architecture with our partners and stakeholders for the enhancement of existing public health infrastructure to support multi-jurisdictional data sharing and collaboration.

Key Deliverables

- Strategically integrate laboratory and epidemiologic surveillance alerts and decision support tools in a common, secure web-based environment, creating the Canadian Intelligence and Outbreak Surveillance Centre (CIOSC). CIOSC will allow for the strategic dissemination of timely laboratory and epidemiology intelligence (including syndromic surveillance, food safety, international disease reports, and other relevant national surveillance information) in a secure web-based environment. CIOSC will focus on key laboratory and epidemiological surveillance data (e.g. PulseNet Canada, respiratory and enteric illnesses, National Enteric Surveillance Program, syndromic surveillance pilots, etc.) as the initial sources of data into the system.
- Enhance key analysis tools (e.g. infectious disease modeling, GIS, simulation and decision support tools) to produce intelligence through the analysis of laboratory and epidemiological surveillance data. Analysis tools will focus on both specific, localized tools (e.g. decision trees, protocols, automated decision support for alerts, etc.) and on larger decision support and simulation exercises for response capacity and capability evaluation (e.g. an outbreak exercise to test resource readiness). Analysis tools will be available to local, provincial, and national stakeholders.
- Coordinate and facilitate national response actions through the creation of a secure operations centre response framework. Operations centre infostructure will enable real-time data collection and integration, data management and manipulation, intelligence organization and display, situational awareness, execution of preplanned response, decision-making, integration of organic and external expertise, command and control, and communications. This framework will support coordination with other key emergency response stakeholders (e.g. DND, RCMP, etc.).
- Provide access to specialized resources for public health stakeholders and first responders, including user-group tools (discussion forums, web-casting, knowledge management), training resources and opportunities, bioinformatics support capacity, simulation and scenario exercises.

Future Outlook

CRTI-02-0041RD

Real-Time Determination of the Area of Influence of CBRN Releases

PROJECT LEAD:
Atomic Energy of Canada Limited, Chalk River Laboratories

FEDERAL PARTNERS:
Environment Canada, Health Canada

AUTHORS:
Dr. Phil Davis

Objectives

CBRN material released to the atmosphere by terrorist activities will form an airborne plume that undergoes advection and dispersion by ambient wind and turbulence fields. A large fraction of this material will be deposited on the ground, particularly if precipitation falls during or after the release. Material deposited on urban or agricultural surfaces will have health and economic consequences long after the primary plume has passed. An appropriate response to this situation requires the best possible knowledge of where and when the material will be deposited, with the shortest possible delay between release and forecast. This information will be vital to decision makers in assessing needs for evacuating populations, determining evacuation routes, implementing protective measures, deploying response teams and planning cleanup activities, all with the aim of minimizing health effects and returning valuable land to service.

The goal of this project is to provide first responders and decision makers with reliable, real-time forecasts of the timing, location and amount of deposited CBRN material. To achieve this goal, a sophisticated computer model is required to address four key areas: forecasting the trajectory and concentration of CBRN material in air; forecasting the location, duration and intensity of precipitation; calculating the amount of airborne material deposited on the ground when it is raining or snowing; and calculating deposition in the absence of precipitation. Such a model is being developed in this project by updating the codes (CANERM and MLCD) currently used in Canada to handle emergency situations.

The short-term precipitation forecasts (nowcasts) required by the models are based on data from weather radar networks, which provide the best estimates of rainfall over large areas for periods of 0-6 hours. Precipitation nowcasting is done using a tracking algorithm that determines the motion field of storms from the evolution of the precipitation in the recent past, and then using this motion field to displace the precipitation pattern to produce a forecast. In the past, forecast times were limited by the useful observation range of a single radar (about 200km). To remove this limit, access has been gained to the raw North American weather radar data, which merges observations from numerous sites in Canada and the United States, and radar composites are now available operationally in real time at the Canadian Meteorological Centre. QA/QC checks are presently being made on the data, and issues involving the presence of ground echo in the Canadian data are being resolved. Work continues on improving the quality of the radar composites, refining the resolution and time step (currently 12 km and 20 minutes, respectively) in the nowcast algorithms, and studying nowcast skill.

Recent Progress

The next step in the process was to modify the MLCD model to accept radar-derived precipitation fields, a task that is now complete. Preliminary tests show that the modified model is working well with a negligible increase in CPU time. Sensitivity tests are being carried out to show how the radar-estimated precipitation fields affect wet deposition.

Work has also begun on developing improved models for dry and wet deposition, which will replace the simple empirical models presently used in CANERM and MLCD. The new models account explicitly for the physical and chemical processes affecting deposition. Wet and dry deposition have some information needs in common, including the vertical concentration profile of the CBRN material, the size distribution and density of the particulates most likely to be released in a terrorist event, the solubility, diffusivity and reactivity of released gases and the meteorological conditions in effect at the time of the release. Additional information is needed for the wet deposition model, including precipitation intensity, which is provided by the radar model, and the drop-size distribution, which is deduced from the forecast intensity. The scavenging rate for each drop size is calculated taking into account the droplet fall speed and the best available estimates of collection efficiency.

Both new deposition models address the evolution in the properties of CBRN material that may occur through interactions with background aerosols and gaseous species such as OH, HO_2 and ozone in the atmosphere. Such interactions may result in gas phase removal of the hazardous material or an effective change in particle size or gas reactivity. The CBRN materials most likely to be released in a terrorist event were determined from the CRTI Consolidated Risk Assessment, and the key properties of those materials (particle size, density, solubility, diffusivity and reactivity) are being determined. Biological agents (viruses, bacteria) would likely be released as very small, light particles with diameters less than 0.1 µm. Chemical agents are most likely to be released as fine droplets from a sprayer, with diameters between 1 and 5 µm. The most likely radioactive isotopes to be used are ^{60}Co, ^{137}Cs and ^{192}Ir, with particle sizes that depend on the method of release.

Preparatory work has been initiated for validation studies that will be done at a later stage of the project. In May 1996, the plume from a large chemical fire in the city of Laval passed over Montréal and was observed by three McGill radars on a cloud-free day. The data collected by the radars will be used to test model predictions of plume transport, plume spread and dry deposition. The data also provide the opportunity to explore the use of meteorological radar in detecting the presence of large toxic particulates in the atmosphere.

Future Outlook

By the end of the project, all this information will have been combined into one integrated system that will have been thoroughly tested. The integrated system will provide an operational tool for predicting the concentration of CBRN material on the ground as a function of space at a sequence of forecast times. In the real event, deposition maps will be generated and distributed to first responders and decision makers to aid in assessing and managing the incident.

CRTI-02-0041TA

Deployable CBRN Monitoring Network

PROJECT LEAD:
Health Canada

FEDERAL PARTNERS:
Canadian Nuclear Safety Commission, Environment Canada

INDUSTRY PARTNERS:
Bubble Technology Industries (BTI), General Dynamics Canada

AUTHOR:
Dr. Kurt Ungar, Radiation Protection Bureau, Health Canada,
775 Brookfield Road, Ottawa,
Ontario, K1A 1C1,
tel: (613) 954-6675,
email: kurt_ungar@hc-sc.gc.ca.

Objectives

Terrorist-initiated and accidental events are unpredictable in place and time. This project fills a gap in Canada's emergency response capability, namely a sophisticated CBRN detection/monitoring network that can be quickly deployed wherever needed, and remotely operated from any location. The network can have any number and type of suitably interfaced sensors covering any sized area; these will deliver detailed quantitative data for use in evaluating emergency response and long-term follow-up. The key to the design is the flexibility inherent in modern technology: flexibility in communications, data handling, and sensor design. The deliverables under the current contract are a suite of leading edge CBRN sensors, one communications node, and complete software for the sensors, node, and remote data reception and control. The software architecture is designed with maximum flexibility to allow future augmentation and integration of the system with other response systems.

This project addresses the CRTI Investment Priority of "Immediate Reaction and Near-time Consequence Management Capabilities" as its primary thrust. Because of its flexibility of deployment and sophisticated data output, it will also impact "Collective C4I Capabilities for CBRN Planning and Response", "Prevention, Surveillance and Alert Capabilities" and "Longer-term Consequence Management Capabilities".

Health Canada is the lead federal partner for this project. Bubble Technology Industries is providing special radiation monitors, interfacing all sensors to a communications node, and developing the software that will control the network and provide both raw data and critical information to the end user. General Dynamics is providing the biological sensor. All federal partners will be providing technical input in the development of the system, as well as testing of the network when it is complete.

The project is scheduled for completion in February 2006. All hardware and software will be completed during 2004, with all system integration and testing completed by November 2005. A commercialization plan will be delivered in September 2005.

Recent Progress

Since November 2003, a Consolidated Hardware/Software Requirements Review report has been completed in consultation with the project partners. The resulting network design enables the deployment of one or many independently operating modules, each configured to meet the needs of the emergency scenario at hand. Each module comprises a node and an associated suite of detectors. The node acts as the communications hub between its sensor suite and a remote control center. Each sensor has GPS and on-board intelligence that presents location, digested data, and raw data on request, to the node. The on-board GPS facilitates either static or mobile deployment of the sensors. Communication between the node and its sensors is wireless, with a modality chosen to suit the spatial extent of the array. Communication between the node and control center can occur by satellite, cellular or landline connection, as appropriate. Data transmission and reception is achieved using Internet technology, with the software architecture and data formats designed to facilitate integration with existing or planned response systems, such as the ARGOS system currently being implemented under another CRTI initiative.

The chemical sensor will be a suitably interfaced commercial unit that will detect both chemical warfare agents and toxic industrial chemicals. The biological agent sensor will be a leading-edge portable system that will detect the four standard biotoxin simulants, and the radiation detectors are specially designed units. A gamma monitor will employ advanced circuitry to allow spectral analysis in high radiation environments, dose and dose-rate calculations, isotope identification, and scenario analysis for complex fission-product releases. A compact portable air monitor will provide spectral analysis of airborne alpha, beta and gamma radiation and will feature automatic or remotely actuated filter advances. In addition to these CBRN detectors, other suitably interfaced sensors (including meteorological, sound, motion and imaging) can be incorporated to meet special needs. Actuators can also be added to initiate actions in response to sensor stimuli or commands from the control center.

Design and construction of the radiation monitors is underway, with completion of these two units on track for the current year. The biological monitor will be the 4WARN Sentry system and will also be delivered in the current year. Two commercial chemical monitor candidates have been identified, with a final impending selection to be based on sensitivity requirements and instrument performance.

Future Outlook

Once the CBRN sensors have been completed, they will be interfaced to a communications node and the operation of the sensors and node will be validated. In parallel, the overall software for the network will be developed, to allow seamless integration of the sensors, data analysis, and data transmission to a remote control center. Special attention will be given to data formats to allow integration with networks being developed in Canada, the USA and Europe. Once the system has been integrated, the federal project partners will conduct extensive performance tests on the network. When the project is completed in February 2006, Canada will be equipped with a powerful, deployable sensor network that will enable early detection and rapid response to CBRN emergencies.

CRTI-02-0043TA

Accelerated Consequences Management Capabilities

PROJECT LEAD:
DRDC Suffield

FEDERAL PARTNERS:
Environment Canada

INDUSTRY PARTNERS:
Vanguard Response Systems Inc.

AUTHORS:
J. Garfield Purdon, Andrew Burczyk and Michele Mayer, DRDC Suffield, PO Box 4000 Stn Main, Medicine Hat, AB, T1A 8K6, tel: (403) 544-4106, email: Garfield.Purdon@drdc-rddc.gc.ca

Objectives

This project will accelerate development of the Blast Guard System, now renamed the Universal Containment System (UCS), a containment/mitigation/decontamination system for chemical/ biological/radiological warfare (CBRW) agents. This system consists of a lightweight, tent-like enclosure of specialized fabric, which is filled with one of several decontaminating foam formulations to absorb blast and fragments, neutralize bio-chemical substances and remove radiological particles from surfaces.

The UCS, currently in service with National and Regional CBRN response teams, can be used in a variety of scenarios such as: discovery of a package suspected of containing BW, CW or radiological agents; an enclosed area which is contaminated by a known C or BW agent or a CBRW terrorist attack on a specific target or event. The decontaminating foams can be used by First Responders to contain, mitigate, or decontaminate areas. In the case of a suspicious package, a portable enclosure erected over it will suppress the explosion when the package is purposely detonated or disrupted and the introduced foam will encapsulate any aerosolized agents present while containing the device fragments. The foam can be applied to a contaminated enclosed area, such as the inside of a room or vehicle, to both contain and decontaminate any CBRW agents. The foams can also be used to decontaminate larger areas, including buildings, equipment, vehicles and terrain in the event of a terrorist attack at sporting, political or high profile events.

More research on these foams is required to address several issues in their performance in CBRW situations. These include environmental effects, operating temperature range limits, performance against a wider spectrum of agents on a variety of surfaces and the extension of the decontamination technology to assess its long term effects and to examine remediation measures. This information will then be applied to the design of an improved product that can be used in further CBRN scenarios. This research is being carried out in five areas:

- Determine decontamination effectiveness of UCS foam formulations when applied to surfaces contaminated with representative CW agents simulating emergency procedures. This is achieved by GC analysis of vapour concentrations of the CW agents, which desorb into a flowing air sweep above a surface, which has been contaminated/decontaminated or residual agents in GC analysis of a liquid extraction of the decontaminated surface. The surfaces are representative of materials in an office environment (e.g. porous surfaces such as alkyd wall paint on dry wall, latex wall paint on drywall, varnished wood, ceiling tile, carpet, concrete and

asphalt, and nonporous surfaces such as Chemical Agent Resistant Coating (CARC) on steel, alkyd paint on steel, window glass, anodised aluminium and vinyl tile). The studies use two CW agents, HD and GD, using both a scrubbing and a non-scrubbing procedure to simulate different field decontamination techniques. This work is being undertaken at Suffield by both DRDC and VRS personnel. (December 2005)

- Determine the liquid-phase rates/stoichiometries/products of reaction of UCS formulations with selected traditional/potential CW agents (e.g.,KCN, HD, L, GA, GB, GD, GF, VX, R33, and T2 toxin) using spectroscopic and chromatographic analysis techniques. The effectiveness of UCS in detoxifying selected BW agents/simulants (including yersinia pestis, vaccina, and anthrax) at predetermined contact times will also be examined. This will be performed at DRDC Suffield by DRDC, O'Dell Engineering Ltd., and VRS personnel. (January 2006).
- Assess the environmental impact of UCS usage to determine the need for any post-treatment or effluent containment. Testing will include both aquatic toxicity and soil toxicity tests undertaken by Stantec Consulting contracted by VRS; the results will be reviewed in consultation with Environment Canada. (November 2004).
- Modify formulation to permit UCS surfactant concentrates to operate over a wider climatic range, more suitable to the Canadian winter environment. This work will be contracted by VRS to McMaster University and Farrington Lockwood Company Ltd (FLCL). Modified formulations will be evaluated by field trials conducted by the RCMP held at and assisted by DRDC Suffield. (December 2005).
- Investigate extension of the UCS for remediation measures. VRS will evaluate the generated data in order to optimize the UCS application equipment, which could allow for mass or wide-area decontamination and remediation. A database of available information on performance against agents on a variety of surfaces will be developed for end users. (February 2006).

Recent Progress

This project commenced on August 1st 2003. At DRDC, most CRTI-funded equipment has been procured, installed, and placed in service. Desorption studies have been carried out on controls of panels of alkyd coated metal, vinyl floor tile, glass, anodized aluminum, ceiling tile and wood and decontaminated (no scrubbing) plates of wood, glass, aluminum and, the worst case thus far, ceiling tile. Methodology development is nearly complete for characterization of liquid reaction solutions for sulfur mustard (HD) and related products using LC-FPD and LC-MSD; the latter resulting in a novel method for detection and quantitation of HD by LC-MSD which will be presented at an international conference on decontamination (May 2004). Arrangements have been made for assessment of BW agent effectiveness on receipt of modified formulations.

Future Outlook

HD desorption studies will continue on the remaining surfaces followed by comparison of effectiveness with/without scrubbing. The investigation will then address similar determinations with GD. Characterization of HD liquid phase reaction is underway and will be followed by similar studies with KCN, L, GA, GB, GD, GF, VX, R33, and T2 mycotoxin. Assessment of effectiveness against yersinia pestis, vaccina and anthrax will be undertaken with original or modified UCS formulations following results from VRS into modifying the surfactant gelling point. Conclusions from the environmental assessment will determine whether additional post-treatment of formulation effluent will be required. A field trial using a formulation incorporating all modifications will be undertaken to verify the effectiveness and utility of the finalized recipe. The result will be a formulation/procedure which will have minimal long-lasting environmental consequences, a lower temperature usability and documented evidence of effectiveness on a variety of civilian and military surfaces against a variety of CBW agents. Information will be developed to estimate the likelihood that UCS formulations could address the long-standing problems of mass or wide-area decontamination and remediation. Information obtained will assist First Responders to use the UCS more effectively for immediate response and near real-time consequences as well as providing essential information for training First Responders in its use. Longer-term consequence management capabilities will be determined and critical information will be obtained from the desorption data on various surfaces, the stability and toxicity of any end products, the environmental impact study and the investigation of the system for further remediation measures. UCS is unique in that it captures the forensic evidence enabling device reconstruction and analysis, and it is expected that this research will be vital in the criminal analysis of the ensuing foam residue, and result in the identification of any agents used.

CRTI-02-0045RD

Forensic Optically Stimulated Luminescence (OSL)

PROJECT LEAD:
DRDC Canada, Department of National Defence

FEDERAL PARTNERS:
Canadian Security Intelligence Service (CSIS), Royal Canadian Mounted Police (RCMP)

INDUSTRY PARTNERS:
Bubble Technology Industry, Inc. (BTI)

AUTHORS:
Marc Desrosiers, Dr. Tom Cousins, DRDC Ottawa, 3701 Carling Ave., Ottawa, Ontario, K1A 0Z4, tel: (613) 949-2739, email: marc.desrosiers@drdc-rddc.gc.ca, tom.cousins@drdc-rddc.gc.ca.

Objectives

Theft or loss of radioactive sources is a situation of major concern to the radiological/nuclear counter-terrorism community. Such sources – even those of moderate activity (few Ci) - are usable in Radiological Dispersal Devices (RDDs) that may contaminate and subsequently paralyze physically large urban infrastructures.

Tracking and attribution in such cases is a difficult problem, as almost all conventional radiation detection methods demand that the sensor be in close proximity to the current physical location of the source in order to find it. Thus the simple act of periodically moving a source will confound existing techniques.

This project seeks to develop, field trial and produce a new system to aid civil authorities in accurately determining *former* radioactive source locations. The method has at its heart the immutable physical property that any substance, when exposed to ionizing radiation, will trap electrons in excited, metastable states. The forced depopulation of these states (via laser irradiation) will result in the concomitant emission of photons. A measurement of these photons (at certain distinct energies) will give proof-positive that a radioactive source was in close proximity to the said substance. Thus this Optically-Stimulated Luminescence (OSL) technique will aid civil authorities in determining former source locations to aid in both tracking source movement (and possibly predicting future movement) and in legal attribution that the source was in an individual's possession.

The first year of the project has been spent on the construction and testing of a pilot lab-based system to study OSL. This provides the opportunity to ascertain the magnitude of the OSL signals of various materials. From these materials, the most promising candidates will be further analyzed and characterized. The knowledge thus garnered will be then used to design and build an upgraded laboratory system optimally suited for forensic OSL. From this laboratory system a field system will be developed and tested.

Recent Progress

The initial stages of this work have concentrated on an examination of *what* materials are most amenable to the OSL technique, and an indication of their *sensitivity*. It is fair to say that effectively all materials tested so far show some degree of OSL-sensitivity. The key to advancing the project is to determine those materials that demonstrate a constructive combination of real-world abundance and OSL-sensitivity. In many ways, the future of the project is the age-old (radiation R&D) issue of improving upon signal-to-noise ratio via shrewd experimentation.

The current materials, which are being or have been investigated, are as follows:

i) TLDs such as: Al_2O_3, LiF:Mg Cu P, CaF_2:Mn, $Li^2B^4O^7$:Mn. All these materials are used commercially in Thermal Luminences Dosemetry (TLD), and were clearly expected to yield strong OSL signals. These materials give us a baseline for testing the equipment and signal processing methods.

ii) Some common "ubiquitous" materials including: pottery, brick, patio stone, assorted rocks, cement, concrete, ceramics, dolomite (lime), gravel, feldspar, sand and scapolite. Most of these materials can contain some quantities of Al_2O_3, SiO_2 or other materials that are known to exhibit strong OSL signals.

iii) Some common building material such as: drywall, ceiling tiles, paint, shingles, cedar, linoleum and plastics (LDPE, HDPE, PTFE, etc…).

iv) A few common household materials including: table salt, dishwasher detergent (dry), hand soap and sugar.

Table 1 summarizes some of the results so far:

Material	Current Threshold for OSL Signal (mGy)
Al^2O^3	0.1
Table Salt	1
Feldspar	10
Sand/Cement	1000

Table 1 : Portions of Current OSL Database

An excellent preliminary OSL database has thus been established.

Future Outlook

Armed with the existing and continuing-to-be expanded database of materials, the project will seek to determine field efficacy. This will be done via a variety of methods:

i) Calculations to determine the length of time a given source must be in proximity to a given candidate material to produce a reliable OSL signal.

ii) Verification of these using well-designed experiments.

iii) Input from investigatory agencies (CSIS and RCMP) as to how the final product should be tailored to meet their needs.

iv) Field trials of prototype Forensic OSL system.

v) Delivery to constabulary.

CRTI-02-0053TA

A Simulation Based Decision Aid for the Optimization of Detection, Protection and Decontamination Systems with Team Structure and Procedures

PROJECT LEAD:
Defence R&D Canada

FEDERAL PARTNERS:
Defence R&D Canada (DRDC), Directorate of Nuclear, Biological & Chemical Defence (DNBCD)

AUTHOR:
David Unrau, Greenley & Associates Inc., 5 Corvus Court, Ottawa, ON K2E 7Z4,
tel: (613) 247-0342 x 205,
email: dunrau@greenley.ca

Objectives

The simulation based decision aid tool is a CBRN Research & Technology Initiative (CRTI) sponsored project to accelerate the integration of technologies that will allow the user to conduct full multidimensional visual simulations of Chemical, Biological, Radiological and Nuclear (CBRN) response across an area of operations. These simulations will incorporate the time varying dispersion of a hazard, the first response personnel, their procedures and their equipment in the context of a specified geographic area. The user will be able to specify, execute and analyze scenario options including the numbers and types of detectors, the protection systems and the decontamination systems within the operational context, along with the number and types of emergency response units and their procedures.

The decision aid will be used to conduct trade-off analyses for acquisition, to plan operations and to conduct training. Throughout the project, first responders from the City of Ottawa, the Ontario Provincial Emergency Response Team, the Canadian Forces Fire Marshals, the Joint Nuclear, Biological and Chemical Defence Company and other groups will be interviewed to solicit their input into the development of the application. These groups will also be engaged in evaluating the application as it is developed to maintain the end user focus of this project.

The decisions that must be made in terms of acquisition, deployment and procedure development must not be taken in isolation due to the interdependencies across systems and across the levels of preparedness. The need for a CBRN decision aid is based on the requirement for the decision maker to be able to:

- Maintain an understanding and appreciation for the CBRN protection construct across the different levels of preparedness or response.
- Develop scenarios for different operations, whereby alternative configurations of detection, protection, decontamination, and procedures can be simulated at the tactical level in the context of different environmental and CBRN threat conditions.
- Conduct 'what if' analyses at the tactical level to allow the user to evaluate the cost/benefit of different configurations of detection, protection, decontamination, and procedures.
- Conduct 'what if' analyses at the technical level, whereby the performance characteristics of different detection, protection, decontamination, and procedural systems can be changed and re-evaluated within the tactical scenarios.

Recent Progress

The project is currently in the requirements definition phase. Members of the first response community at the municipal, provincial and federal levels have been interviewed to solicit their input for the project. Task flows describing the user's actions and defining their interaction with the software application have been developed and will be validated by members of the user community. Technical development has begun on the integration of the various software systems required to implement the decision aid application. Interfaces have been developed to scientifically support dispersion models, and an initial capability to visualize hazard dispersion information in 3D has been demonstrated. Current technical work is focused on the development of a simulation framework suitable for the simulation of CBRN hazards and response related activities. Collaboration with the City of Ottawa's Geographic Information Systems (GIS) Department has lead to the development of an initial 3D simulation of the City of Ottawa.

In the near future, the core user requirements for the decision aid system, expressed as task flow diagrams, will be reviewed and validated by the user community. On this basis, technical development will proceed until the fall of 2004, when an initial version of the system will be operated and evaluated by representatives of the user community. This evaluation will enable the user requirements and system design to be further refined. Based on this refined design, development of the final system will proceed until the spring of 2005, when the final system will be reviewed by the user community.

Future Outlook

Significant outputs of the project will include:

- **The Decision Aid Software Application,**
- **An initial database characterizing CBRN detectors, protective clothing and response related equipment for simulation purposes, and**
- **A framework for simulation of CBRN response, populated with an initial selection of equipment and entity models.**

The decision aid software application will be of direct utility to members of municipal, provincial and federal first responders, especially those in Emergency Operations Centre and Training roles. Future application of this technology could include support of operations, in addition to the support of planning, analysis and training activities.

CRTI 02-0057TA

Canadian Radiation Alert / Expert System for Critical Infrastructure Monitoring

PROJECT LEAD:
Health Canada

FEDERAL PARTNERS:
CCRA

AUTHORS:
Ed Korpach,
tel: (613) 952-5658,
email: ed_korpach@hc-sc.gc.ca;

Kurt Ungar,
tel: (613) 954-6675,
email: kurt_ungar@hc-sc.gc.ca;

Grant Gallant,
tel: (613) 941 9552,
email:
Grant.Lab.Gallant@ccra-adrc.gc.ca.

Objectives

Development of this project will create a comprehensive expert alert system to process and evaluate continuous isotopic and radiation field measurements, which alarms with high sensitivity and low false alarm rates. It will provide event classification and efficient information distribution to assist Laboratory Cluster management of radionuclide incidents. The goals of this initiative are the development of real-time alarming, isotope/incident identification, automated, high-sensitivity numerical full spectrum analysis, high-speed data/results transmission to multiple remote sites and secure web access to network information for first responders and central decision makers. This innovation will provide cluster support allowing critical emergency information sharing among multiple users. Output would be linked to decision support systems for agriculture, environment, and infrastructure using GIS maps to coordinate municipal, provincial and federal responses. It will provide measurement capabilities that facilitate early detection and rapid assessments of radionuclide contamination.

Recent Progress

Real time isotope identification has been completed and delivered to CCRA. In addition to the identification, an audio enunciator has been added to improve the system's operation with a single driver / operator. The real time isotope identification for mobile applications has been successful and is being actively tested at 2 ports. The project is looking into utilizing the identification software in helicopter search / survey applications.

Real time alarming software has been successfully deployed into some of the existing monitoring stations. The data server has the ability to receive incoming calls and notify the operator visually that an alarm has occurred.

Future Outlook

This work will continue with the integration of the real time isotope identification system and the real time alarming software. More sophisticated and sensitive alarm protocols will be developed. Integration of the alarm with a notification procedure will be created.

These innovations will allow for the development of a complete radiation alert, radionuclide-monitoring product capable of low false alarm rate, high sensitivity detection of unusual releases of radioactive materials and detection of unusual traffic of radioactive materials. The information technology component will integrate the alarms into the operational response to a nuclear incident and provide timely reporting and analysis to first responders and central decision makers.

CRTI-02-0066RD

Development of Simulation Programs to Prepare against and Manage Bioterrorism of Animal Diseases

PROJECT LEAD:
Canadian Food Inspection Agency

FEDERAL PARTNERS:
Environment Canada

OTHER PARTNERS:
United States Department of Agriculture, Animal and Plant Health Inspection Service, Ontario Ministry of Agriculture and Food, University of Guelph, Colorado State University.

AUTHOR:
Dr Caroline Dubé, 174 Stone Rd W., Guelph, Ontario, N1G 4S9,
email: dubecm@inspection.gc.ca

Objectives

The intentional release of highly contagious agents of livestock and poultry could have serious consequences to Canada's agriculture and economy. Successful control and management of such outbreaks require that adequate response strategies be developed beforehand. Simulation models have been used in the past in veterinary medicine to evaluate optimal control strategies for diseases of livestock. These models enable decision-makers and emergency preparedness personnel to explore the "What if?" scenarios and determine the effects of various control measures, such as vaccination, on the size, duration and cost of outbreaks. Such models require good quality data on farms, and on the spread of disease agents. Effective emergency management systems that store such data in "peacetime" and record information on the progression of an outbreak are able to provide the required data for disease simulation models.

The objective of this project is the development of simulation models to plan and predict the extent of outbreaks, using data from an animal health emergency management system used by CFIA field personnel during a terrorist-mediated outbreak of an animal disease. This is a 4-year project that started in July 2003 and will end in December 2007. The first year was dedicated to the development of a stochastic simulation model for the spread of some potential bioterrorist agents of animals: Foot-and-Mouth Disease, Classical Swine Fever (Hog Cholera), Highly Pathogenic Avian Influenza and Exotic Newcastle Disease. Two versions of this model were developed. The first version was developed by the USDA-APHIS to work on portable computers and desktops. This model was first developed in 1999 and was subsequently modified as part of this project by USDA-APHIS and Colorado State University with collaboration of all project partners. The second version is a supercomputer model developed by the University of Guelph as part of this project with input from all project partners, with USDA-APHIS providing the source code of its model.

The second year of the project will be dedicated to the development of a wind dispersion model by Environment Canada to predict the spatial spread of agro-terrorism agents of animals that can be spread by wind. Validation of the stochastic simulation model will also take place in the second year and includes expert panel reviews, comparison of model outputs with past outbreaks and comparison with other existing models. The third and fourth year will include the testing and implementation of the animal health emergency management system. This system will have a desktop and remote component enabling field inspectors to quickly enter data into the system while on the farm. It is composed of a core group of applications that track animal health records, farm location data, inspection dates and laboratory results, and includes another application specialized in the management of emergencies that will be linked to the core group. All these applications are critical to provide the disease simulation models with accurate and timely information.

The identification, through modelling, of critical factors of large outbreaks in North America, and the creation of a North American bank of hypothetical outbreak scenarios with pre-identified optimal control measures will also be accomplished in the third and fourth year of the project.

It is expected that the tools developed in this project will increase North America's preparedness against intentional release of animal disease agents through improved preparedness, decision-making and outbreak management tools.

Recent Progress

The stochastic simulation models were developed and programmed during the first year of the project. Testing of the two versions against each other was achieved through a series of test suites developed by the University of Guelph to ensure that the two versions included the same concepts and would give approximately the same results when using the same input parameters. The University of Guelph developed an approach to infectious disease model validation that could be used by other model developers in the world. Such work will be presented at the GISVET conference in Guelph, Ontario, June 23-25.

An expert panel that includes disease and modelling experts from around the world was put together. These experts have agreed to meet June 14-18, 2004 to validate the assumptions, processes and programming used in the stochastic simulation models. The objective is to further increase the model's credibility internationally and domestically, increasing decision-makers' confidence in the uses and results obtained from the model.

A North American Modelling Team was created within the North American Animal Health Committee to collaborate on the use of the stochastic simulation model within Canada, the United States and Mexico. The team was trained in February and May of 2004.

Environment Canada completed the review of various existing wind dispersion models for animal diseases and is in the process of including biological parameters for disease agents in their generic particle dispersion models.

Future Outlook

The following activities are planned:

- **Validation of the stochastic simulation models between April 2004 and January 2005;**
- **Development of the beta version of the wind dispersion model by July 2004;**
- **Sensitivity analyses of the stochastic simulation model and identification of critical factors of large outbreaks in Canada between January 2005–January 2006;**
- **Finalized version of wind dispersion model by September 2005;**
- **Evaluation of various release and outbreak scenarios with identification of optimal control measures, creating the bank of scenarios for Canada: January 2006–June 2007;**
- **Deployment of core group of applications of animal health emergency management system: July 2006;**
- **Deployment of remote version of core group applications of animal health emergency management system: January 2007;**
- **Deployment of emergency management application within animal health emergency management system: May 2007;**
- **Implementation and installation of animal health emergency management system nationally: December 2007.**

CRTI-02-0067RD

Restoration of Facilities and Areas after a CBRN Attack

PROJECT LEAD:
Environment Canada

FEDERAL PARTNERS:
Health Canada, Environment Canada, DRDC

INDUSTRY PARTNERS:
Science Applications International Corporation, United States Environmental Protection Agency, VLN Ottawa, Vanguard Stoney Creek, and Hytec Calgary

AUTHORS:
Merv Fingas, Environment Canada,
335 River Ottawa,
tel: 998-9622,
email: Fingas.merv@etc.ec.gc.ca;

Dr. Stefan Wagener, Canada Science Centre, Winnipeg, Man.,
tel: (204) 789-2029,
email: Stefan_Wagener@hc-sc.gc.ca;

Dr. Tom Cousins, DRDC-O,
Ottawa, ON,
tel: (613) 998-2312,
email:
Tom.Cousins@DRDC-RDDC.gc.ca.

The objective of the project is to gather and compile information on and subsequently test and validate all known procedures for restoration of areas including buildings, exteriors of buildings, the interior contents of buildings, and areas adjacent to buildings, such as parking lots, lawn, vehicles, etc. This includes the air inside the building and the surfaces contaminated. The restoration includes pickup, neutralization, decontamination, removal and final destruction/deposition of the contaminant, cleaning/neutralization of material and contaminated detritus resulting from the act. Further, the project is intended to develop new ideas and test existing ideas for application to the restoration process.

This project is an R&D effort, and deals with chemical, biological and nuclear contamination. The object is to develop a suite of methods to decontaminate and restore buildings and areas after a CBRN attack. At least 16 methods will be tried. These include pickup of the contaminant, neutralization or encapsulation, concentration or separation and final disposal. Some methods may involve more than one process and may include neutralization or destruction as well as pickup. Concepts will be collected from a variety of sources including the extensive work conducted in the United States. This work includes extensive linkages to several government agencies in the United States and their private contractors. The procedure includes a wide-sweeping survey and then a test of the best candidates on a laboratory scale. All selected chemical target items will be tested, as well as at least one model biological candidate and at least one nuclear isotope. This effort will also assess those methods that apply across the CBRN spectrum.

The first phase consists of laboratory scale experiments in which the efficacy of some of the proposed concepts will be tested, using standard laboratory techniques. The second phase, which consists of the radiological portion, will proceed along the premise that radiation decontamination is a two-step proposal consisting of removal and concentration/removal of the radioisotopes from the removal fluid. The third phase is the testing of the remaining candidate procedures on a small scale. NATO and DRDC-Suffield have developed 'standard' tests with a small surface token. The testing of chemicals will be conducted at the Environment Canada facility in Ottawa, the biologicals at the Health Canada facility in Winnipeg and the radiologicals at the radiological facility at DRDC-Ottawa. The fourth phase is the preparation of procedures for decontamination and restoration. A trade-off decision basis will be developed to provide information on abandonment and quarantine versus cleanup. The fifth phase of the project is the preparation of a detailed report covering all phases of the work. The report will form the basis of a detailed manual for restoration of facilities.

The work includes twenty steps, four of which have now been completed:

1. Review available literature on the topic of chemical, radiological and biological decontamination methods including the processes of contaminant pickup, neutralization, encapsulation, concentration, separation and final disposal of the cleaning materials and detritus left by the incident. Personnel involved in decontamination projects world-wide will be engaged for ideas and previous experience in the topic.
2. Use the priority CBRN substances/organisms and threats and characterize the most probable types, and cluster the substances/organisms into classes for decontamination evaluation purposes. Characterize the most probable types and the characteristics of the contamination and waste that would be created for each of the chemical, biological and radiological facets.
3. Meet with all available Canadian and US agencies with expertise and experience in the restoration aspects noted in statement one above.
4. Review the concepts already in progress, such as some of the patented decontamination materials and procedures already used on previous CBRN attacks.

Objectives

1. Research and test new methods for restoration of areas and facilities after a CBRN attack;
2. Collect known methods of restoration and evaluate those concepts;
3. Prepare manuals of procedures for the restoration of buildings and other areas;
4. Develop new ideas for the restoration of areas;
5. Evaluate and test all ideas for the restoration of facilities in the laboratory and on a small scale; and
6. Develop procedures for contaminant pickup, neutralization, encapsulation, concentration or separation and final disposal.

Recent Progress

The first year of the project is now complete and largely consisted of literature review and some controlled laboratory experimentation. The literature search turned up about 300 documents, far more than originally estimated. However, many of the documents are not quantitative. Numerous general papers on the topic exist. The literature, which did not turn up significant new ideas, has now been distilled into a detailed literature review along with summary tables. Several new ideas were generated by the work group, particularly in the chemical portion. Studies of Fenton's reagent as a general decontaminant have been conducted both at Environment Canada's laboratories and at DRDC-S. This shows that there may be great potential for this reagent in general decontamination, particularly chemical and biological.

A laboratory study was completed on the feasibility of decontaminating various building materials such as: wood, ceiling tile, arborite, carpet, wallboard, etc. The results of this study are forthcoming, but will show the future of attempting to decontaminate these surfaces. The conclusions may be one of two, either that the particular surface can be decontaminated in-situ or that thorough decontamination is not possible and that material would have to be removed and dealt with by other means (e.g. incineration or strong decontamination in a process stream).

The remaining steps include:

5. Generate new ideas for the various restoration actions and consolidate these through a series of brainstorming sessions.
6. Collect and evaluate all the concepts from work statements 1 to 5 above into a major report. Assess this information in the context of specific potential applicability, advantages and disadvantages for the priority substances/organisms identified in the CRTI clusters as priority items. Identify acceptable and best available technology and practices that could be used to meet the threat with some confidence. Identify and summarize areas of deficiency.
7. Conduct laboratory experiments on the ideas to ascertain the feasibility of approaches and effects on selected substrates.
8. Re-evaluate new concepts based on the laboratory tests.
9. Design small-scale tests for all three target groups - chemical, biological and radiological. For radiological decontamination, a surrogate item may be chosen for preliminary tests, but live agents are the only approach to validate any technique. The target groups for each cluster will include all top priority substances, organisms and threats from each of the CBRN assessment threats. Standard tests such as those implemented at DRDC-Suffield for chemical and biological agents will be reviewed for applicability here. Items tested at Suffield will not be re-tested, but results incorporated into this series.
10. Subject the best concepts for each set of target CBRN items to small-scale tests for process of contaminant pickup, neutralization, encapsulation, concentration, separation and final disposal of the cleaning materials and detritus left by the incident.
11. Review the test results through each of the matrix of agents and restoration processes. Re-evaluate the possible restoration processes and any new ideas. Treat the data to develop estimates of cleanup potential for each method. Compare these values to known or published cleanup objectives.
12. Re-test new ideas on a small scale and any first-stage ideas that require re-testing.
13. Develop procedures based on the small-scale and mid-scale tests. Incorporate procedures from other studies noted in the earlier steps above.

Future Outlook

14. Develop a cost estimate for the methods and prepare a decision tree on restoration methods including a decision method to decide between cleanup and quarantine.
15. Re-evaluate the cleanup methodologies against the list of priority substances/organisms.
16. Re-visit key sources of information in Canada, U.S. and elsewhere to verify information, gather new information and seek comment on current findings.
17. Prepare a final report on all findings and information.
18. Prepare a detailed manual on restoration procedures.
19. Review all reports or manuals, and make necessary changes.
20. Disseminate the reports to those who can use or further disseminate the literature.

CRTI 02-0069RD

Molecular Epidemiology of Biothreat Agents

PROJECT LEAD:
National Microbiology Laboratory, Health Canada

FEDERAL PARTNER:
Defence R&D Canada- Suffield

AUTHORS:
Louis Bryden, National Microbiology Laboratory, Winnipeg, MB,
tel: (204) 789-2000,
email: louis_bryden@hc-sc.gc.ca;

Dr. M. Mulvey, National Microbiology Laboratory, Winnipeg, MB,
tel: (204) 789-2133,
email: michael_mulvey@hc-sc.gc.ca;

Doug Bader, Defence R&D Canada – Suffield, Medicine Hat, AB,
tel: (403) 544-4650,
email: doug.bader@drdc-rddc.gc.ca;

Dr. A Kabani, National Microbiology Laboratory, Winnipeg, MB,
tel: (204) 789-6056,
email: amin_kabani@hc-sc.gc.ca;

Eric Leblanc, Infectious Diseases Research Center, St. Foy, QC,
tel: (418) 654-2705,
email: eric.leblanc@crchul.ulaval.ca;

Michel G. Bergeron, Infectious Diseases Research Center, St. Foy, QC,
tel: (418) 654-2705,
email: michel.g.bergeron@crchul.ulaval.ca.

Objectives

The National Microbiology Laboratory, in conjunction with Defence R&D Canada–Suffield, is establishing a national molecular typing capability for *Bacillus anthracis*, *Francisella tularensis* and *Yersinia pestis*. Rapid detection and characterization of human pathogens is critical to minimize the impact of bioterrorism events and to facilitate a microbial forensic investigation. The capability of providing strain-level DNA signature identification will allow us to conduct an epidemiological investigation to trace the possible source of an outbreak resulting from the deliberate release of a biowarfare agent and to provide forensic investigational capability during a biocrime investigation.

Molecular typing technologies such as multi-locus variable-number tandem repeat analysis (MLVA), multi-locus sequence typing (MLST) and single nucleotide polymorphism (SNP) genotyping are being implemented for strain level signature generation of isolates involved in an event. MLVA is a highly discriminatory subtyping method that characterizes genetic loci that change at a high frequency, and is useful for determining whether one strain is related to another over a relatively short period of time. MLST will be developed to characterize genetic loci that evolve at a slower but steady rate and can be used to subtype the organism into a larger clonal group. Single nucleotide polymorphisms (SNPs) provide useful targets as genetic markers for molecular, population and evolutionary studies especially in clonal bacterial species and are amendable to high throughput analysis.

Recent Progress

No information was known about the molecular diversity of the Health Canada bacterial collection of *B. anthracis*, *Y. pestis*, or *F. tularensis* isolates. Minisatellite markers were identified in all three pathogens and multi-locus variable-number tandem repeat analysis (MLVA) was used to type the isolates in the collection. MLVA sequence analysis of the eight *B. anthracis* loci classified 6 isolates in the A1.a global grouping, 1 isolate in the A3 group and 1 isolate as a new genotype differing at the vrrC1 locus (Keim et al., 2000. Journal of Bacteriology 182:2928-2936). Eleven distinct genotypes for *F. tularensis* were identified, based on the analysis of 9 VNTR loci, and we identified that all 6 *Y. pestis* isolates were unique based on sequence analysis of 14 polymorphic loci. High throughput procedures are currently being developed based on size-separation of marker alleles using an ABI 3100 Genetic Analyzer for *B. anthracis*. The use of Bionumerics software has enabled the creation of a reference database to facilitate outbreak tracking.

An assessment has been conducted on the utility of using SNPs identified in the virulence plasmid pX01 of *B. anthracis* as potential markers for genetic discrimination of these isolates. Comparative sequence analysis of these markers revealed no sequence variation with the genetically distinct Canadian isolates. Comparison of the Canadian sequences with the "Florida" strain sequence revealed seven SNPs, whereas ten SNPs were observed in comparison with the "Sterne" strain. The lack of variation in the pX01 plasmid SNP markers did not correlate with the genetic variability established by MLVA analysis for the Canadian isolates and thus are of limited value as markers of species diversity. Other potential markers based on pX02 SNPs and INDELS in the *B. anthracis* chromosome will be investigated.

Future Outlook

The objective for the year to come is to complete MLVA subtyping for all *B. anthracis, Y. pestis* and *F. tularensis* isolates found in the national collections (Health Canada and DRDC Suffield), and to create a Bionumerics database of typed strains based on sequence and MLVA fragment profiles. The team will continue to assess identified SNP targets on the plasmids and in the genome of *B. anthracis* and will begin screening potential markers that are suitable for MLST analysis of *Y. pestis* and *F. tularensis* isolates as well as identify potential targets for SNP analysis. The final outcomes of the project will include the capability for high resolution subtyping of *B. anthracis, Y. pestis* and *F. tularensis* with high discriminatory indicies at two federal sites, the capability for high throughput characterization of isolates in a bioterrorist event situation and the creation of a national database of typed strains.

CRTI 02-0080RD

Psychosocial Risk Assessment and Management (RAM) Tools to Enhance Response to CBRN Attacks and Threats in Canada

PROJECT LEAD:
Institute of Population Health, University of Ottawa

FEDERAL PARTNERS:
Health Canada, Canadian Food Inspection Agency (CFIA)

AUTHORS:
L. Lemyre, 1, Stewart st. (#312), Ottawa, ON, K1N 6N5, tel: (613) 562-5800 x1196 (assist.x2321), email: louise.lemyre@uottawa.ca;

M. Clément, W. Corneil, R. Clarke, & D. Krewski.

Objectives

The Psychosocial Risk Assessment and Management (RAM) Tools project is an initiative of the Institute of Population Health of the University of Ottawa, led by Drs. Lemyre, Krewski and Clarke, along with the Institute for Risk Research of the University of Waterloo, in partnership with Health Canada, the Canadian Food Inspection Agency and the City of Ottawa. The project will provide an integrated framework for managing psychosocial aspects of CBRN risks with specific guidelines for CBRN agent risk assessment, perception and evaluation as well as risk communication. It will yield to practical bilingual field-based training tools to enhance the capability of key responders in Canada to mitigate the psychosocial and human health impacts of CBRN threats and attacks.

Canada is facing the heightened need to improve its preparedness to cope with the short-term, mid-term and long-term responses of CBRN threats or attacks. Research indicates that the behavioral and psychological impacts of CBRN terrorism may well be the most widespread, long-lasting and costly consequences. As the response to a CBRN terrorist event is unique depending on the agent and its expression, there is an emerging realization that the response can be conducted by an array of non-traditional first responders including local public health authorities, front-line health care providers, food inspectors, and lay responders. Adequate training of all key responders is crucial to managing the acute and long-term psychological effects of CBRN terrorism.

The project objectives are:

- A Canadian CBRN integrated psychosocial risk management framework articulating risk assessment with public perception and psychosocial dimensions to strengthen the capacity to rapidly launch effective response strategies to CBRN threats and attacks.
- A set of RAM tools and training with strategies, decision-trees and guidelines. The Psychosocial Modules will include evidence-based literature reviews and survey results that assess the perceptions of CBRN risks and psychosocial impacts of CBRN terrorism on the general public and first responders. The work will focus on various classes of agents, vectors and target populations, for both threats and actual attacks.

Recent Progress

Four evidence-based literature reviews were recently completed to serve as the basis to identify best practices in the field. These reviews focused on four different topics:

1) Review of CBRN agents with reference to behavioral impacts,
2) Review of the psychosocial impact of CBRN threats and attacks,
3) Review of the psychosocial interventions for CBRN threats and attacks, and
4) Review of the risk communication literature.

These documents will be later reformatted to better meet the needs of responders for ease of consultation, and an independent panel of experts will overview a set of derived recommendations.

A first network of responders and collaborators was established:

1) With the City of Ottawa and their emergency services initiatives
2) The Region of Waterloo and their community of first responders

A link to the following networks was also established:

a) The Biosecurity Summit Conference in Washington, DC,
b) The Counter-terrorism and Public Health Conference in Toronto,
c) A consultant and reviewer of the World Health Organization strategy,
d) The Australia-Canada Conference on Population Health at the University of Ottawa, Session on the Psychosocial Preparedness to Terrorism and Disasters, with Pr Beverley Raphael, and
e) A roundtable discussion with responders and policy-makers in CBRN Preparedness and Responsiveness.

Future Outlook

A second roundtable with key responders is scheduled, followed by thorough consultation with two test sites: Ottawa and Waterloo.Field trips are also scheduled to gather current psychosocial practices and needs.

Further literature reviews will be conducted to gather a list of emergency protocols that are currently available as well as a lexicon of terms and acronyms used in the field.

Based on literature synthesis and observation of practices, an Integrated Psychosocial RAM will be designed and tested with various communities of responders.

A needs assessment protocol will be followed for emergency services from focus groups and questionnaires.

A general public risk perception and needs assessment study will be completed through a major national survey supplemented by focus groups.

Then, in the third and fourth year of our program, a curriculum, supported by a panel of experts, will be established for the training modules. These will then be tested using on-site experiments, and revised accordingly.

Recent partnerships with the Canadian Red Cross, the Canadian Psychological Association, the Canadian Public Health Association as well as the Social Sciences and Humanities Research Council will also enable us to extend the results of our research.

CRTI 02-0091TA

Clostridium botulinum type A genomic DNA microarray

PROJECT LEAD:
Bureau of Microbial Hazards, Health Products and Food Branch, Health Canada

FEDERAL PARTNERS:
Institute of Biological Sciences, National Research Council of Canada

INDUSTRY PARTNERS:
Institute of Food Research, Norwich Research Park, Norwich, UK

AUTHORS:
Marjon H.J. Bennik[1], Michael W. Peck[1] and John W. Austin[2]

[1] Institute of Food Research, Norwich Research Park, Norwich, UK (tel 44-1603-255251; email Mike.Peck@bbsrc.ac.uk, Marjon.Bennik@bbsrc.ac.uk) and

[2] Bureau of Microbial Hazards, Health Products and Food Branch, Health Canada, Tunney's Pasture, Ottawa, ON (tel 613 957-0902; email John_Austin@hc-sc.gc.ca)

Objectives

By the creation of a total genomic DNA microarray for *Clostridium botulinum* type A, this project will provide (1) a rapid method to detect the presence of botulinum neurotoxin (BoNT) structural genes within microorganisms (addressing prevention, surveillance and alert needs), (2) a subtyping method based on comparative genomics (addressing forensic needs) and (3) a tool for gene expression studies in *C. botulinum* type A (addressing basic research needs).

The initial stage of the project involves the production and validation of the microarray for *C. botulinum*. All work to date has been carried out at the Institute of Food Research (IFR) in the UK.

The key milestones of the project are:

- Design of primers (October 2003)
- Synthesis of primers (March 2004)
- PCR amplification of the genes (July 2004)
- Printing of microarray (October 2004)
- Validation of the microarray (December 2004)
- Distribution of microarray slides (January 2005)
- Development of genome typing for *C. botulinum* (September 2006)
- Development of a microarray-based rapid detection and identification assay for BoNT producing clostridia (December 2007)

Recent Progress

Research at IFR is currently ahead of schedule. The genome sequence of *C. botulinum* Type A strain ATCC 3502 (Hall A strain) has been recently completed (http://www.sanger.ac.uk/Projects/C_botulinum/). The genome is 3,886,916 bp in size and reveals 3,649 genes. There is also a 16,344 bp plasmid, and the average G+C content is 28.2%. The microarray is based on this sequence, and also on the sequence of other available neurotoxin genes.

Primers have been designed in collaboration with Dr. Al Ivens (Sanger Institute), and have now been synthesized. These primers have been used for PCR amplification of the genes. A first printing of the microarray is due to take place in April at IFR. This microarray will contain 3,453 genes of the Hall A strain, and unique 5' and 3' sequences of the *C. botulinum* type B, C, D, E, F and G, and C. *baratii* type F neurotoxin genes.

Future Outlook

Further work is required to produce the first generation of *C. botulinum* microarray slides. This includes (i) testing and validation of the microarray, and (ii) further tests to include genes currently missing from the microarray. It is anticipated that the existing milestones will be completed on or before the agreed deadlines. Once the microarray has been completed, future research will involve use of the microarray for detection of clostridia containing structural genes for botulinum neurotoxin(s), genome typing of strains of *C. botulinum* and expression profiling.

A rapid microarray-based assay will be developed for detection and discrimination of all seven serotypes (A through G) of the botulinum neurotoxin producing clostridia. This will include all *C. botulinum* strains, *C. butyricum* strains producing type E neurotoxin, *C. barati* strains producing type F neurotoxin and *C. argentinense* strains producing type G neurotoxin. This assay should allow unambiguous identification of all seven serotypes of *C. botulinum* based on sequence differences in the botulinum neurotoxin structural genes. The array may also detect genetically modified neurotoxins. This microarray assay should be a simple, rapid, and robust method that would also be a potentially valuable tool for identification and characterization of botulinum neurotoxin producing clostridia.

The *C. botulinum* type A genome microarray will be used for genome-genome comparison studies with food, environmental and clinical isolates. Phylogenetic relationships between and among serotypes are of great interest, as this will provide insights into genomic differences underlying environmental distribution, growth and survival in foods. This will also allow typing of isolates at the individual gene level, providing a powerful tool for forensic investigation.

Additionally, the microarray will be used to study gene expression in *C. botulinum*. This will provide information regarding the molecular basis for important phenotypes of *C. botulinum* including germination and sporulation, and the response to altered environmental conditions.

Once developed, the microarray will be used at both the Botulism Reference Service for Canada and the IFR laboratories, and will also be used in other laboratories in Finland and France. The microarray will also be made available, at cost of production, to laboratories across the world.

CRTI 02-0093RD

Advanced Emergency Response System for CBRN Hazard Prediction and Assessment for the Urban Environment

PROJECT LEAD:
Canadian Meteorological Centre - Environment Canada

FEDERAL PARTNERS:
Defence Research and Development Canada (Suffield) - DND, Radiation Protection Bureau - Health Canada, Atomic Energy Canada Limited

INDUSTRY PARTNERS:
Kosteniuk Consulting Limited, University of Alberta (J.D. Wilson and associates), University of Waterloo (Waterloo CFD Engineering Consulting Inc).

AUTHOR:
Michel Jean, Canadian Meteorological Centre, Environment Canada, 2121 TransCanada Highway, North Service Road, Dorval, Québec, Canada, H9P 1J3,
email: Michel.Jean@ec.gc.ca .

Objectives

The release of chemical, biological, radiological, or nuclear (CBRN) agents by terrorists or rogue states in a North American city (densely populated urban centre) and the subsequent exposure, deposition, and contamination are emerging threats in an uncertain world. The transport, dispersion, deposition, and fate of a CBRN agent released in an urban environment is an extremely complex problem that encompasses potentially multiple space and time scales (e.g. a chemical agent may have a hazard range of only several to tens of kilometers, a biological agent may pose hazards over a range of several hundreds of kilometers, whereas radiological and nuclear agents may result in a hazard range of several to tens of thousands of kilometers). The availability of high-fidelity, time-dependent models for the prediction of a CBRN agent's movement and fate in a complex urban environment can provide the strongest technical and scientific foundation for support of Canada's more broadly based effort at advancing counter-terrorism planning and operational capabilities.

The objective of this project is to develop and validate an integrated, state-of-the-art, high-fidelity multi-scale modeling system for the accurate and efficient prediction of urban flow and dispersion of CBRN materials. Development of this proposed multi-scale modeling system will provide the real-time modeling and simulation tool to predict injuries, casualties, and contamination and to make relevant decisions (based on the strongest technical and scientific foundations) to minimize the consequences based on a predetermined decision making framework. This major undertaking has been divided into 5 major tasks.

Recent Progress

Component 1.
Models for the prediction of the complex flow in the urban environment at the micro-scale have been developed, implemented, and validated against a number of comprehensive and detailed data sets obtained from wind tunnel and water channel simulations of flow over and through various building arrays. The models are based on a Reynolds-averaged Navier-Stokes (RANS) approach with the hierarchy of turbulence correlation closure models based on a phenomenological two-equation model for the turbulence kinetic energy *(k)* and viscous dissipation rate (_). This two-equation closure model for turbulence was used with linear and non-linear eddy viscosity formulations for the Reynolds stresses (the former based on a Boussinesq type of eddy viscosity formulation and the latter based on a general quadratic constitutive relation between the Reynolds stress tensor and the mean velocity gradient field). The two-equation formulation is appealing because it provides

the transport equations for the turbulence that help account for some non-local and history effects but, at the same time, is not overly computationally intensive.

The predictive capabilities of the RANS equations for urban flow on the micro-scale, used with a two-equation turbulence closure model incorporating either linear or non-linear eddy viscosity formulations for the Reynolds stresses, have been validated against very detailed and comprehensive wind tunnel and water channel data sets for flow over and through two-dimensional and three-dimensional building arrays. Quantitatively, it was found that the prediction performance of these various RANS models was generally good—the quantitative agreement for the mean velocity is good, although the turbulence kinetic energy is generally underestimated by the models. An important conclusion of this study is that the standard *k*-_ turbulence-closure model with a linear eddy viscosity is perhaps the simplest complete turbulence model that could be used for the prediction of urban flows on the micro-scale. This model may be useful as a general purpose simulator of small-scale urban flows because it is robust and simple enough to be tractable numerically, and hence does not require excessive computing time. It is conceivable that this urban flow simulation model could provide all the statistics of the disturbed wind flow in a building array required as input to a physically-based model for the prediction of contaminant dispersion in the urban environment.

In the models described above, all the buildings in the cityscape were resolved explicitly in the sense that boundary conditions were imposed at all walls and roofs of every building. To reduce the computational cost of this approach, the project team investigated also the utility of representing groups of buildings in the cityscape in terms of a distributed drag force. To this purpose, the mathematical model for the prediction of flows within and over a building array based on an aggregation of groups of buildings in the array into a number of drag units, with the ensemble being treated as a continuous porous medium, has been developed. In particular, the team showed how a modified k-_ model for the prediction of the time-mean spatially-averaged wind and turbulence fields in an urban canopy can be derived systematically by time-averaging the spatially-averaged Navier-Stokes equation. This procedure ensures the mathematical and logical consistency of the source/sink terms in the mean momentum equations and in the supporting transport equations for the turbulence quantities.

Component 2

The work required to include the effects of urban terrain in the sub-grid scales of a mesoscale meteorological model (the Global Environmental Multiscale Limited Area Model or GEM-LAM) through an urban parameterization has been initiated. The development of this parameterization, called Town Energy Budget or TEB, is being developed with Environment Canada' Mesoscale Compressible Community model (MC2 model) and will be ported to the GEM-LAM later on. This parameterization is being developed in order to account for the area-averaged effects of form drag, increased turbulence production, heating and surface energy budget modification due to the presence of buildings/obstacles and urban landuse within the urban complex. The "urbanized" mesoscale model will be coupled downwards with the urban micro-scale flow models developed in component 1.

Component 3

Component three involves coupling the urban micro-scale flow models developed in component 1 with the "urbanized" mesoscale models developed in component 2. The interface between the urban micro-scale flow models and the "urbanized" GEM-LAM model is demanding in that the information transfer between the two models must honor physical conservation laws, mutually satisfy mathematical boundary conditions, and preserve numerical accuracy, even though the corresponding meshes might differ in structure, resolution, and discretization methodology. Inter-grid communication allows the coarse mesh solution obtained by the GEM-LAM model to impose boundary

conditions on the fine mesh of the urban micro-scale flow model (one-way interaction), and furthermore permits feedback from the fine mesh to the coarse mesh (two-way interaction). The coupled system can be interpreted as a hybrid RANS/VLES system where the "very large eddy simulation" (VLES) represented by the mesoscale model (GEM-LAM) will use information from RANS for high-resolution simulation of flows near and around buildings, but allows spatial fluctuations to develop and evolve on the larger scales.

Component 4
Component four will involve using the mean flow and turbulence predicted by the multi-scale flow model completed in component 3 to "drive" a Lagrangian Stochastic (LS) model for the prediction of urban (and atmospheric) dispersion of CBRN agents. The application of LS models to atmospheric dispersion in general (and urban dispersion in particular) is recommended because LS models are (1) (in principle) the most flexible and the most easily able to incorporate all the known statistical details on the complex urban flow and (2) physically transparent, and easily adapted to handle particulates, biological or radioactive decay, dry and wet depositions, and other source and sink mechanisms.

Component 5
Component five involves the verification and validation of the multi-scale modeling system for both the flow and dispersion components. In the model validation effort, past and future (planned) comprehensive urban flow and dispersion experiments will be leveraged (e.g. Urban 2000, Mock Urban Setting Test, Joint Urban Trial 2003). The validation effort will enable a whole system test of the modeling system for both flow and dispersion, and will provide the user with information of the accuracy and fidelity of the model predictions for flow and dispersion over the complex urban environment.

Future Outlook

Successfully implementing the research methodology described above will result in a high-fidelity multi-scale CBRN modeling system that will be fully operational at the Environmental Emergency Response Division at Canadian Meteorological Centre. This resource can serve as a nation-wide general problem-solving environment for first responders involved with CBRN incidents.

CRTI 02-0093TA

Recherche sur les polymères avancés pour une application destinée à l'équipement de protection personnelle

CHEF DE PROJET:
Acton International Inc.

PARTENAIRES FÉDÉRAUX:
RDDC

AUTEURS:
Julie Tremblay-Lutter DSTHP 4, DRDC,
email:
Julie.Tremblay@drdc-rddc.gc.ca;

Jef Stewart,
email: jef.stewart@airboss-acton.com;

Céline Michaud,
email:
celine.michaud@airboss-acton.com;

Acton International,
881 Landry, Acton Vale, Qc,
tel: (450) 546-2776.

Objectifs

L'objectif de ce projet est de développer une nouvelle formulation polymère à usages multiples pour maximiser les fonctions de protection CBRN, ainsi que la résistance à la flamme, aux huiles et autres substances nocives.

Au cours d'événements CBRN, les premiers intervenants n'ont souvent pas le temps d'analyser le type d'agent en cause avant de revêtir leur équipement de protection. Ils comptent donc sur une protection complète et adéquate pour une durée de temps plus ou moins limitée.

Progrès récents

Résultats de la Phase I
(Étude de marché et comparaison des gants, des appareils respiratoires et des protège-chaussures en matière de protection NBC.)

Lors de cette phase, les produits actuellement sur le marché ont subi un ensemble de tests pour comparer les performances de chacun. L'accent était mis sur les propriétés des polymères au cours de tests physiques et de tests portant sur la résistance chimique et la résistance aux agents HD (gaz Moutarde) et GB (Sarin).

À la suite de l'analyse des résultats de la phase I du projet, on remarque qu'aucun des produits actuellement sur le marché ne satisfait complètement aux besoins en terme de protection CBRN. Jusqu'à présent, les produits évalués n'offrent qu'une protection limitée contre certains types d'agents. À titre d'exemple, les bottes militaires offrent une excellente protection contre les agents HD et GB, mais peu de résistance aux huiles et au vieillissement. Par contre, les bottes des premiers intervenants n'offrent que peu ou alors aucune résistance aux agents HD et GB, mais offrent une grande protection contre les huiles et certains liquides industriels.

De plus, il est très difficile de comparer et d'analyser des polymères en faisant abstraction du produit fini. Lors d'analyses, les échantillons de polymères étaient prélevés sur des produits finis. Les résultats ont donc été grandement influencés par la conception et la fabrication du produit.

De façon générale, l'épaisseur du polymère est directement proportionnelle au niveau de protection. Par contre, cette épaisseur est inversement proportionnelle aux performances ergonomiques du produit. Certains des produits évalués étaient deux fois plus épais que d'autres, pour des utilisations similaires.

Il est aussi difficile de comparer les propriétés des polymères ayant des traitements de surface différents. Les performances en matière de protection proviennent-elles uniquement du polymère ou en partie du traitement de surface (par oxydation par exemple), et ce dans quelle mesure?

La conception du produit influence aussi beaucoup la perception de l'utilisateur en ce qui a trait au niveau de protection. Un produit confortable donnera une impression de légèreté. Un produit lourd donnera une impression de grande protection. Même la couleur du produit contribue à la perception psychologique du confort et de la performance.

Lors de certains tests, le produit complet devait être évalué. Par exemple, la résistance à la pénétration des gaz, ainsi que l'étanchéité des masques à gaz ont été testées sur des mannequins. Il est donc difficile d'identifier les performances reliées au polymère seul, car il peut y avoir des points faibles à plusieurs autres endroits (système de communication, de vision, de filtration).

Pour les raisons énumérées ci-dessus, nous croyons qu'avant de développer tout nouveau produit, il est important d'en identifier les performances souhaitées.

Perspectives d'avenir

Au cours de la phase II, un cahier de charges sera établi conjointement avec les utilisateurs pour déterminer les performances de protection minimales. De plus, une matrice de développement de différents polymères sera mise au point. Les recherches se concentreront principalement sur 5 types de polymères différents : Halo-Butyl, Epichlorohydrin, Nitrile, Carboxylated Nitrile ainsi que le Polyuréthane. Cette phase se terminera à la fin du mois de juillet 2004.

Au cours des phases subséquentes, ces nouvelles formulations de polymères seront mélangées, testées et validées selon le cahier de charges. Comme ces nouveaux polymères seront tous à l'état de mélange et non de produits finis, il sera facile de faire des tests quantitatifs et comparatifs. Les résultats des tests seront plus adéquats pour comparer les performances de protection des polymères seuls.

De plus, l'impact de l'ajout du traitement de surface pourra être validé. Un même polymère pourra être évalué selon plusieurs traitements de surface différents. Entre autres, l'oxydation de la surface par le chlore et le brome ainsi que les traitements possibles au plasma seront analysés.

À la fin du projet, en novembre 2005, les nouvelles formulations seront utilisées pour la production de gants et de bottes. Les échantillons seront soumis à l'ensemble des tests de la phase I. Le rapport de ces tests deviendra le document technique de référence des produits. Une évaluation sur le terrain sera aussi effectuée pour valider les performances de ces échantillons par les utilisateurs en situations opérationnelles réelles ou simulées.

CRTI Biology Cluster Acquisition Projects

AUTHOR:
Helen Spencer, Defence Research & Development Canada, 305 Rideau St, Ottawa, Ontario, K1A 0K2,
tel: (613) 998-6418,
email: Helen.Spencer@drdc-rddc.gc.ca.

Objectives

CRTI Acquisition Projects are selected to close critical gaps in the laboratory cluster's capability and capacity to respond. Gaps are closed by establishing or enhancing the infrastructure and equipment available to the federal laboratories involved in responding to an incident. Acquisitions typically involve "off-the-shelf" technologies and are completed within one year.

During the first round of CRTI selections for the Biology Laboratory Cluster, thirteen acquisition projects were funded at a total value of $9,900K. This includes $4,851K in CRTI funds, along with $5,049K "contribution-in-kind" from participating federal government departments. This provides a ratio of 49% CRTI funds to 51% in-kind contributions, a significant leverage of the program requirement for a minimum of 33% in-kind contribution.

These projects will address a broad range of Biology Laboratory Cluster gaps. Overall objectives are as follows:

- Surveillance/Detection
- Diagnostics
- Decontamination
- Treatments & Prevention

Recent Progress

Projects from the first round are the following:

BIO 001AP: "Inactivation / Decontamination of Human and Animal Bioterrorism Agents and Analysis of Suspicious Materials with Mixed Hazards" led by Dr. S. Wagener of Health Canada, addresses Cluster objectives 2 and 3.

BIO 002AP: "Charcoal Filter on CL3 Biological Safety Cabinet at CL3 lab, Tunney's Pasture" led by Ms. M. Heisz of Health Canada, addresses Cluster objective 2.

BIO 003AP: "National Real-Time Network for Identification of Bioterrorism Agents" led by Dr. M. Mulvey of Health Canada, addresses Cluster objective 2.

BIO 004AP: "Upgrade of Contamination Areas (CL3 & CL4) to Test for Bioterrorism Agents" led by Dr. H. Feldmann of Health Canada, addresses Cluster objective 2.

BIO 005AP: "Chemical / Biological Forensic Reference Lab - Major Lab Equipment" led by Dr. B. Kournikakis of Defence Research & Development Canada, addresses Cluster objective 2.

BIO 006AP: "Laboratory Response Network, Participation in US and Canadian Initiatives" led by Ms. M. Heisz of Health Canada, addresses Cluster objectives 1 and 2.

BIO 007AP: "Gamma Cell for Irradiation of Biological Agents" led by Dr. B. Kournikakis of Defence Research & Development Canada, addresses Cluster objective 3.

BIO 008AP: "Data Standards for Shared Information" led by Dr. J. Hockin of Health Canada, addresses Cluster objective 2.

BIO 009AP: "Direct Fluorescent Antibody Assays for Viruses & Bacteria" led by Dr. L. Nagata of Defence Research & Development Canada, addresses Cluster objective 2.

BIO 010AP: "Virus Culture and Purification" led by Dr. L. Nagata of Defence Research & Development Canada, addresses Cluster objectives 2 and 3.

BIO 011AP: "Acquisition of a Crisis Information Management System" led by Mr. C. Heyes of the Canadian Food Inspection Agency, addresses Cluster objectives 1 and 2.

BIO 012AP: "Emergency Management Response System (EMRS) and the Canadian Animal Disease Emergency Response System" led by Mr. D. Hayes of the Canadian Food Inspection Agency, addresses Cluster objectives 1 and 2.

BIO 013AP: "State-Transition Model software for Animal Health and Zoonosis Threat Assessment" led by Dr. R. Morley of the Canadian Food Inspection Agency, addresses Cluster objectives 1 and 2.

During the second round of CRTI selections for the Biology Laboratory Cluster, six acquisition projects were funded for a total value of $6,955K. This includes $2,181K in CRTI funds, along with $4,774K "contribution-in-kind" from participating federal government departments. This provides a ratio of 32% CRTI funds to 68% in-kind contributions, tremendous leverage of CRTI funds and virtual reversal of program requirements of 67% CRTI to 33% in-kind.

BIO 014AP: "CAN / US Counter terrorism R&D MOU - Aerosol Sampling Equipment Retention" led by Dr. B. Kournikakis of Defence Research & Development Canada, addresses Cluster objectives 1 and 3.

BIO 016AP: "Penside and Rapid Diagnostic Tests for FMD, Hog Cholera, and Avian Influenza" led by Dr. P. Kitching of the Canadian Food Inspection Agency, addresses Cluster objectives 1 and 2.

BIO 017AP: "Network GIS" led by Ms. C. Doan of the Canadian Food Inspection Agency, addresses Cluster objective 1.

BIO 018AP: "Public Health Map Generator" led by Dr. J. Hockin of Health Canada, addresses Cluster objective 1.

BIO 019AP: "Upgrade of Hybridoma Facilities" led by Ms. E. Fulton of Defence Research & Development Canada, addresses Cluster objectives 2 and 4.

BIO 020AP: "Rapid Identification and Detection of Plant Pests & Pathogens" led by Mr. L. Foster, addresses Cluster objectives 1 and 2.

Future Outlook

The Biology Cluster will continue to access gaps and select Technology Acquisition Projects reflecting needs in those remaining areas.

AUTHOR:
NormanYanofsky, Portfolio Manager
Chemistry, CRTI, 305 Rideau Street,
Ottawa, Ontario, K1A 0K2.
Tel: (613) 998-6417,
email:
norman.yanofsky@drdc-rddc.gc.ca.

Objectives

CRTI Acquisition of Technology projects address the following Chemical Cluster objectives:

1. Improve integration of data/ info management systems for operational needs;
2. Improve analytical approaches to the rapid detection of hoaxes;
3. Identify lead laboratories for all chemicals on the priority substances list;
4. Address gaps in lead laboratory capabilities for chemicals on the list;
5. Develop improved capabilities for field detection of chemicals on the list;
6. Improve mobile analytical capability to provide direct support to responders.

Recent Progress

In the first round of Chemical Cluster project selection, twelve Acquisition of Technology projects were funded for a total value of $6961K. This breaks down as follows: $4161K from CRTI and $2800K in in-kind contribution from participating departments. This gives a leverage ratio of 60% CRTI to 40% in-kind which is somewhat better than the CRTI program requirement of 67% CRTI to 33% in-kind.

First Round Projects are the following:

1. Merv Fingas of Environment Canada is leading "Field Response - Re-equiping Vehicle Portable Analytical Systems", which addresses Cluster Objectives 5 and 6.
2. Merv Fingas of Environment Canada is leading "Field Response - Person Portable Analytical Equipment", which addresses Cluster Objectives 5 and 6.
3. Eva Dickson of Royal Military College is leading "Relocation of Chemical Vapour Protection Test Facility", which addresses Cluster Objective 6.
4. Gary Lombaert of Health Canada is leading "Chemical Containment Lab Assessment", which addresses Cluster Objective 4.
5. Joe Deak of the RCMP is leading "Raman for Rapid Characterization of Unspecified Materials Recovered from Terrorist Incidents" which addresses Cluster Objectives 2 and 5.
6. Joe Deak of the RCMP is leading "Micro XRF for Rapid Identification of Unspecified Materials Recovered from Terrorist Incidents" which addresses Cluster Objectives 2 and 5.
7. Joe Deak of the RCMP is leading "X-Ray Diffraction (XRD) to Identify Unknown Particulates for Presentation as Evidence" which addresses Cluster Objectives 2 and 5.
8. Pat Rasmussen of Health Canada is leading "Facility for Gravimetric Analysis of Airborne Particulate Matter" which addresses Cluster Objectives 4 and 5.
9. Paul d'Agostino of Defence R&D Canada-Suffield is leading "Analysis of Chemical Warfare Agents in Samples Collected in Support of Counter-Terrorism" which addresses Cluster Objectives 3 and 4.
10. Messrs Graham and Brous of the Canadian Food Inspection Agency are leading "Microscope for FTIR" which addresses Cluster Objectives 2, 4 and 5.
11. Garth Burns of the Canadian Food Inspection Agency is leading "Toxic Element

Contamination - ICP/MS for Toxic Element Analysis" which addresses Cluster Objective 4.

12. Ralph Oncuil of the Canadian Food Inspection Agency is leading "Enhanced Capability for Identification of Chemical Residues in Foods, Feeds and Fertilizers" which addresses Cluster Objective 4.

In the second round of Cluster project selection, seven Acquisition of Technology projects were funded for a total value of $3261K. This breaks down as follows: $1562K from CRTI and $1699K in in-kind contribution from participating departments. This gives a leverage ratio of 48% CRTI to 62% in-kind which is even better than either the program requirement of 67% CRTI to 33% in-kind or the first year leverage ratio noted above.

The projects are the following:

1. Elaine Fulton of DRDC-Suffield is leading "High Throughput Multiplexed Identification of Ricin and other Biological Toxins". (Cluster Objective 4.)
2. Carmela Jackson Lepage of DRDC-Suffield is leading "Standard Atmospheres for Chemical Warfare Agents Detector Challenge and Evaluation." (Cluster Objective 4.)
3. Merv Fingas of Environment Canada is leading "Response Gap Equipment". (Cluster Objectives 4, 5 and 6)
4. Ralph Oncuil of the Canadian Food Inspection Agency is leading "Emergency Analytic Capability for Pesticide Residues & Other Chemical Contaminants in Food, Animal Feed, and Fertilizers." (Cluster Objective 4.)
5. Gary A. Lombaert of Health Canada is leading "Chemical Containment Lab Study." (Cluster Objective 3, 4.)
6. Garth Burns of the Canadian Food Inspection Agency is leading "Saxitoxin and Other Marine Toxins." (Cluster Objective 4.)
7. Merv Fingas of Environment Canada is leading "Decontamination Equipment." (Cluster Objectives 4, 5 and 6)

While the above two listings give an indication of the breadth of the first two rounds of Chemical Cluster acquisitions, some individual projects will be highlighted here to illustrate some of the specific capabilities acquired. Dr. Elaine Fulton's project, cited above, addresses Defence R&D Canada Suffield Laboratory's mandate to identify ricin and other biological toxins in sample unknowns, such as botulinum toxin and staphylococcal enterotoxin B. The accepted methodology is antibody-based assays. As was seen in the US during the anthrax event in the fall of 2001, government laboratories were overwhelmed by the need to analyze multiple samples. The challenge is the capacity to do multiple samples with multiple toxin agents, and the adaptation of high throughput, multiplex protein suspension arrays will provide the capacity. The Defence R&D Canada Suffield Laboratory has a mandate to act as Canada's principal facility for the identification of chemical warfare agents.

Future Outlook

Dr. Paul D'Agostino's project, the acquisition of a Nuclear Magnetic Resonance (NMR) spectrometer, allows Suffield to identify chemical warfare agents unambiguously by using the NMR in conjunction with existing mass spectrometry techniques.

In addition to chemical warfare agents and biological toxins, there are many toxic industrial chemicals that pose a potential terrorist threat. Dr. Merv Fingas' project of equipping field response personnel with Person Portable Analytic Equipment adds surge capacity to Environment Canada in addressing CBRN threats. This builds on 20 years of experience of the Emergencies S&T Division in researching and evaluating the field equipment of first responders.

CRTI Radiological-Nuclear Cluster Acquisition Projects

AUTHOR:
Mr. Ted Sykes, Defence Research & Development Canada, 305 Rideau St, Ottawa, Ontario, K1A 0K2,
tel: (613) 998-6418,
email: Ted.Sykes@drdc-rddc.gc.ca.

CRTI Acquisition Projects are selected to close critical gaps in the laboratory cluster's capability and capacity to respond. Gaps are closed by establishing or enhancing the infrastructure and equipment available to the federal laboratories involved in responding to an incident. Acquisitions typically involve "off-the-shelf" technologies and last one year.

During the first round of CRTI selections for the Radiological-Nuclear Cluster, six acquisition projects were funded at a total value of $17,705K. This includes $6,198K in CRTI funds, along with a $11,507K "contribution-in-kind" from participating federal government departments. This provides a ratio of 35% CRTI funds to 65% in-kind contribution, which represents a significant leverage over and above the minimum program requirement of a 33% in-kind contribution.

Acquisition projects will address a broad range of Radiological-Nuclear Laboratory Cluster gaps. Overall objectives are as follows:

1. Improve Canada's Radiological-Nuclear surveillance capabilities;
2. Establish a capability to notify and activate the Federal Labs;
3. Improve the integration and sharing of data across the Radio-Nuclear Cluster; and
4. Close high-priority gaps in human and environmental measurement capability.

The six first-round acquisition projects for the Radiological-Nuclear Cluster are as follows:

RN001AP: "Fixed-Point Surveillance System for Canada", led by Dr. K. Ungar of Health Canada, addresses Cluster objective 1. Partners include Atomic Energy of Canada Limited and the Canada Boarder Services Agency. This project will establish a network of fixed-point sensors around Canada's five nuclear power plants and in ten major population centres.

RN002AP: "Aerial Surveillance for Radiological-Nuclear Incidents", led by Mr. R. Schives of Natural Resources Canada, will address Cluster objective 1. This project will acquire a system comprised of three rapidly deployable semi-quantitative gamma ray spectrometers that provide Phase 1 detection and identification; along with two more sensitive Phase 2 systems that map nuclide deposition patterns. The system will be capable of real-time radio-telemetry of data to a ground-based receiver with a sophisticated GUI and GIS display.

RN003AP: "Whole Body Monitoring of Radiological Contamination", led by Dr. G. Kramer of Health Canada, will meet Cluster objective 4. Defence R&D Canada is a partner. This project will acquire deployable, portable facilities to assist first responders in quickly identifying and assisting contaminated individuals; as well as high resolution fixed facilities to identify complex mixtures of fission and activation

products. The latter will facilitate treatment and risk projection activities.

RN004AP: "Biological Dosimetry for Radiation Exposure", led by Dr. D. Wilkinson of Defence R&D Canada, will meet Cluster objective 4. Partners include Health Canada and Atomic Energy of Canada Limited. This project will acquire a "Luminex" System capable of providing rapid analysis of many cytokines in the blood plasma of potentially exposed individuals. It will be an automated system with enhanced instrumentation that provides more rapid sample analysis with an improved measurement capability than had previously been available.

RN005AP: "Nuclear Cluster Emergency Alerting and Notification System", led by Mr. Brian Ahier of Health Canada, will meet Cluster objective 4. This project will acquire an Emergency Information Management Network/Portal for hosting shared information. It will also acquire the requisite information management applications. Features include an Automated Emergency Notification System, with a Web and Interactive Voice Response capability.

RN006AP: "Networking Laboratory Results", led by Dr. S. Johnson of Health Canada, will meet Cluster objective 2. This project will acquire a Laboratory Information Management System (LIMS) allowing for the automatic input of results, from various instruments, directly into a centralized database. It will assist in forensic analysis of samples and Chain of Custody tracking.

Second round acquisition project selection for the Radiological-Nuclear Cluster resulted in five federal government departments and agencies partnering to request CRTI funds in support of one collaborative project. The total value of the project is $2.6M. It includes $1.5M in CRTI funds, along with a $1.1M "contribution-in-kind" from participating federal government departments. This provides a ratio of 58% CRTI funds to 42% in-kind contribution, which once again represents a significant leverage over the minimum program requirement of a 33% in-kind contribution.

RN007AP: "Deployable Analytical Facilities to Support Expert Response to Radiological/Nuclear Incidents", led by Dr. T. Cousins of Defence R&D Canada, will meet Cluster objective 3. Partners include Atomic Energy Canada Limited, National Research Council, Canadian Nuclear Safety Commission, Canadian Boarder Services Agency, Health Canada and Department of Fisheries and Oceans. This project will acquire mobile field sampling and analytical tools necessary to establish cross-Canada technical response capabilities to a Radiological-Nuclear incident, on land or in water. Four Mobile Nuclear Laboratories (MNLs) will be acquired and stored in British Columbia, Manitoba, Ontario and Nova Scotia respectively. Each will be comprised of a vehicle equipped with a suite of data acquisition, analysis and communication equipment. So equipped, the MNLs will allow scientific teams to identify the nature and extent of radiological contamination at the site of an incident, and predict the dispersion pattern of contamination.

CRTI CHEM009AP

Analysis of Chemical Warfare Agents in Samples Collected in Support of Counter-Terrorism

PROJECT LEAD:
DRDC Suffield

AUTHORS:
Paul A. D'Agostino,
tel: (403) 544-4670,
email:
paul.dagostino@drdc-rddc.gc.ca;

James R. Hancock,
Carmela R. Jackson Lepage and
Claude L. Chenier, DRDC Suffield,
P.O. Box 4000, Station Main,
Medicine Hat, AB, T1A 8K6.

Objectives

More than 150 State Parties have ratified the Chemical Weapons Convention (CWC) and agreed not to develop, produce, stockpile, transfer or use chemical weapons, and to destroy their own chemical weapons and production facilities. The CWC has reduced the likelihood of chemical weapons use by State Parties, but there remains a serious concern that other parties may make use of these weapons against civilian or military targets. Analytical methods need to be developed to ensure that suspect samples collected under these scenarios can be analyzed for the presence of chemical warfare agents in a timely manner.

The DRDC Suffield analytical research laboratories provide the Canadian Forces and Solicitor General (RCMP) with a national capability for the identification of chemical warfare agents in suspect samples. Mass spectrometry plays an important role in the confirmation of these compounds in collected samples. A new Micromass/Waters QTOF Ultima tandem mass spectrometer, received at DRDC Suffield as part of a CRTI Technical Acquisition, is presently being used to develop new analytical methods for chemical warfare agents and is available at short notice to support the analysis of forensic samples suspected to contain chemical warfare agents.

Recent Progress

Liquid chromatography electrospray tandem mass spectrometry (LC-ESI-MS/MS) methods were developed for the analysis of chemical warfare agents, their degradation products and related compounds in aqueous samples and extracts using the high resolution quadrupole/time-of-flight (QTOF) tandem mass spectrometer purchased through CRTI. The developed methods have been applied to a variety of different environmental samples containing chemical warfare agents including:

i) Spiked aqueous samples that were generated to simulate the type of sample that might be expected during retrospective identification of chemical warfare agents.

ii) Aqueous extracts of soil samples collected at a former mustard storage site as part of an on-going environmental assessment.

iii) Tabun samples containing numerous related synthetic and/or degradation products.

iv) Autoclaved aqueous extracts of soil samples suspected to contain chemical and/or biological warfare agents.

LC-ESI-MS/MS data were acquired for the chemical warfare agents and related compounds with a resolution of 9000, enabling accurate mass determination of $(MH)_+$ precusor ions and structurally significant product ions. Acquired ESI-MS/MS was used to confirm the presence of chemical warfare agents and their hydrolysis products and for the identification of novel related compounds not previously associated with chemical warfare agent determinations.

Future Outlook

DRDC Suffield, as an active partner in the CRTI Chemical Cluster, maintains responsibility for the analysis of chemical warfare agents in forensic (or other) samples suspected to contain these compounds. Readiness in the event of an emergency is maintained by on-going chemical warfare agent research and development efforts within the analytical laboratory at DRDC Suffield. New sample preparation and analysis methods based on solid phase microextraction, gas chromatography, liquid chromatography, mass spectrometry and tandem mass spectrometry will continue to be developed to insure that DRDC Suffield can respond to the analytical requirements of the Canadian Forces. These methods may be used in the future for the analysis of suspect samples collected by the Solicitor General (RCMP), during Chemical Weapons Convention inspections or in support of the CRTI Chemical Cluster mandate.

CRTI RN-003AP

Whole Body Monitoring for Radiological Contamination

PROJECT LEAD:
Health Canada

FEDERAL PARTNERS:
Department of National Defence, Atomic Energy of Canada Limited

AUTHORS:
Dr. Gary H. Kramer, Human Monitoring Laboratory, Radiation Protection Bureau, 775 Brookfield Rd, PL6302D1, Ottawa, Ontario, K1A 1C1,
tel: (613) 954-6668,
email: gary_h_kramer@hc-sc.gc.ca.

Dr. Anthony Waker, Radiation Biology and Health Physics, Atomic Energy of Canada Ltd, Chalk River Laboratories, Chalk River, Ontario K0J 1J0,
tel: (613) 584-8811 x3611,
email: wakera@aecl.ca.

Objectives

Monitoring capability following the intentional release of radioactive material in an urban center has been identified (by CSIS) as extremely poor. All the radiological scenarios (with a reasonable probability of occurring) would result in internal contamination of first responders and the Canadian public, possibly in a large number.

Deployable, *portable facilities* were required at the Radiation Protection Bureau (RPB) and Atomic Energy of Canada Limited (AECL) for first responders to quickly separate affected individuals into those internally contaminated and those uncontaminated. The capability of field identification of internally deposited radionuclides would greatly enhance subsequent risk estimates and accurate consequence management of the affected personnel.

High-resolution facilities were required for first responders to identify internal complex mixtures of radioactive material in the contaminated persons. Upgrading the RPB-based fixed facilities to a high resolution Whole Body Counter permits the analysis of a complex internal burden resulting from the release of fission and the identification of activation products. Upgrading the RPB Lung Counter to larger, more reliable detectors permits the accurate analysis of an actinide (uranium, plutonium, neptunium etc.) intake.

Recent Progress

Originally, this project was planned as a one-year technology acquisition; however, the complexity of the monitoring systems, and the fact that the required germanium detectors are at the forefront of current manufacturing capabilities, caused delays in both designing and meeting specifications and organizing the multi-vendor components. As a result, a cascading delay occurred as one vendor had to wait upon another vendor's response before the final package could be built and delivered.

The graded Z liner in the lung-counting chamber has been completed. It consists of a layer of tin covered by a layer of copper. These layers were installed over the existing layer of lead that lines the thick steel walls of the lung counting chamber. An air handling system containing a HEPA filter has been installed in the lung counting chamber in an attempt the reduce the high radon background, but this has proved to be minimally successful. Nevertheless, this has dramatically improved the air quality in the chamber during the counting of contaminated persons.

Equipment has been purchased and the lung counter has been installed and is now operational. Background characterization has been completed and efficiency calibrations have commenced. The latter is a lengthy procedure

as multiple lung sets must be measured at multiple chest wall thickness values and the counts are generally in excess of 50,000 seconds to obtain good counting statistics.

The whole body counter is in early commissioning phases and background characterization, resolution capabilities, and calibration will commence later this year.

The portable monitoring capabilities have been enhanced by the acquisition of more P3 monitors so that up to 1000 persons per hour can be screened. Other hand-held equipment has been acquired to enhance field capabilities, including high-resolution capabilities. The latter instrument can also be used as a high resolution field deployable whole body counter as persons contaminated following an intentional release of radioactive material are likely to have easily detectable quantities of radionuclides either on or in them.

The role adopted by Chalk River Laboratories (CRL) is to complement the large-scale screening capability of Health Canada and the Department of Defence by providing a transportable monitoring and internal dose assessment system for evaluation of contaminated first responders and victims of a radiological event.

In order to establish a dose assessment and medical support capability, CRL has procured equipment to setup a transportable, rudimentary bioassay laboratory with wound monitoring capability and basic quantitative in-vivo monitoring. Capabilities include: transportable gamma spectrometers, wound monitoring detector systems, transportable liquid scintillation counter, survey equipment, data management, and computing modeling software.

The immediate activities are to calibrate the fixed facilities. The lung counter's background must be fully characterized and the detectors calibrated using both the Lawrence Livermore Torso phantom with two lung shapes, and the Japanese Atomic Energy Research Institute Torso Phantom. This will provide person-size calibration data sets. Minimum detectable activities must be established for a variety of radionuclides including ^{239}Pu, ^{241}Am, and natural and enriched uranium.

The whole body counter must be calibrated using the BOMAB phantom family that includes a 4-yr-old, a 10-yr-old, a 5-percentile male, a reference female, a reference male, and a 95-percentile male. Detection limits must be established and the multi-channel scaling characteristics of the new counter must be established.

The portable high-resolution monitoring equipment will be calibrated using a combination of experimental and Monte Carlo techniques. All the field deployable equipment must be tested under realistic exercise conditions; this is planned for later in 2004.

Future Outlook

Anticipated Non-Emergency Use: RPB's P3 monitors are being used to monitor persons entering and leaving the building. They will also be used in emergency exercises (Federal & Provincial). AECL's equipment will be used in international intercomparisons, emergency response exercises, annual staff training, and routine standby use at AECL. The fixed facilities will be used to support research and development programs as well as intercomparison programs, to measure international standards, and to measure Canadians who may be potentially contaminated through accidental or occupational exposure.

It is anticipated that all the equipment described above will be fully characterized and functional by early 2005.

CRTI RN 006AP

Networking Laboratory Results from a Certified National Laboratory

PROJECT LEAD:
Health Canada, Radiation Protection Bureau

FEDERAL PARTNERS:
None

AUTHORS:
Sonia Johnson, Jeff Haydt, Jeff Whyte, Radiation Protection Bureau, 775 Brookfield Rd, Ottawa, ON, K1A 1C1
email: sonia_johnson@hc-sc.gc.ca, tel: (613) 954-6677;

email: jeff_whyte@hc-sc.gc.ca, tel: (613) 941-2736;

email: jeff_haydt@hc-sc.gc.ca, tel: (613) 954-8585).

Objectives

This project deals with the CRTI investment priority of Immediate Reaction and Near-Term Consequence Management Capabilities.

Real-time reporting of laboratory results of environmental samples to first responders and the Canadian public is essential for ensuring public confidence in the event of a nuclear emergency. Inputting these results into the decision-making framework ARGOS (Accident Reporting and Guidance Operational System), operated in support of the Federal Nuclear Emergency Plan, along with other relevant information such as meteorological modelling and aerial surveys, provides an overall perspective on radioactive plume dispersion and deposition, and dose evaluation. The outputs from ARGOS will be used by decision-makers and first responders for safe and effective response to the radiological/nuclear emergency.

To accomplish the goal of rapid, shared reporting of quality laboratory results, laboratory certification and a Laboratory Information Management System (LIMS) are necessary. Laboratory certification ensures that international standards for generating reliable and reproducible laboratory data are followed. This is critical for providing credibility on the quality of the results that are reported from the laboratory. LIMS provides rapid reporting of laboratory results and increased efficiency via centralized resource management and automation of workflow steps. The LIMS platform can also be used to share information with other clusters and partners.

Recent Progress

The LIMS development project has realized enormous success over the past fiscal year. The core LIMS group at RPB is responsible for the leading edge development of an independent LIMS environment, which includes the successful completion of the Threat Risk Assessment (TRA). The TRA investigates the risks involved in hosting a separate, independent network.

This project has also achieved the successful installation of LIMS software, the establishment of the standalone network of user PCs and instrument PCs, and the configuration of 2 key laboratory processes, including instrument interfacing. This configuration is the result of extensive consultation services and training of HC personnel. These 2 processes are in the testing stage of the pilot, and will go live by March 2005.

The laboratory is working towards ISO 9001 certification. The Quality Manual has been written and reviewed by a Quality consultant to ensure that ISO 9001 elements have been addressed. The work instructions that supplement the Quality Manual are being written by CRMN technical staff.

For the LIMS implementation, the following stages will occur:

- **Parallel use of the LIMS and existing work methods. This will highlight any glitches in the LIMS-based platform that will require further configuration and development.**
- **Validation of the LIMS configuration.**
- **Complete rollout of the LIMS platform for use in the day-to-day production environment, with transfer of relevant information to the Nuclear Emergency Preparedness and Response Division's (NEPRD) ARGOS system in a timely fashion.**

For the ISO certification project, the work instructions for the Quality System will be completed. The Quality System will then be implemented and tested for a period of time (3-6 months) prior to the internal audit, a prerequisite for obtaining external certification.

Future Outlook

The project is expected to be completed no later than Quarter 1 of FY 05/06, with the deliverables of: a laboratory certified to ISO 9001; key processes executed on a LIMS-based platform; and sharing of high quality radioactivity measurements with key partners. Transfer of radioactivity measurement data to a central repository (i.e. ARGOS) during routine situations will ensure that pertinent information required during an emergency will be available in a rapid and reliable fashion. This enhanced preparedness is essential for decisive response in an emergency, which will aid and protect first responders, emergency workers, and the Canadian public.

The LIMS / ISO projects of the Canadian Radiological Monitoring Network (CRMN) laboratory can be used as a model for other laboratories or clusters having the same needs and requirements for data sharing.

Recovery of Physical Evidence from Crime Scenes Contaminated with Chemical or Biological Warfare Agents

PROJECT LEAD:
Royal Canadian Mounted Police

FEDERAL PARTNERS:
Defence Research & Development Canada (Suffield), Health Canada

AUTHORS:
Dr. Della Wilkinson, Room 503, NPS Building, 1200 Vanier Parkway, Ottawa, ON, K1A 0R2.
tel: (613) 993-3059.
e-mail: della.Wilkinson@rcmp-grc.gc.ca

Objectives

In 2001, when the Anthrax letters were circulating in the US, forensic identification specialists (FIS) had no standard operating procedures (SOPs) for examining this type of physical evidence for fingerprints or DNA. The main objective of this project is to determine SOPs for evidence recovery from a crime scene that has been contaminated with chemical (CW) or biological warfare (BW) agents. The milestones involved in reaching this objective are:

1. Observation of the effects of decontamination agents (CASCAD and MODEC) on fingerprints and DNA (completed);
2. Determination of the effect of CW agents on the recovery of fingerprint evidence using current chemical detection procedures (completed);
3. Determination of the effect of CW agents on the integrity of DNA evidence (completed);
4. Determination of the stability of selected CW agents to DNA extraction protocols (ongoing);
5. Determination of the robustness of non-pathogenic bacteria to FTA® swabs used to collect and store forensic DNA samples (completed);
6. Determination of the robustness of non-pathogenic bacteria to DNA extraction protocols (completed);
7. Determination of the effect of BW agents on the integrity of DNA evidence and of the robustness of the bacteria to DNA extraction protocols (ongoing).

The resulting SOPs will provide FIS, who are part of the CBRN first responder community, with information on the most effective method for recovering fingerprint, DNA, footwear, and hair and fibre evidence. This information addresses shortcomings in the CRTI investment priority: "Criminal Investigation Capabilities".

Recent Progress

The decontamination agents are destructive to both fingerprint and DNA evidence. In the presence of selected CW agents, all standard chemical fingerprint detection procedures were performed with the exception of blood peroxidase methods. This initial research was completed in 2000 through collaboration with scientists at DRDC Suffield. Further work with DRDC Suffield and a contract with the University of British Columbia, completed in 2003, identified four CW agents that inhibit the ability to recover forensic DNA profiles.

The stability of non-pathogenic bacteria to standard DNA collection and extraction protocols was explored in 2003 through a contract with the University of Ottawa's Centre for Research in Environmental Microbiology (CREM). The results showed that FTA® swabs could not inactivate the selected bacteria. However, they were destroyed by the presence of Lysis buffer in the DNA extraction procedure with the spore forming bacteria requiring the addition of heat (95 °C for 30 minutes) for total kill.

Future Outlook

The stability of the CW agents, which did not inhibit DNA profiling, is being studied through an ongoing contract with scientists at The Netherlands Organization of Applied Scientific Research-Prins Maurits Lab (TNO-PML).

An MOA with scientists at Health Canada's Centre for Emergency Preparedness and Response has been designed to continue the DNA research using pathogenic bacteria.

Chemical Incident Simulator: A New Approach for Deriving Passive Defence Requirements

AUTHORS:
M.J.G. Linders, C.A. van Beest, P. Brasser, L.F.G. Geers, G. van 't Hof, R.A. Rumley-van Gurp, R.P. Sterkenburg, S.C. van Swieten, H.W. Zappey, A.R.T. Hin and M.W. Leeuw, TNO – Prins Maurits Laboratory, PO Box 45, 2280 AA Rijswijk, The Netherlands,
+31 15 284 3303,
email: brasser@pml.tno.nl.

Objectives

Traditionally, passive defence has been the preferred way to counter the BC threat. Passive defence encompasses the whole array of measures that are available to the soldier: detection and identification, physical protection, medical countermeasures and decontamination.

Modelling and simulation are increasingly important instruments and these approaches could have an enormous impact in the area of passive defence. Traditionally, the assessment of the chemical threat using the concept of challenge levels has been the primary focus. As the threat picture is changing, assessment of biological threats as well as threats exerted by (industrial) toxic compounds (including releases other than attack) have become important issues as well.

Threat assessment has been used as a starting point to define the requirements for a passive defence system. In the past, such requirements were determined on a more or less *ad hoc* basis. The TNO Prins Maurits Laboratory has started a scenario-based systemic approach to model the complete chain of passive defence measures, in order to derive challenge levels and casualty levels. This enables the study of the effects of passive defence requirements upon these levels, thus improving the selection process.

The Chemical Incident Simulator, CIS, simulates events that encompass the passive defence against chemical warfare agents. The model starts in 'release, transport and dispersion' mode, where agent release in an incident scenario is simulated. The model generates concentration-time exposure profiles for the detectors, mask, suit, filters and people present in the scenario. In addition, challenge levels to the whole target are calculated. In the next step the model is in detection mode; as soon as the release of a chemical agent is detected, an alarm is generated. These detection alarms and the exposure profiles are input for the next mode, where the skin and respiratory protection models are triggered. These models calculate the amount of protection offered by the protective material. This results in exposure profiles for lung, eye and skin to liquid, vapour and aerosols. In the final mode, the toxic-effects model translates the exposure profiles into casualty probabilities for the personnel. Scenarios (i.e. attacks or incidents) are needed as input for the model. Over the years an extensive number of scenarios has been collected. For easy retrieval of scenarios a database has been built. The scenario takes into account all relevant factors necessary to calculate challenge levels (i.e. target data, weapon characteristics, chemical agent properties and meteorological effects). Furthermore, different NBC-alert states (Mission Oriented Protective Posture – MOPP) can be selected. These states range from 'low', meaning no protective clothing or mask is worn, to 'high', meaning

the soldier is completely protected. Each alert status is characterized by time intervals that define how long it takes before the mask and suit are worn, thus offering their respective protection. The resulting challenge levels, dosage fields and deposition fields, are stored in the database as well.

Recent Progress

The Chemical Incident Simulator simulates the dispersion of chemical warfare agents (and in the future also industrial agents and biological warfare agents), detector responses, the effects of protective equipment, and the human toxicological responses for many scenarios. The calculations start by defining the scenarios: incident properties such as target, terrain, climate, weapons, agent, etc.; personnel deployments – type of protection available (mask, suit); detector deployments – single detector, array of detectors, location; and NBC-alert state. Subsequently, the 'agent release and transport' in the scenario is calculated, which results in concentration-time profiles at the locations of detectors and personnel.

For release, transport and diffusion, the simulation program RAP2000, developed by TNO-PML, is used. The engine of RAP2000 consists of a series of models that predict physical quantities like concentration and surface deposition as function of time and location, given a chemical or biological release scenario. A major premise of RAP is that every chemical or biological attack, including line-shaped spray releases, can be split up in single sources. A single source is defined as a cloud of vapour and liquid drops with a three dimensional Gaussian mass distribution. The single source itself is split up in an initial vapour puff and a number of puffs containing droplets with the same size.

The detector model is capable of simulating both vapour and liquid detection systems. For vapour detection, there are three aspects that are modelled: sensitivity, response time, and regeneration. The liquid detector model simulates the behaviour of detection papers, which are in operational use by the Dutch and many other Defence forces. It simulates whether or not a paper will show a visible coloration, depending on deposition density and droplet sizes. The theoretical detector display outputs are corrected for operational detector procedures and residual contamination.

The skin protection model, or suit model, calculates the concentration of warfare agents, which penetrates the NBC-clothing. The vapour is adsorbed on the carbon, which is present in the NBC-protective clothing. The breakthrough concentration is calculated by the model on the basis of the type of NBC-protective clothing material, the outside concentration, the temperature, the wind speed, the time of exposure, the type of vapour etc. Next to vapour contamination, the suit model also includes a basic liquid drop model.

The respiratory protection model, or mask model, consists of two parts: a carbon filter model and a mask leakage model. The carbon filter model predicts the vapour breakthrough through the filter as a function of time. The model is valid for the adsorption of a vast number of physisorbed organic contaminants. Climatic aspects like temperature and humidity are important parameters in this respect. The leakage model is deduced from protection factor measurements of people wearing gas masks in the field. The final vapour concentration that a soldier inhales and to which the eyes are exposed, is a fraction-based mean of the breakthrough through the filter and of the leakage at the sides of the mask.

The toxic effects model uses concentration-time profiles from the respiratory and skin protection models as input to estimate casualty probabilities. This model translates the exposure profiles into casualty probabilities for the personnel, assuming a probabilistic dose-effect relationship. The casualty levels and spectra can be obtained for various types of health effects, e.g. eye effects, inhalation, percutaneous effects, subdivided in two levels (incapacitating and lethal), and various protection levels, e.g. no protection, suit only, mask only, mask and suit, and collective protection.

All input parameters, scenario definitions and results are stored in a database for easy access and retrieval. Analysis of individual scenario results and statistical analysis over all scenarios (or any subset) is possible. Typical individual scenario results are deposition, dosage and casualty level on the attacked target. Typical statistical analysis results are dosage and deposition threat spectra, and casualty spectra.

Thus, the Chemical Incident Simulation model largely eliminates the subjectivity involved in scenario studies, and procurement of protective and detector equipment. CIS can simulate the effect of the complete passive defence chain in a consistent way. The strength of CIS is that it can simulate this effect for a huge number of different situations and thus is able to establish passive defence requirements in a systematic way. The proof of principle for simulating the complete protection chain has been given. Extensive work has to be done to refine this approach, so that CIS can be an effective tool to set requirements for real life situations.

Future Outlook

In the near future, while the CIS module matures, it is foreseen that a so-called CIS user group will be initiated, which NATO countries can join.

The system will ideally provide an analysis tool to support planning and decision making. The system will eventually support operations in an analytical mode as well as interface with an integrated warning and reporting network to provide real-time analysis capability. Ideally, the system should also be capable of interfacing with other models that simulate the effects of blast, fragmentation, fire, nuclear events and combinations thereof.

Finally, it should be noted that the systemic approach could also play a role in defining research policies, as it will be capable of pointing out relative weaknesses in the passive defence system, which needs improvement.