

6 August 2009

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

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Chem-Bio News – S&T Edition

1. MODELING APPROACH TO ASSESS CLUSTERING IMPACT ON RELEASE RATES OF PESTICIDES FROM MICROENCAPSULATED PRODUCTS:

"The net pesticide release rate from microcapsule clusters was calculated from superposition of smaller decomposed cluster geometries for which release rates were determined by numerical solution to the mass balance governing equation, thereby coupling the self-assembly of microcapsule and microcapsule clustering with environmental release rate predictions.."

2. DEVELOPMENT OF REAL-TIME PCR TESTS FOR DETECTING BOTULINUM NEUROTOXINS A, B, E, F PRODUCING CLOSTRIDIUM BOTULINUM, CLOSTRIDIUM BARATII AND CLOSTRIDIUM BUTYRICUM:

"Adoption of these PCR assays is a step forward a reliable and rapid detection of these clostridia in food samples."

3. ESTABLISHMENT OF AN ADULT MOUSE MODEL FOR DIRECT EVALUATION OF THE EFFICACY OF VACCINES AGAINST VIBRIO CHOLERA:

"Studies with mutant strains unable to produce cholera toxin or toxin-coregulated pili revealed that neither factor contributed significantly to colonization potential. Protection against V. cholerae challenge was shown to be serogroup restricted, and significant inverse correlations were detected between serum and intestinal anti-lipopolysaccharide antibody responses and the levels of excretion of challenge organisms."

4. KINETICS OF LETHAL FACTOR AND POLY-D-GLUTAMIC ACID ANTIGENEMIA DURING INHALATION ANTHRAX IN RHESUS MACAQUES:

"This study emphasizes the value of LF detection as a tool for early diagnosis of inhalation anthrax before the onset of fulminant systemic infection."

5. SYNTHETIC PEPTIDE VACCINE TARGETING A CRYPTIC NEUTRALIZING EPITOPE IN DOMAIN 2 OF BACILLUS ANTHRACIS PROTECTIVE ANTIGEN:

"These results highlight the potential importance of this immunologically cryptic neutralizing epitope from PA as a target for alternative and adjunctive vaccines for anthrax."

6. HARVESTING CANDIDATE GENES RESPONSIBLE FOR SERIOUS ADVERSE DRUG REACTIONS FROM A CHEMICAL-PROTEIN INTERACTOME:

"In conclusion, SADR targets and the patient-specific off-targets could be identified through a systematic investigation of the CPI, generating important hypotheses for prospective experimental validation of the candidate genes."

7. RESEARCH SUGGESTS THAT ZINC ACTIVATES A KEY PROTEIN ON T CELLS NEEDED TO FIGHT INFECTIONS:

"Now, a new research study in the August 2009 print issue of the Journal of Leukocyte Biology suggests that zinc may be pointing the way to new therapeutic targets for fighting infections."

8. BUILDING DISEASE-SPECIFIC DRUG-PROTEIN CONNECTIVITY MAPS FROM MOLECULAR INTERACTION NETWORKS AND PUBMED ABSTRACTS:

"Further development of computational molecular connectivity maps to cover major disease areas will likely set up a new model for drug development, in which therapeutic/toxicological profiles of candidate drugs can be checked computationally before costly clinical trials begin."

9. ISOLATION OF GENETICALLY DIVERSE MARBURG VIRUSES FROM EGYPTIAN FRUIT BATS:

"The genetically diverse virus genome sequences from bats and miners closely matched. These data indicate common Egyptian fruit bats can represent a major natural reservoir and source of Marburg virus with potential for spillover into humans."

10. WORLD SHORTAGE OF MOLYBDENUM MAY HINDER NUCLEAR MEDICINE SERVICES:

"Problems with the world production and supply of molybdenum are leading to shortages of radioisotopes for nuclear medicine imaging tests, according to an editorial in the journal Nuclear Medicine Communications, official journal of the British Nuclear Medicine Society (BNMS)."

CB Daily Report

Chem-Bio News

MODELING APPROACH TO ASSESS CLUSTERING IMPACT ON RELEASE RATES OF PESTICIDES FROM MICROENCAPSULATED PRODUCTS

Agriculture Week
July 30, 2009

"The pesticide release rate from polymer-encapsulated microcapsules is controlled by diffusion across the polymer membrane, membrane thickness, and pesticide loading. However, conditions for microcapsule clustering following conventional application practices and the impact of clustering on the overall release rate are often ignored."

"Microcapsules are delivered to target surfaces using water droplets as a carrier, and capillary-driven velocities arise within the deposited sessile drop as the water evaporates. This work describes experimental observations and a quasi-static mathematical approach used to elucidate microcapsule clusters that remain following the drop-drying process, along with the anticipated patterns for pesticide release as a function of cluster geometries. The dynamic behavior of a drying sessile drop was modeled as a trajectory of constrained quasi-static equilibrium shapes having a fixed contact line. Observed monolayer clustering of microcapsules was a function of the initial contact angle and size of the sessile drop, evaporation rate, and the microcapsule number density within the drop."

"The net pesticide release rate from microcapsule clusters was calculated from superposition of smaller decomposed cluster geometries for which release rates were determined by numerical solution to the mass balance governing equation, thereby coupling the self-assembly of microcapsule and microcapsule clustering with environmental release rate predictions.."

The full article can be found at: (S.A. Cryer, et. al., "Modeling Approach To Assess Clustering Impact on Release Rates of Pesticides from Microencapsulated Products". Journal of Agricultural and Food Chemistry, 2009;57(12):5443-5451). Link not available.

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DEVELOPMENT OF REAL-TIME PCR TESTS FOR DETECTING BOTULINUM NEUROTOXINS A, B, E, F PRODUCING CLOSTRIDIUM BOTULINUM, CLOSTRIDIUM BARATII AND CLOSTRIDIUM BUTYRICUM

TB & Outbreaks Week
August 4, 2009

"To develop real-time PCR assays for tracking and tracing clostridia responsible for human botulism. Real-time PCR assays based on the detection of the genes *ntnh* encoding the nontoxin-nonhaemagglutinin (NTNH) proteins or the most homologous regions of the botulinum neurotoxin (*bont*) genes have been developed together with four real-time PCR assays, each being specific of the genes *bont/A*, *bont/B*, *bont/E*, *bont/F* and enables a toxin type-specific identification."

"The specificity of the assays was demonstrated using a panel of botulinum toxin producing clostridia (29 strains), nonbotulinum toxin producing clostridia (21 strains) and various other bacterial strains. The toxin type-specific assays had a sensitivity of 100 fg-1000 fg of total DNA in the PCR tube (25-250 genome equivalents) which correspond to 10(3) to 10(4) cells ml(-1). After a 48 h enrichment in

anaerobic conditions, these PCR assays allowed the detection of Clostridium botulinum type A in a naturally contaminated sample of 'foie gras' suspected in a C. botulinum outbreak. These PCR tests are specific and reliable for detection of heterogeneous BoNT producing clostridia responsible for human botulism."

"Adoption of these PCR assays is a step forward a reliable and rapid detection of these clostridia in food samples."

The full article can be found at: (P. Fach, et. al., "Development of real-time PCR tests for detecting botulinum neurotoxins A, B, E, F producing Clostridium botulinum, Clostridium baratii and Clostridium butyricum". Journal of Applied Microbiology, 2009;107(2):465-73). Link not available.

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ESTABLISHMENT OF AN ADULT MOUSE MODEL FOR DIRECT EVALUATION OF THE EFFICACY OF VACCINES AGAINST VIBRIO CHOLERA

Drug Week

August 7, 2009

"We describe here a new animal model that offers the prospect of using conventional adult mice for direct evaluation of the protective potential of new cholera vaccines. Pretreatment of adult mice with oral streptomycin allowed intestinal colonization by streptomycin-resistant Vibrio cholerae strains of either the O1 or the O139 serogroup."

"Bacteria were recovered in greatest numbers from the cecum and large intestine, but recoveries from all regions of the gut correlated significantly with bacterial excretion in fresh fecal pellets, which thus provides a convenient indicator of the extent and duration of gut colonization. Mice immunized mucosally or systemically with viable or inactivated V. cholerae were shown to be comparatively refractory to colonization after challenge with the immunizing strain. Several variables were examined to optimize the model, the most significant being the size of the challenge inoculum; surprisingly, a smaller challenge dose resulted in more consistent and sustained colonization."

"Studies with mutant strains unable to produce cholera toxin or toxin-coregulated pili revealed that neither factor contributed significantly to colonization potential. Protection against V. cholerae challenge was shown to be serogroup restricted, and significant inverse correlations were detected between serum and intestinal anti-lipopolysaccharide antibody responses and the levels of excretion of challenge organisms."

The full article can be found at: (E. Nygren, et. al., "Establishment of an adult mouse model for direct evaluation of the efficacy of vaccines against Vibrio cholera". Infection and Immunity, 2009;77(8):3475-84). Link not available.

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KINETICS OF LETHAL FACTOR AND POLY-D-GLUTAMIC ACID ANTIGENEMIA DURING INHALATION ANTHRAX IN RHESUS MACAQUES

Health & Medicine Week

August 3, 2009

"We report the characterization of LF, PA, and PGA levels during the course of inhalation anthrax in five rhesus macaques. We describe bacteremia, blood differentials, and detection of the PA gene (pagA) by PCR analysis of the blood as confirmation of infection. For four of five animals tested, LF exhibited a triphasic kinetic profile. LF levels (mean \pm standard error [SE] between animals) were low at 24 h postchallenge (0.03 ± 1.82 ng/ml), increased at 48 h to 39.53 ± 0.12 ng/ml (phase 1), declined at 72 h to 13.31 ± 0.24 ng/ml (phase 2), and increased at 96 h (82.78 ± 2.01 ng/ml) and 120 h (185.12 ± 5.68 ng/ml; phase 3). The fifth animal had an extended phase 2. PGA levels were triphasic; they were nondetectable at 24 h, increased at 48 h ($2,037 \pm 2$ ng/ml), declined at 72 h (14 ± 0.2 ng/ml), and then

increased at 96 h (3,401 ±8 ng/ml) and 120 h (6,004 ±187 ng/ml). Bacteremia was also triphasic: positive at 48 h, negative at 72 h, and positive at euthanasia. Blood neutrophils increased from preexposure (34.4% ±0.13%) to 48 h (75.6% ±0.08%) and declined at 72 h (62.4% ±0.05%). The 72-h declines may establish a 'go/no go' turning point in infection, after which systemic bacteremia ensues and the host's condition deteriorates."

"This study emphasizes the value of LF detection as a tool for early diagnosis of inhalation anthrax before the onset of fulminant systemic infection."

The full article can be found at: (A.E. Boyer, et. al., "Kinetics of lethal factor and poly-D-glutamic acid antigenemia during inhalation anthrax in rhesus macaques". *Infection and Immunity*, 2009;77(8):3432-41). Link not available.

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SYNTHETIC PEPTIDE VACCINE TARGETING A CRYPTIC NEUTRALIZING EPITOPE IN DOMAIN 2 OF BACILLUS ANTHRACIS PROTECTIVE ANTIGEN

Medical Letter on the CDC & FDA
August 9, 2009

"Current evidence suggests that protective antigen (PA)-based anthrax vaccines may elicit a narrow neutralizing antibody repertoire, and this may represent a vulnerability with PA-based vaccines. In an effort to identify neutralizing specificities which may complement those prevalent in PA antiserum, we evaluated whether sequences within the 2beta2-2beta3 loop of PA, which are apparent in the crystal structure of heptameric but not monomeric PA, might represent a target for an epitope-specific vaccine for anthrax and, further, whether antibodies to these sequences are induced in rabbits immunized with monomeric PA."

"We evaluated the immunogenicity in rabbits of a multiple antigenic peptide (MAP) displaying copies of amino acids (aa) 305 to 319 of this region. Overall, four out of six rabbits vaccinated with the MAP peptide in Freund's adjuvant developed high-titer, high-avidity antibody responses which cross-reacted with the immobilized peptide sequence comprising aa 305 to 319 and with PA, as determined by an enzyme-linked immunosorbent assay, and which displayed potent and durable neutralization of lethal toxin (LeTx) in vitro, with peak titers which were 452%, 100%, 67%, and 41% of the peak neutralization titers observed in positive-control rabbits immunized with PA. Importantly, analysis of sera from multiple cohorts of rabbits with high-titer immunity to PA demonstrated a virtual absence of this potent antibody specificity, and work by others suggests that this specificity may be present at only low levels in primate PA antiserum."

"These results highlight the potential importance of this immunologically cryptic neutralizing epitope from PA as a target for alternative and adjunctive vaccines for anthrax."

The full article can be found at: (J. Oscherwitz, et. al., "Synthetic peptide vaccine targeting a cryptic neutralizing epitope in domain 2 of Bacillus anthracis protective antigen". *Infection and Immunity*, 2009;77(8):3380-8). Link not available.

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HARVESTING CANDIDATE GENES RESPONSIBLE FOR SERIOUS ADVERSE DRUG REACTIONS FROM A CHEMICAL-PROTEIN INTERACTOME

By Lun Yang, Jian Chen, Lin He
PLoS Computational Biology
July 24, 2009

"Identifying genetic factors responsible for serious adverse drug reaction (SADR) is of critical importance to personalized medicine. However, genome-wide association studies are hampered due to the lack of case-control samples, and the selection of candidate genes is limited by the lack of

understanding of the underlying mechanisms of SADR. We hypothesize that drugs causing the same type of SADR might share a common mechanism by targeting unexpectedly the same SADR-mediating protein. Hence we propose an approach of identifying the common SADR-targets through constructing and mining an in silico chemical-protein interactome (CPI), a matrix of binding strengths among 162 drug molecules known to cause at least one type of SADR and 845 proteins. Drugs sharing the same SADR outcome were also found to possess similarities in their CPI profiles towards this 845 protein set. This methodology identified the candidate gene of sulfonamide-induced toxic epidermal necrolysis (TEN): all nine sulfonamides that cause TEN were found to bind strongly to MHC I (Cw*4), whereas none of the 17 control drugs that do not cause TEN were found to bind to it. Through an insight into the CPI, we found the Y116S substitution of MHC I (B*5703) enhances the unexpected binding of abacavir to its antigen presentation groove, which explains why B*5701, not B*5703, is the risk allele of abacavir-induced hypersensitivity. In conclusion, SADR targets and the patient-specific off-targets could be identified through a systematic investigation of the CPI, generating important hypotheses for prospective experimental validation of the candidate genes."

"Author Summary Top

Why do tragedies caused by Vioxx or Avandia only happen to certain individuals? The unexpected bindings among drugs and human proteins might play important roles in such serious adverse drug reactions (SADRs). To mine these unexpected chemical-protein interactions, 162 drug molecules known to cause SADRs are 'hybridized' onto 845 proteins to construct a chemical-protein interaction matrix, from which two aspects of the information, the binding strength and the binding conformation, are disclosed. Followed by the data-mining strategies, the unexpected bindings that mediate SADRs are identified. For example, abacavir is found to bind to the antigen presentation groove of MHC I molecule in patients carrying the B*5701 allele but not B*5703, which explains why HLA-B*5701, not B*5703, is the risk allele of abacavir hypersensitivity. This research could explain to the public that SADR happens when some of the innocent proteins are attacked by drugs unexpectedly, and variances in certain people's genome make their proteins more sensitive to the drug. By pre-therapy screening, the susceptible people could be protected. Furthermore, new drugs or modified drugs will be designed to avoid these patient-specific unintended bindings, in a step toward realizing personalized medicine."

The full article can be found at: <http://www.ploscompbiol.org/article/info%3Adoi%2F10.1371%2Fjournal.pcbi.1000441;jsessionid=6392AC7A835D166DD7EF38C35EFA2AEB>
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RESEARCH SUGGESTS THAT ZINC ACTIVATES A KEY PROTEIN ON T CELLS NEEDED TO FIGHT INFECTIONS

News-Medical.net
July 31, 2009

"Now, a new research study in the August 2009 print issue of the Journal of Leukocyte Biology suggests that zinc may be pointing the way to new therapeutic targets for fighting infections.

Specifically, scientists from Florida found that zinc not only supports healthy immune function, but increases activation of the cells (T cells) responsible for destroying viruses and bacteria.

"It has been shown that zinc supplementation significantly reduces the duration and severity of childhood diarrhea, lower respiratory infections, and incidence of malaria in zinc-deficient children," said report co-author, Robert Cousins, Ph.D., who also is the director of the Center for Nutritional Sciences within the Food Science and Human Nutrition Department at the University of Florida. "Age-related declines in immune function have also been related to zinc deficiency in the elderly."

The full article can be found at: <http://www.news-medical.net/news/20090731/Research-suggests-that-zinc-activates-a-key-protein-on-T-cells-needed-to-fight-infections.aspx>
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BUILDING DISEASE-SPECIFIC DRUG-PROTEIN CONNECTIVITY MAPS FROM MOLECULAR INTERACTION NETWORKS AND PUBMED ABSTRACTS

By Jiao Li, Xiaoyan Zhu, Jake Yue Chen

PloS Computational Biology

July 31, 2009

“Abstract

The recently proposed concept of molecular connectivity maps enables researchers to integrate experimental measurements of genes, proteins, metabolites, and drug compounds under similar biological conditions. The study of these maps provides opportunities for future toxicogenomics and drug discovery applications. We developed a computational framework to build disease-specific drug-protein connectivity maps. We integrated gene/protein and drug connectivity information based on protein interaction networks and literature mining, without requiring gene expression profile information derived from drug perturbation experiments on disease samples. We described the development and application of this computational framework using Alzheimer's Disease (AD) as a primary example in three steps. First, molecular interaction networks were incorporated to reduce bias and improve relevance of AD seed proteins. Second, PubMed abstracts were used to retrieve enriched drug terms that are indirectly associated with AD through molecular mechanistic studies. Third and lastly, a comprehensive AD connectivity map was created by relating enriched drugs and related proteins in literature. We showed that this molecular connectivity map development approach outperformed both curated drug target databases and conventional information retrieval systems. Our initial explorations of the AD connectivity map yielded a new hypothesis that diltiazem and quinidine may be investigated as candidate drugs for AD treatment. Molecular connectivity maps derived computationally can help study molecular signature differences between different classes of drugs in specific disease contexts. To achieve overall good data coverage and quality, a series of statistical methods have been developed to overcome high levels of data noise in biological networks and literature mining results. Further development of computational molecular connectivity maps to cover major disease areas will likely set up a new model for drug development, in which therapeutic/toxicological profiles of candidate drugs can be checked computationally before costly clinical trials begin.

Author Summary

Molecular connectivity maps between drugs and a wide range of bio-molecular entities can help researchers to study and compare the molecular therapeutic/toxicological profiles of many candidate drugs. Recent studies in this area have focused on linking drug molecules and genes in specific disease contexts using drug-perturbed gene expression experiments, which can be costly and time-consuming to derive. In this paper, we developed a computational framework to build disease-specific drug-protein connectivity maps, by mining molecular interaction networks and PubMed abstracts. Using Alzheimer's Disease (AD) as a case study, we described how drug-protein molecular connectivity maps can be constructed to overcome data coverage and noise issues inherent in automatically extracted results. We showed that this new approach outperformed both curated drug target databases and conventional text mining systems in retrieving disease-related drugs, with an overall balanced performance of sensitivity, specificity, and positive predictive values. The AD molecular connectivity map contained novel information on AD-related genes/proteins, AD candidate drugs, and protein therapeutic/toxicological profiles of all the AD candidate drugs. Bi-clustering of the molecular connectivity map revealed interesting patterns of functionally similar proteins and drugs, therefore creating new opportunities for future drug development applications.”

The full article can be found at: <http://www.ploscompbiol.org/article/info%3Adoi%2F10.1371%2Fjournal.pcbi.1000450;jsessionid=F3DCA55F051962C9069D14B170E070B9>

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ISOLATION OF GENETICALLY DIVERSE MARBURG VIRUSES FROM EGYPTIAN FRUIT BATS

By Jonathan S. Towner, Brian R. Amman, Tara K. Sealy, Serena A. Reeder Carroll, James A. Comer, Alan Kemp, Robert Swanepoel, Christopher D. Paddock, Stephen Balinandi, Marina L. Khristova, Pierre B. H. Formenty, Cesar G. Albarino, David M. Miller, Zachary D. Reed, John T. Kayiwa, James N. Mills,

Deborah L. Cannon, Patricia W. Greer, Emmanuel Byaruhanga, Eileen C. Farnon, Patrick Atimnedi, Samuel Okware, Edward Katongole-Mbidde, Robert Downing4, Jordan W. Tappero, Sherif R. Zaki, Thomas G. Ksiazek, Stuart T. Nichol, Pierre E. Rollin
PloS Pathogens
July 31, 2009

“Abstract

In July and September 2007, miners working in Kitaka Cave, Uganda, were diagnosed with Marburg hemorrhagic fever. The likely source of infection in the cave was Egyptian fruit bats (*Rousettus aegyptiacus*) based on detection of Marburg virus RNA in 31/611 (5.1%) bats, virus-specific antibody in bat sera, and isolation of genetically diverse virus from bat tissues. The virus isolates were collected nine months apart, demonstrating long-term virus circulation. The bat colony was estimated to be over 100,000 animals using mark and re-capture methods, predicting the presence of over 5,000 virus-infected bats. The genetically diverse virus genome sequences from bats and miners closely matched. These data indicate common Egyptian fruit bats can represent a major natural reservoir and source of Marburg virus with potential for spillover into humans.

Author Summary

Marburg virus, similar to its close cousin Ebola virus, can cause large outbreaks of hemorrhagic fever (HF) in rural Africa with case fatalities approaching 90%. For decades, a long-standing enigma has been the identity of the natural reservoir of this deadly virus. In this report, we identify the cave-dwelling Egyptian fruit bat (*Rousettus aegyptiacus*) as a natural host of Marburg virus based on multiple lines of evidence which include, for the first time ever, the isolation of virus directly from wild-caught and apparently healthy bats. The species *R. aegyptiacus* is common throughout Africa with distribution into the eastern Mediterranean and Middle East. Our finding of active virus infection in approximately 5% of *R. aegyptiacus* bats and their population exceeding 100,000 in Kitaka cave in Uganda suggests there are likely over 5,000 Marburg virus-infected bats in this cave, which is only one of many such cave populations throughout Africa. Clearly, these bats could serve as a major source of virus with potential to initiate human epidemics, and the implications for public health are striking. Additionally, we found highly divergent (21%) genome sequences among viruses circulating in these bat populations, a level of diversity that would result from a long-term association with a suitable reservoir host of large population size.”

The full article can be found at: <http://www.plospathogens.org/article/info%3Adoi%2F10.1371%2Fjournal.ppat.1000536;jsessionid=C513F832F4525A21A2F4C76A21AE339C>

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WORLD SHORTAGE OF MOLYBDENUM MAY HINDER NUCLEAR MEDICINE SERVICES

The Medical News

August 05, 2009

“Problems with the world production and supply of molybdenum are leading to shortages of radioisotopes for nuclear medicine imaging tests, according to an editorial in the journal *Nuclear Medicine Communications*, official journal of the British Nuclear Medicine Society (BNMS).”

Over the years, production and distribution of molybdenum—used to produce radiopharmaceuticals containing technetium-99m, widely used in nuclear medicine—has been highly reliable. However, problems have arisen due to the age of the commercial reactors that make molybdenum. All but one of the six reactors worldwide are more than 40 years old.

In the last two years, shutdowns of reactors in Canada and the Netherlands have led to disruptions in the supply of molybdenum to hospital nuclear medicine departments in the United Kingdom, Europe, and North America. With supplies reduced to 30 percent of normal, departments have had to adapt to make the most effective and efficient use of their supplies.

Earlier this year, the Nuclear Energy Agency convened an international workshop of molybdenum producers and distributors, nuclear medicine societies (including the BNMS), and clinicians to discuss the problem. "In the foreseeable future there will be further disruptions in molybdenum supplies and the cost will increase significantly," workshop attendees agreed. At the same time, they pledged to address the problems through exchange of materials, information, and strategic support."

The full article can be found at: <http://www.news-medical.net/news/20090805/World-shortage-of-molybdenum-may-hinder-nuclear-medicine-services.aspx>

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