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VISION

ABSA, the leader in the profession of biological safety.

MISSION STATEMENT

The American Biological Safety Association is dedicated to expanding biological safety awareness to prevent adverse occupational and environmental impact from biohazards.

GOALS

- Expand professional and public awareness of biological safety through effective communication.
- Participate in the development of biological safety standards, guidelines, and regulations.
- Develop ABSA as the recognized resource for profession and scientific expertise in biological safety.
- Advance biological safety as a scientific discipline through education, research, and professional development.
- Develop and maintain standards for biological safety professionals.
IN MEMORIUM

In Appreciation
John Howard Richardson
1930-1998

The world biological safety community lost a quiet giant when John H. Richardson died February 10 at his home in Atlanta, Georgia. He had lung cancer.

Dr. John Richardson was a remarkable individual who made an enormous contribution to the field of biological safety. John had many fine qualities that formed the foundation for his full and distinguished career. Among these were a strong academic background in veterinary medicine and public health, an uncompromising belief in the goodwill of his colleagues, the value he placed on using his abilities in service to others, and a deep inner spirit that was nurtured by the love he shared with his family and friends, his colleagues, his church, and with nature.

John received his doctorate in veterinary medicine from the University of Georgia in 1954. He then joined the U.S. Public Health Service as a commissioned officer and was assigned to the Epidemiology Intelligence Service at the Communicable Disease Center, the predecessor organization of the Centers for Disease Control and Prevention (CDC). His career path included service in the state health departments of Delaware, Kansas and Georgia, and a stint at the Tulane University School of Public Health where he was awarded the M.P.H. degree in 1963. After an assignment with the CDC foreign quarantine program, John joined the CDC Office of Biosafety in 1970 and served as its director from 1975 until his retirement from the Public Health Service in 1984. For many years he was a consultant with the World Health Organization and directed the WHO Collaborating Centre for Applied Biosafety Programmes and Research at CDC. In 1985, John began a career in academia at Emory University where he was appointed as an associate professor in environmental health. He became the director of the University’s Environmental Health and Safety Office in 1989 and served in that capacity until 1992 when he chose to conclude his formal professional career.

Everyone who knew John respected his intelligence, enjoyed his keen wit, benefited from the openness with which he shared his knowledge, admired his capacity to work, was reassured by his calm demeanor, and trusted his impeccable judgment. John possessed the uncanny ability to distill the simple truths from the complexities of life’s experiences. He used this special talent to bring about a new code of practice for safeguarding the health of laboratory personnel who work with infectious agents.

John conceived the idea for an authoritative code of biosafety practice and lead the CDC-NIH collaboration that resulted in the 1984 publication of Biosafety in Microbiological and Biomedical Laboratories. With persistence and determination over a seven year period, he forged a common bond among disparate groups of biomedical scientists and clinical microbiologists that made consensus possible. The enormity of this achievement can best be appreciated by reviewing the names of the guest editors, panel chairmen, and special contributors listed in the first edition of the Guidelines.

John also made significant contributions to the American Biological Safety Association. He served as president in 1987-1988, delivered the Arnold G. Wedum Memorial Lecture at the 32nd Biological Safety Conference in New Orleans, Louisiana, and worked on numerous committees. He believed in the ideals of professionalism and was extraordinarily helpful in establishing the certification program of the American Academy of Microbiology for biological safety professionals.

John will be missed and always remembered for his contributions to the field of biological safety and for the joy he brought to the hearts of everyone he touched.

W. Emmett Barkley
PRESIDENT’S PAGE

Biological agents (Infectious agents and toxins) are hot stuff with the media in today’s world. Top headlines for the past three months go to Iraq’s deadly arsenal of chemical and biological weapons. Where are they manufactured? Where are they stored? How do we destroy them? In the last weeks of February the arrest of two people, one with links to a right wing racial group, for possessing large amounts of anthrax, elbowed its way onto the front page of newspapers and magazines and into one minute sound bites on the news shows. On one news show retired Senator Bill Bradley talked about the vulnerability of our society to biological terrorists and illustrated this vulnerability by referring to the possibility of crop duster airplanes spraying anthrax over New York City. A year ago retired Senator Sam Nunn also discussed on national TV our vulnerability to biological terrorists. The Discovery Channel’s SpyTec presented in detail the murder of a Bulgarian patriot by the Bulgarian KGB using an umbrella gun that injected an iridium pellet containing the toxin ricin into the leg of the patriot.

However, the role of biological agents in threatening our food, water and air also regularly makes it way into the news media. In the past several weeks special feature TV shows addressed bovine spongiform encephalopathy (BSE) and the Hong Kong flu.

Biological agents are also a favorite topic of book authors. Just wander over to the science section of your local bookstore. About 2% of the books are on infectious agents. These books include The Hot Zone (Richard Preston), Virus Hunter (C.J. Peters), The Coming Plague (Laurie Garrett), Level 4 (Joseph McCormick and Susan Fisher-Hoch), Virus X (Frank Ryan), Deadly Feasts (Richard Rhodes), Emerging Viruses (Leonard Horowitz). You might also find The Forgotten Plague (Frank Ryan), The Dancing Matrix (Robin Marantz Heydig) and Emerging Viruses (Stephen S. Morse).

If you need some riveting stories on the use of biological agents to kill, harm or terrorize large numbers of people wander over to the fiction book section. You might be able to feel the terror after reading such books as Outbreak and Contagion (Robin Cook), Carrier (Peter Lynch), The Stand (Stephen King), The Cobra Event (Richard Preston), Executive Orders (Tom Clancy), The Pandemic (Pierre Oullette), The Eleventh Plague (John S. Mann and John Baldwin), and Code Red (Nancy Fisher).

If you were to wander over to the magazine section you would find headlined on the front cover of Newsweek (February 23) the Flu Hunters. On February 8, Parade magazine headlined on its front cover “The Virus Hunters.” Just three months ago (November 24, 1997), Newsweek, Time, and US News and World Report all had stories related to microbes (two on biological warfare and one on antibiotic resistance) on their front cover.

As the media continue to sensitize the public to biological agents the requirements for biological laboratories to reassure the public that they have qualified workers in appropriately designed facilities under safe work conditions will grow. In today’s climate of biological terrorism the laboratories also have to assure the public that they are performing legitimate work that is in the public interest and that they can maintain their biological agents securely so that they cannot end up in the wrong hands. Unsafe laboratories are newsworthy. Laboratory acquired infections are newsworthy. Nobody wants an unsafe laboratory in their backyard. Nobody wants an unauthorized biological laboratory in their backyard. The best assurances that a laboratory can provide the public is to demonstrate that they have a complete biological safety program managed by trained biological safety professionals that is in accordance with all federal and state regulations and guidelines. ABSA needs to become recognized as a national resource and the national leader for information, training, education and certification in all biosafety program elements.

Richard Knudsen
Centers for Disease Control
Atlanta, Georgia
EDITOR'S PAGE

Class II biological safety cabinets are the most widely used safety devices for protection against biological hazards. When combined with recommended good working practices, they provide a safe working environment for biosafety levels (BL) 1, 2, and 3. The construction, testing, and certification of class II biological safety cabinets are detailed in NSF International's Standard 49, entitled, "Class II (Laminar Flow) Biohazard Cabinetry." It is a consensus standard prepared by a committee composed of cabinet manufacturers, users, certifiers, and public interest representative and adopted by the NSF Board of Trustees. It is anticipated that NSF Standard 49 will be adopted by the American National Standards Institute (ANSI) and by the International Standards Institute (ISO).

NSF 49 was first issued in June 1976. The standard development process was initiated at the request of the National Institutes of Health (NIH) to formalize (1) cabinet designs that had previously been developed by NIH (Type A cabinets) and the National Cancer Institute (Type B cabinets), (2) test protocols and (3) acceptance criteria. NSF 49 has been revised, as prescribed, at roughly 5-year intervals and currently is under study for the next revision. In general the successive revisions are gradually converting the document from a construction (how to build it) to a performance (what it must do) standard in order to provide the widest possible scope for designers and manufacturers to incorporate innovative designs, improved materials, and more efficient construction methods into their NSF-certified cabinets. This has been effective and the process continues.

It has been recommended from the start that field certification of NSF-certified cabinets be conducted immediately after initial installation, after a cabinet has been moved, following repairs that require removal of access panels, and at least annually to verify that the cabinet continues to operate as it did in the testing laboratory at NSF when it was certified.

In the latest revision of NSF 49-1992 field test procedures and acceptance criteria are identified explicitly for the first time. They appear as "Annex F, Field Tests," accompanied by this notation, "This Annex is not part of Standard 49 but is provided for information only." This may suggest that NSF has taken a trip to Wonderland with Alice, but in fact, it was a stratagem devised by NSF to obtain approval of the 1992 revisions in the face of a badly fractured standards committee. Most of the field certifier representatives on the committee adamantly opposed making Annex F mandatory because of their unwillingness to conduct cabinet leak tests "when [a] cabinet is newly installed, relocated, or after maintenance procedures that require removal of [interior access] panels." The reasons given were (1) Type B cabinets have negative pressure contaminated ducts and plenums and hence cabinet leaks are not a problem (no reason was provided for not field testing Type A cabinets that do have positive pressure contaminated ducts and plenums) (2) competitors were not doing it and hence they would be at a cost disadvantage if they insisted on doing cabinet leak tests in addition to the ventilation and filter leak test procedures. In rebuttal it was pointed out (1) negative pressure Type B cabinets as well as positive pressure Type A cabinets will both emit formaldehyde during decontamination when the cabinets are not leak tight (2) were Annex F to be adopted as a mandatory part of NSF 49, every cabinet certifier would have to perform this safety check at the designated times and there would be a level playing field for everyone.

Why is this an important matter for the entire community that depends on Class II cabinets for maintaining safe operations? Because NSF 49 is in the process of undergoing its mandatory 5-year review and is open for public comment on this as well as on any other part. It is not only important for the users of this equipment to understand how and why it functions as a safety device but also to appreciate the importance of the field certification process as an important safety check and to insist that it be conducted in all its phases by well-trained, experienced, and conscientious technicians. I must report that I continue to hear (sometimes under oath) about slack field certification procedures and what I perceive to be safety lapses by some field
EDITOR'S PAGE (continued)

certification personnel. Therefore, I say to all biosafety cabinet users, be on your guard. Learn about the field certification procedures in NSF 49. Observe your field certification technicians closely while they are at work. Ask lots of questions and insist on clear, rational answers. Field certification is not a ritual, every user’s health and safety are at stake.

The writer wishes to be fair to readers by identifying himself as a member of past NSF 49 standard preparation committees and the principal author of Annex F.

Melvin W. First
Harvard School of Public Health
Boston, Massachusetts

CORRECTION:

JABSA regrets the omission of the name of one of the three Nobel Laureates who have addressed ABSA. The Editorial on page 6 in Volume 2, Number 4, 1997 stated that two Nobel Laureates have addressed ABSA. However, at the 28th Annual Meeting in 1985 in La Jolla, California, the 10th Arnold G. Wedum Memorial Lecture was delivered by Nobel Laureate Dr. Renato Dulbecco who discussed “Risk of Containment of Viruses.” Thus, three Nobel Laureates have addressed the Association to date, rather than two. The JABSA appreciates Richard Kruse’s observation of this oversight.

CORRECTION:

JABSA regrets omitting the name of one of the authors of the article entitled, “Handling of Large Experimental Animals Infected with a Risk Group 4 Virus” which was published in Volume 2, Number 4, 1997. The authors are affiliated with the Australian Animal Health Laboratory in Geelong, Victoria, Australia. The full list of authors is:

G. Abraham
P. Hooper
M. Williamson
J. Muschialli
D. Martin
I. Duff
S. Edwards
LETTER TO THE EDITOR

The Journal of the American Biological Safety Association has presented a number of informative articles on the history of biosafety. Since the ABSA logo was under intense scrutiny in the last issue of JABSA, I thought the time was right to reprint the origin of the center of the ABSA logo—the biohazard symbol.

Karen B. Byers, MS, RBP

BIOHAZARDS SYMBOL: DEVELOPMENT OF A BIOLOGICAL HAZARDS WARNING SIGNAL

ABSTRACT

The need for a symbol to warn of potential infection hazards became apparent during Public Health Service contract work on the development of containment facilities for virus-leukemia research. A program of direct inquiry and a search of the literature revealed that there was no universally used signal and that scientific and safety organizations concurred in the need for one. Criteria for symbol design were established, and final section was based on "uniqueness" and "memorability." The National Institutes of Health is recommending use of the symbol as a warning of biological hazard.

The scientific community, engaged in infectious disease research, has accepted as unfortunate, but unavoidable, the occasional accidental infection of microbiology laboratory personnel and associated nonlaboratory personnel. Since the mid-1940's, the seeming inevitability of such accidents has received an increasing amount of study. The eventual consensus was that perhaps most of these accidents need not happen, providing proper precautionary measures are taken and enforced. The last decade, in particular, saw great strides in the development of containment systems and in the design of safety equipment to protect the laboratory worker, his work, and the exterior environment from contamination by infectious agents. A new science of containment, founded on the concept of continuous agent control through the increasing of intelligently designed barrier systems, has emerged. Design of these barriers is based on a rational assessment of risk; the barriers may be created in the form of solid walls, pressure differentials to control movement of air, controlled movement of personnel and materials, or inactivation of the infectious agents themselves. In the maintenance of the barrier systems, one essential factor is that, at all times, the locations of te infectious agents must be known in order not to inadvertently violate the barrier systems. Each person in the vicinity must know what equipment, glassware, rooms, corridors, and ducts are contaminated by the infectious agents and that thereby, they constitute a biological hazard.

Unfortunately, such biological hazards, like radiation hazards, are usually impossible to detect by cursory examination only. Being invisible, odorless, and tasteless, they require special procedures for detection. It seems logical, then, to mark the location of "biohazards", as they are commonly called, with a suitable warning sign that is readily noticed and easily recognized.

During investigations of biological control and containment conducted under contract for the National Cancer Institute, the need for such a symbol became apparent to the Dow biohazards research and development team. A search of the literature revealed that, while certain biological warning signs are used by various agencies, a universal symbol to warn of danger from infectious or potentially infectious agents—a symbol whose immediate significance is known to all—does not exist. Colleagues in the field of biological research concurred, in reply to direct query, that such a warning symbol is needed.

Universally accepted symbols for hazards that are not readily detectable have already been established, such as those used in denoting radioactive areas. Similar warning notices are being sought to point out danger due to laser emission. In biology laboratories, however, a number of different symbols are in use; none of these has been universally accepted, and none imply or encompass all possible biohazards. For example, an inverted blue triangle bearing the term "BIO" is used by the Army to warn of biological contamination; a rectangular "hot-pink" label, with radiating yellow bands, is used by
LETTER TO THE EDITOR (continued)

U.S. Navy laboratories in areas containing infectious organisms; a red and black sign is used by the National Institutes of Health to mark restricted areas; and the white snake-and-staff imprint on a violet field is sponsored by the Universal Postal Convention to mark infectious materials during transit.

In formulating the design for the proposed biohazards symbol, six criteria were established, mainly dealing with the psychology of recognition and retention. These criteria, in order of their importance, are that the symbol be (i) striking in form in order to draw immediate attention; (ii) unique and unambiguous, in order not to be confused with symbols used for other purposes; (iii) quickly recognizable and easily recalled; (iv) easily stenciled; (v) symmetrical, in order to appear identical from all angles of approach; and (vi) acceptable to groups of varying ethnic backgrounds. Dow artists created more than 40 symbol designs, of which six were selected for testing. A survey to ascertain acceptability of the six symbols was conducted among Dow employees. This survey was directed toward determining uniqueness and memorability.

To select the final symbol, a nationwide survey, based on precepts well established in mass-psychology experimentation, was conducted in two parts. First, the candidate symbols were tested for uniqueness by determining which had the least prior association for the viewer. Three hundred subjects, males and females, from 25 cities and with various amounts of income and formal education were shown the six symbols along with 18 other commonly used symbols. They were asked what each symbol meant, or was used for. Participants were also encouraged, if uncertain, to guess at the meaning. A "meaningfulness score" was obtained for each symbol based on the percentage of respondents who offered any association whatever to the symbol. Since the purpose was to determine the least meaningful symbol, the smaller scores identified the most desirable symbols.

One week after the initial survey had been conducted, participants were revised for a "memorability" test. The original respondents were shown a group of 60 symbols which included the 24 seen during the first test. They were asked to identify those symbols which they had been shown on the first interview. Each symbol was given a "memorability score" that depended on the percentage of participants who correctly identified the symbol as having appeared in the earlier test.

Identical memorability scores were obtained for two of the six test symbols, and these scores exceeded the average for the other 18 symbols tested. Since one of the two also obtained the lowest score in the meaningfulness test, it emerged as the one symbol best qualified as being both unique and memorable (Figure 1).

![FIGURE 1](Biohazard warning symbol. The design color stipulated in the standard form is fluorescent orange-red.)
LETTER TO THE EDITOR (continued)

Having evolved a suitable symbol, the next step was to attach the designed significance to it. It became important to define as clearly as possible how and under what circumstances the symbol should be used. A use standard was therefore prepared. This standard stipulates that the symbol “shall be used to signify the actual or potential presence of a biohazard and shall identify equipment, containers, rooms, materials, experimental animals, or combinations thereof which contain or are contaminated with viable hazardous agents. It also defines the term “biohazard”, for the purpose of the standard, as being: “those infectious agents presenting a risk or potential risk to the well-being of man, either directly through his infection or indirectly through disruption of his environment.”

This symbol and the recommendations regarding usage have been submitted to the United States of America Standards Institute for inclusion in their next revision of the “Standard Specifications for Industrial Accident Prevention Signs,” Z35.1 code.

This symbol, in fluorescent fire-orange color, has been evaluated during a 6-month period at the National Cancer Institute and other selected laboratories engaged in studies involving hazardous agents. These cooperating research groups included the U.S. Army Biological Laboratories and the U.S. Department of Agriculture laboratories, as well as a number of commercial and academic laboratories working under National Institutes of Health research grants and contracts.

In view of its acceptance by the scientists during this evaluation, the National Institutes of Health is recommending that this symbol be used as a general biological hazard warning.

Note: The subject material of this paper was presented at the 6th Annual Technical Meeting of the American Association for Contamination Control, Washington, D.C., 18 May 1967. The work was performed for the National Cancer Institute under contract No. PH 43-65-1045.

7 July 1967


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A HISTORY OF THE AMERICAN BIOLOGICAL SAFETY ASSOCIATION. 
PART III: SAFETY CONFERENCES 1978-1987

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INTRODUCTION

The biological safety conferences continue to grow in attendance and the number of presentations. Many topics that were matters of concern at the first biological safety conference in 1955 continue to interest those attending: biosafety cabinets; air sampling; laboratory-acquired illnesses; and decontamination. Improvements have occurred as described in A History of the American Biological Safety Association Part I (Barbeito 1997) and Part II (Kruze 1997). Today, more infectious microorganisms are studied by an increased number of scientists and laboratory personnel, but laboratory-acquired infections have decreased. Biological safety personnel must aggressively continue to pursue basic and applied research applicable to biosafety. Class II biosafety cabinets are replacing Class I biosafety cabinets. Data presented in biological safety conferences have been instrumental in significant changes in microbiological safety. The biological safety conference’s eulogy to Dr. Wedem in the form of an Annual Memorial Lecture had an auspicious beginning with Dr. Karl M. Johnson delivering the first lecture.

21st Biological Safety Conference

The twenty-first biological safety conference, sponsored by the Center for Disease Control Hepatitis Laboratories, Phoenix, was held November 6-8, 1978 at the Safari Hotel Convention Center, Scottsdale, Arizona. After a Sunday night cocktail party many attendees left to enjoy Mexican food.

Dr. John Jaugstetter, Centers for Disease Control (CDC), discussed a revision of the Classification of Etiological Agents on the Basis of Hazards. It included an expanded list of etiologic agents, a detailed explanation of containment levels, and a list of containment levels commensurate with the risk of the etiologic agent.

Vincent Oviatt, World Health Organization (WHO), discussed four special programs established in 1987 by WHO: laboratory safety elements; emergency services; shipment of infectious substances; and maximum containment laboratories. A WHO brochure Public Health Aspects and Safety Regulations in General Experimentation was published.

Dr. John Forney described changes over the past 18 years at CDC. Most important were modifications of buildings, procedural changes, and the added number of personnel handling infectious material. In 1977, an extensive survey and questionnaire received 99% response from bench microbiologists who believed training programs needed strengthening. Most employees were not cognizant of the Safety Manual.

Ralph Kuehne described the special containment facilities available at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) to study highly infectious viruses (Ebola, Lassa, Machup, Marburg, etc.) for which there are no effective prophylaxis or therapy. USAMRIID is the only facility in the United States capable of transporting, isolating, and providing medical care to persons exposed to high hazard microbes.

Dr. Martin Favero, CDC-Phoenix, discussed disagreements among space scientists in bringing Martian "soil" to earth. Some scientists believe the risks are enormous, some state contamination technology is inadequate and join others stating the mission should be cancelled. Dr. Favero believes the United States has the capability and technology to fully contain a sample from Mars.

James Lauer described 15 clinical laboratory cases of hepatitis that occurred in 1976 at the University of Minnesota in high-risk areas; eight in

*Note: See article “Mars Sample Return and Biocontainment” in this issue of JABSA.
the hemodialysis-transplantation area, and seven in the clinical laboratories. Of 76 samples of environmental surfaces, 26 were positive for hepatitis B surface antigen.

Dr. Waldemar F. Kirchheimer delivered the 2nd Arnold G. Wedum Memorial Lecture entitled, *Recent Advances in Experimental Leprosy*. Dr. Kirchheimer was Dr. Wedum's assistant until he joined the staff of the U.S. Public Health Service's (USPHS) hospital in Carville, Louisiana. He compared treatment of leprosy from Biblical times to today's patient care. Dr. Kirchheimer described the hospital and laboratories at Carville, and discussed new research he was instrumental in perfecting. The nine-banded armadillo (*Dasypus novemcinctus Linn.*) always gives birth to identical quadruplets, and provides a system for the study of immunological factors which control development of the disease. *Mycobacterium leprae* has been cultivated to $10^9$ viable organisms in the armadillo.

Dr. Jerome Landy, Germfree Laboratories, Inc., described a new total exhaust laminar flow biosafety cabinet in which air is not recirculated.

John Harb, University of North Carolina (UNC), discussed the effectiveness of formaldehyde gas at various humidities and contact times. Paper strips impregnated with *Bacillus subtilis* subsp. *niger* (*BG*) were placed in a desiccator and exposed to formaldehyde gas at relative humidities (RH) of 33%, 53%, and 75% and contact times of 1, 4, 7, 10, 16, and 24 h. He reported that only at 75% RH and 10-h contact time were $10^6$ *BG* spores completely killed. At 33% and 53% RH, complete kill did not occur even after 24-h contact time. The discussions that ensued suggested that, if his data were correct, everything learned or practiced on decontamination procedures up to this time was wrong.

Mr. Everett Hanel, Jr., described the methodology used to successfully decontaminate an animal holding facility in which animals infected with slow viruses had been housed for several years. Sodium hypochlorite adjusted to pH 11.5 was used as a primary decontaminant followed by depolymerization of paraformaldehyde.

**22nd Biological Safety Conference**

The twenty-second biological safety conference, sponsored by National Cancer Institute (NCI), met October 15, 1979 in Bethesda, Maryland. Dr. Robert Stevenson, Litton Bionetics, moderated a seminar on the Ethical and Legal Issues in the Laboratory Workplace. The participants: Samuel Gorovitz, University of Maryland; Sheldon Samuels, AFL-CIO; Estell Ramey, Georgetown University School of Medicine; and Daniel Singer, Attorney, Washington, DC, described hazards found in the scientific laboratory in addition to etiological agents. They further stated that laboratory personnel faced inconsistent regulations and real risks that might interfere with their scientific freedom and privacy rights.

Dr. Previn Blatt described the development of safety guidelines at Yale University. Individual investigators had assumed responsibility for safety until 1970 when a university-wide Safety Advisory Committee developed uniform policies and procedures. In 1978, the Department of Biological Safety was established.

Dr. Hotse Bartlema, European Molecular Biology Laboratory, Heidelberg, Germany, described a U-shaped tunnel approximately 19 m with a diameter of 30 cm that is used to test HEPA filters.

On Tuesday afternoon, the safety conference moved to Fort Detrick for the Dr. Arnold G. Wedum memorial. The event took place in front of Building 550, the headquarters of Industrial Health and Safety Division when the Post was under the command of the U.S. Army Chemical Corps. Dr. William Payne, NCI, introduced Everett Hanel, Jr., Frederick Cancer Research Center (FCRC), who introduced the platform guests. After a brief statement, Major General Kenneth Dirks, U.S. Army, presented the dedicatory flag to Mrs. Wedum and Eric, Dr. Wedum's son. Donal S. Frederickson, Director of National Institutes of Health (NIH), gave the dedication speech followed by remarks of Dr. Arthur Upton, Director of NCI. Many friends of Dr. Wedum and personnel who worked in Industrial Health and Safety Division attended.

After the ceremony, a trip to Cozy Restaurant in Thurmont, Maryland. After dinner, Dr. Clarence J. Gibbs, NIH, delivered the 3rd Arnold G. Wedum Memorial Lecture entitled, Transmissible Virus Dementias of Man, an Overview. The viruses causing kuru, Creutzfeldt-Jakob disease, and scrapie are called "subacute spongiform virus encephalopathies." They are very resistant to ultravio-
let and ionizing radiation, heat, and formaldehyde. The diseases may have an incubation period of 90 months before the onset of symptoms. Clinical, pathological, and epidemiological data on the diseases were discussed.

Dr. David Stuart, The Baker Company, described concentration of toluene vapor in a Class II, Type B biosafety cabinet. His data showed work should be performed toward the rear of the work area.

Dr. Jerry Walker, Plum Island Animal Disease Center (PIADC), discussed the escape of foot and mouth disease virus from a high containment laboratory that infected normal cattle on the island. After all the animals were transferred into laboratories, all areas were disinfected. An investigation showed that the pressure differential in a high containment laboratory had changed from negative to positive, and the filters leaked around the gaskets. Another probable source of viral escape may have been from liquid seepage under a temporary partition erected during construction.

Vincent Oviatt, WHO-Geneva, discussed essentials for developing biosafety programs. Programs were implemented in member countries, and occupationally-acquired illnesses now will be reported to WHO.

23rd Biological Safety Conference
The twenty-third biological safety conference, sponsored by MEDI, Inc., was held October 12-15, 1980 at the Campbell House Inn, Lexington, Kentucky.

Everett Hanel, Jr., presented the first 25 years of biosafety conferences. Dr. Jerry Tulis reviewed the first year of the Bioscience Program at University of North Carolina's (UNC) graduate educational program established under Chairman John E. Larsh, Jr., in the School of Public Health, to confer M.P.H. and Dr.P.H. degrees. Dr. Albert Balows, CDC, and President, American Society for Microbiology (ASM) stated that the failure of many scientists to adhere to microbiological safety practices is a pressing problem.

Kenneth Brow, NCI, analyzed time and cost required to build or renovate small laboratory suites, research animal facilities, P-3 laboratories, and large multistoried buildings. Dr. Howard Larsh, University of Oklahoma, described procedures used to decontaminate and remove 15-year-old wooden safety cabinets from the Missouri State Chest Hospital in Mount Vernon that had been used for isolating and identifying M. tuberculosis and pathogenic systemic fungi such as Histoplasma capsulatum and Blastomyces dermatitidis. Dr. Arthur DiSalvo described South Carolina's 93,000 ft² Public Health Laboratory Building. The laboratories have one-pass airflow with a separate air system for the animal quarters. Stanley Nagle, NIH, described the extensive modifications needed to construct the P-4 laboratory in Building 550, Fort Detrick. Dr. Sol Miller, Abbott Laboratories, described new safety laboratories with entry and exit air locks to each module, HEPA-filtered supply air, HEPA- and charcoal-filtered exhaust air.

Back then...
An old-fashioned North Carolina Bar-B-Que was planned for dinner on Monday. Saturday night people met at Bourbon Arabians. The pigs were slowly cooked over hickory embers from dusk Saturday to dawn Sunday. A good time was had by Irene and Mac Vandiviere; Cathy, Markita and Norm Goodman; Eleanor and Ken Schatzle; Connie and Serf Guerra; Eve and Roy Hubbard; the Bob Rushes; the Desmond Robinsons; Dan Lieberman; Jim Sullivan; and Bob Everett who joined the cooks Ann and Tom Christenberry. Sunday, at noon, many sleepy individuals enjoyed lobsters, compliments of The Baker Company, cooked and served by chef Robert Rush.

Dr. Susan Rubinstein, University of Alberta, discussed Canada's laboratory safety regulations and guidelines. In 1977 the Medical Research Council published guidelines for recombinant DNA molecules, animal viruses and cells, and demanded compliance by researchers they funded. Dr. Desmond Robinson, Department of Health and Social Security, London, discussed methods by which the United Kingdom's safety recommendations were initiated, implemented and enforced. Wally Guntherope, London School of Hygiene and Tropical Medicine, described the British Standard for Microbiological Safety Cabinets. Dr. Hotse Bartlema described the European Molecular Biology Laboratory's procedure for biotesting laminar flow biosafety cabinets. The protocol varies from the procedures employed in the United States and
England in that a person sits in front of the cabinet during aerosolization of the test organism. He believed this procedure to be more realistic than the use of a metal cylinder on the work surface. Dr. Barbara Page-Roberts, Vickers Limited, described a negative pressure, flexible heavy gauge plastic isolator system that provided primary containment similar to a Class III biosafety cabinet.

Dr. Alfred Wallbank, University of Manitoba, showed that formaldehyde (2 to 10%) was effective against poliovirus (Sabin L Sc, 2ab) in 8.5% bovine serum albumin (Difco, Detroit, MI). Skim milk (Difco, Detroit, MI) neutralized the reaction.

Dr. Riley D. Housewright, former Scientific Director of Fort Detrick, and Past President of ASM, presented the 4th Arnold C. Wedum Memorial Lecture entitled, Safe Drinking Water and Health. He discussed his long-time association with Dr. Wedum and the many meetings in which more protection for personnel was advocated by Dr. Wedum.

Dr. Peter Gerone, Delta Regional Primate Research Center, described hazards of using experimental animals in relation to their potential impact on human health, the quality of scientific work, and environmental contamination. Dr. S.S. Kalter, Southwest Foundation for Research and Education, discussed experiments with primates, and stated that a vigorous and rigid husbandry protocol must be implemented to prevent animal and human illness. Dr. A.E. New, NCI, discussed the increased use of specific pathogen-free rodents in research. Gnotobiotic animals were defined by Dr. Perry Mathews, National Animal Disease Center (NADC), and he discussed the many ways these animals were used in research.

Lola Bilowich, M.D. Anderson Hospital and Tumor Institute, reviewed the many problems she encountered while establishing a biological safety program.

Irene Melvin, University of Kentucky Medical Center, presented a history of tuberculosis. Case rates show that tuberculosis was far from being eradicated and remains a hazard to personal health and financial security. She emphasized the need for more specific skin test materials.

Dr. Emmett Barkley, NIH, described revisions to the Guidelines for the Laboratory Use of Chemical Carcinogens, issued June, 1979, that recommend three procedures and safeguards applicable to the laboratory to minimize exposure to carcinogenic substances.

At the business meeting, Dr. Emmett Barkley appointed Edward Lazear, Jerry Tulis, David Stuart, Kenneth Jones, Manuel Barbeito, and Richard Kruse to serve on a steering committee charged with coordinating plans for the formation of a formal safety organization.

24th Biological Safety Conference

The twenty-fourth biological safety conference, sponsored by the University of Georgia, CDC, and U.S. Department of Agriculture's (USDA) Southeast Poultry Research Laboratory, was held October 5-7, 1981 at the Center for Continuing Education, University of Georgia, Athens.

Lowell Muse, University of Georgia, described the results from monitoring personnel exposed to isotopes of tritium and iodine. Dr. Julio Rivera, NIH, discussed the protocols for medical surveillance from NIH's Guidelines for Laboratory Use of Chemical Carcinogens. Lola Bilowich reviewed the genotoxic potential of cancer therapeutic agents. A study, using mutagenic activity in urine, confirmed that pharmacy personnel were absorbing these agents. Mary Woebkenberg, National Institute for Occupational Safety and Health (NIOSH), described a passive monitoring method based on Fick's law of diffusion.

Dr. Marshall Levine, Johns Hopkins Medical Institutions, in Considerations in Developing a Medical Surveillance Program for Laboratory-Associated Diseases, stated that programs should be designed as an integral part of the overall laboratory safety program, and should include: establishment of goals, close cooperation between health and safety departments, health education, and periodic evaluation of program effectiveness.

Stephen Pijar summarized 15 years experience with biological safety cabinets, from Class I biosafety cabinets manufactured in the early 1950s to the current Class II biosafety cabinets. Dr. Melvin First, Harvard School of Public Health, described a 2-week course Certification of Biological Safety Cabinets sponsored by NIH. Ten classes were held between June 1979 and August 1981, and 115 attendees completed written and practical examinations. Additional courses will be offered.
Back then...

Monday night, everyone traveled to Charlie William’s Lodge where Georgia Bar-B-Que was served. The fellowship between the students of Dr. Tulis’ class mingling with the “old timers” was outstanding.

Janet Macher, Harvard School of Public Health, discussed studies on the collection efficiency of old and new biological air samplers.

Joseph Songer reported that biohazards exist in all environments. They may be toxic or allergenic substances. Examples are: animal toxins from snakes, bees, wasps, and ants; plant toxins from poison ivy, oak, and sumac; allergens from fungi in air conditioners; and grain dust and fungi from hay on farms. Norman Petersen described the increased risk of viral hepatitis by clinical laboratory personnel handling blood and blood derivatives. Although many biological and biomedical assays are now automated, care must still be taken while unpacking, decapping, transferring, and disposing of specimens.

Tuesday night, after a social hour and dinner, the 5th Arnold G. Wedum Memorial Lecture entitled, Responsibility for Scientific Response to Nonspecific Judgments in the Public Arena was delivered by Dr. Fred Davidson, President of the University of Georgia. He said, “Honest responses and straightforward answers always should be given. Never cover up an accident or illness, but be truthful and timely.”

25th Biological Safety Conference

The twenty-fifth biological safety conference, sponsored by the Massachusetts Institute of Technology (MIT), Harvard School of Public Health, and Harvard University, was held November 4-6, 1982 at 57 Park Plaza Hotel, Boston.

The keynote address Genetic Manipulation with Retroviral Vectors was presented by Nobel Laureate Dr. David Baltimore, Director, Whitehead Institute for Biomedical Research. The retroviruses first came into prominence as causes of cancer in animals. Their genetic strategy made them ideal vectors for carrying new genetic information into cells.

C.A. Schlegel, The Baker Company, discussed the handling of antineoplastic agents. A horizontal airflow clean work station should never be used.

She stated that an assessment of risk should be performed: identify and characterize the agents used; describe normal activities; and identify possible exposure. Appropriate disposal of antineoplastic-chemotherapy waste was essential.

Keith Allner discussed the Centre for Applied Microbiology and Research that formerly was the Ministry of Defense Establishment, United Kingdom. Their work was oriented toward health care and public health research involving vaccine production, biotechnology, genetic manipulation, environmental microbiology, and biological safety.

E.C. Cole, UNC, using a 6-stage Andersen Sampler, demonstrated the presence of high concentrations of bacteria and fungi in a poultry farm’s coop air.

Back then...

After the business meeting, the attendees traveled to the New England Aquarium for a reception. It was extremely interesting and the sights were, as one attendee stated, “terrific.”

Dr. John Richardson, CDC, discussed risk assessment and precautions against HIV. The illness, acquired immunodeficiency syndrome (AIDS), first came to medical attention in 1981, and is characterized by immunosuppression of undetermined origin, a biopsy-proven Kaposi’s sarcoma, or culture-proven opportunistic infection in previously healthy individuals.

C. Welty, Channing Laboratory, Boston, discussed the booster effect of serial tuberculin skin tests. The booster phenomenon increased as an individual aged. The mean age of the subjects was 64 yr. At a VA Hospital, 457 patients received the initial PPD skin test with 106 positive reactions. At two weeks, to evaluate booster effect, 322 patients, who initially had negative PPD skin tests, received the second PPD testing. The booster effect is an increase in size of the tuberculin skin test reaction by serial skin testing thought to be due to stimulation of the immune skin test. The booster effect was at least twice that observed in younger populations. Having worked with experts in mycobacteriology, namely Drs. Mac Vandiviere and George Kubica, Richard Kruse asked if these data were realistic because PPD is a mixture of several tuberculo-proteins, each of which could cause a delayed hypersensitivity reaction.
Dr. D.A. Giard described the large-scale Cell Culture Center established in 1974 at MIT. The facility produces approximately 400 L of suspensions and 2,000 roller bottles per month.

Friday was poster session day. Fran Wimberly, University of Georgia, displayed a micro-computer system that provided current status, location, date of last survey, and air velocity of chemical fume hoods on the campus. Robyn Gershon displayed an outline of the expanded medical surveillance program at Yale University. Shigeo Hino, Nagasaki University School of Medicine, displayed a modified biosafety cabinet for handling animals. Thomas Allen, Flanders Filters, displayed a technique to improve safety when contaminated HEPA filters are removed and bagged. Dr. Robert Olcerst, Mercy College, New York, displayed results of microbial assays performed on the surface of microscope oculars and showed that pathogenic, or potentially pathogenic, microorganisms were recovered from 28% of the oculars. Robert Gross, Medical Repair Laboratories, compared the sensitivity of the DOP leak test, the halogen leak test, and other leak tests for Class II biosafety cabinets and concluded that the halogen leak test was unnecessarily stringent. He claimed that DOP could be used satisfactorily for leak testing the biological safety cabinet but NSF rejected the procedure.

The 6th Arnold G. Wedum Memorial Lecture, entitled, *Biosafety—A Discipline in Transition* was delivered by Dr. W. Emmett Barkley.

Dr. Melvin First, Terry Webb, and Shigeo Hino compared characteristics of various nebulizers used at National Sanitation Foundation (NSF). All three stated the 6-jet Collison consistently delivered a more uniform aerosol than the DeVilbis 40, or glass and plastic Vaponefrin nebulizers. Dr. First stated the Collison should be modified so its discharge velocity would approximate that of the DeVilbis 40 nebulizer used by NSF for cabinet testing. The presenters constantly mentioned DeVilbis 40 nebulizer, but this nebulizer was not used for the initial microbiological tests (National Sanitation Foundation 1976).

The final session was devoted to “Waste Management” that included *Integrated Approach to Waste Management* by Judith Gordon, Gordon Resources Consultants; *Destruction and Recovery of Waste Solvents* by Mark Jensen, National Animal Disease Center (NADC); *Destruction of Chemical Carcinogens* by Dr. Eric Sansone, FCRC; *Decontamination of Microorganism Waste by Steam Sterilization* by William Ruta, UNC, and *Methods for Decontamination of T-2 Mycotoxins* by Ralph Kuehne.

A conference proceedings, with the text of most presentations, was prepared and distributed to the attendees. This was the second time conference proceedings were distributed. Becton, Dickinson and Company prepared proceedings of the 17th biological safety conference.

**26th Biological Safety Conference**

The twenty-sixth biological safety conference, sponsored by M.D. Anderson Hospital and Tumor Institute, was held October 17-19, 1983 at the Warwick on the Park Hotel in Houston.

S. Lindell discussed the results of a survey questionnaire sent to 544 University of Iowa faculty who used hazardous biologicals and chemicals. Ninety-five percent responded and the most common complaint was improper storing and handling of chemicals.

Alex McIntosh described microbiological safety practices at the University of Strathclyde, Glasgow, Scotland. George Harper, Porton Down, England, used dynamic microbiological tests to assess the safety of all sealed containers in laboratory centrifuges.

Trevor Menzies, Ultraviolet Supplies at Victoria, Australia, described a biosafety cabinet and room in Australia used exclusively to prepare cytotoxic drugs.

Dr. David Stuart, The Baker Company, proposed a performance envelope to be used to certify biological safety cabinets. NSF Standard Number 49 specifies that a biosafety cabinet's airflow be set within ± 5 fpm for NSF certification tests. Microbial tests were performed using four airflow settings: (1) high inflow-high down-flow; (2) high inflow-low down-flow; (3) low inflow-low down-flow; and (4) low inflow-high down-flow to ascertain if the cabinet contained the test microorganisms. Barbara Rake, Contamination Control, Inc. (CCI), also evaluated the performance envelope concept. She tested 16 different airflow combinations and found an acceptance range of ± 25% from designated setpoint values for the personnel protection test and an
even larger range for product protection and cross contamination tests. Lynn Harding, Harvard University, evaluated effects of operator activity on airflow and microbial tests in Class II biosafety cabinets.

Back then...

After Happy Hour, compliments of the Winfield Corporation, the attendees became cowgirls and cowboys and traveled to Mickey Gilley’s Club in nearby Pasadena. People forgot their safety training and attempted to ride the bucking broncho at top speed. After a few “long necks” the bruises were forgotten.

William Brubaker (USAMRIID) described procedures employed to decontaminate a 20,000 ft² research laboratory. Class II and Class III biosafety cabinets, aerosol chambers, vacuum lines, filter plenums etc. were treated by depolymerized paraformaldehyde or liquid formalin in the entire research laboratory. Filter paper patches, impregnated with 10⁶ BG spores were placed at various locations throughout the area. After 18-h contact time, each patch was placed in a tube of thioglycolate broth (Difco Laboratories, Detroit, MI) and the tube was incubated at 37°C to verify decontamination effectiveness. Lawrence Gibbs, University of Connecticut, discussed problems encountered in the disposal of waste scintillation counting fluids. An automated recovery process was developed that removed radioactivity and purified the organic solvent.

After a social hour and dinner, the 7th Arnold G. Wedum Memorial Lecture entitled, AIDS was presented by Dr. Peter W.A. Mansell, Department of Cancer Prevention, M.D. Anderson Hospital and Tumor Institute.

Dr. Daniel Liberman, MIT, described three converging research areas as researchers search for the molecular basis of cancer: oncogene and retroviral biology, and amphotropic virology.

William H. Puckett, Jr., M.D. Anderson Hospital and Tumor Institute, showed that antineoplastic drugs can be carcinogenic. Studies have shown mutagenic activity in the urine of personnel who prepared certain antineoplastic drugs in a horizontal airflow clean bench.

27th Biological Safety Conference

The twenty-seventh biological safety conference, sponsored by the School of Public Health, University of North Carolina, was held October 14-17, 1984 at the North Raleigh Hilton Hotel. Joseph Songer delivered the keynote address entitled, Risk Management in the Laboratory and emphasized that assessment of risk is an essential part of safety.

Robyn Gershon described the first laboratory-acquired infection with Rocio virus. The researcher became ill with fever, severe headaches, malaise, and meningismus. Serology showed a 2-fold rise in titer to Rocio virus. The infection was of unknown origin.

Charles Miller presented an epidemiological review of zoonotic infections incurred at NADC. It was estimated that 46% of employees were exposed to infectious microorganisms. Syringe use and animal necropsy were responsible for the majority of exposures.

Terry Webb, WHO Collaborating Centre for Biosafety, Canada, compared the dioctylphthalate-forwarding light-scattering photometer (DOP) method and the sodium chloride-hydrogen flame photometer (NaCl) method for testing HEPA filters.

Barbara Rake evaluated a 2-ft Class II biosafety cabinet. She modified NSF Standard Number 49 microbial tests by (1) placing a manakin in front of the cabinet, and (2) having a person sit in front of the cabinet and perform normal microbial techniques.

Back then...

Monday night we heard oomph-pah-pah music from the ballroom and assembled there for a buffet of German dishes. The Little German Band, dressed in Lederhosen and Tyrolean hats played polkas and other music. Larry Taylor of paraformaldehyde fame, was now Reverend Taylor and gave the blessing. The dance floor was full, the food scrumptious, the music superb, and a great time was had by all. Thank you, Jerry.

Keith Allner, Porton Down, England, discussed formaldehyde decontamination of smallpox isolation hospitals that were closed by The Department of Health and Social Security. Wayne Thomann, Duke University Medical Center, described the airflow dynamics in a 20-bed Hematology-Oncology Unit. Using an Andersen Sampler he demonstrated that the supply air was consistently negative for
Aspergillus spp., but the problem of fungal contamination was exacerbated by excessive Aspergillus spp. in the air of 11 of 12 rooms. J.L. Lauer discussed methods used to control Aspergillus spp. in a Bone Marrow Unit at the University of Minnesota Hospitals. In-room recirculating HEPA filters were installed and reduced nosocomial aspergillosis from 18% to 5.4% in immunosuppressed patients.

Deborah Wilson, NIH, described a dynamic aerosol unit to expose animals to continuously generated infectious aerosols. Studies varying exposure times of guinea pigs to aerosols of M. tuberculosis were carried out that ascertained minimum exposure time for 100% infection.

David Brantley, E.I. DuPont, and Joseph Van Houten, Schering Corporation, discussed biosafety programs at their respective companies. Each company adopted NIH's Guidelines for Research Involving Recombinant DNA Molecules as corporate practice.

Dr. Emmett Barkley discussed Biosafety in Microbiological and Biomedical Laboratories, published after 7 yr. research. Four biosafety levels were established that provided a risk assessment, data on laboratory hazards, and recommendations. Vincent Oviatt discussed WHO's Laboratory Safety Manual that had guidelines for international application.

Dr. Donald E. Gardner, Northrop Services, Inc., Research Triangle Park, North Carolina delivered the 8th Arnold G. Wedum Memorial Lecture entitled, Pulmonary Infections in a Compromised Host. He explained how false data were derived from the death of animals from nosocomial infections, not from the organism under study. Proper methods for housing the animals were discussed.

Mary Ellen Kennedy, Centre for Laboratory Control, Canada, moderated a session on Infectious Waste. Dr. Jonathan Richmond discussed methods used at NIH; Roy Hubbard, Con-Test Ltd., described actions of the Environmental Protective Legislation in the Province of Ontario; Dr. James Vacik, University of South Alabama, described the school's new facilities for infectious wastes; Lawrence Gibbs described methods used at Yale University; and Dr. Eric Sansone, FCRC, discussed decontamination and destruction of chemical carcinogens.

28th Biological Safety Conference

The twenty-eighth biological safety conference, sponsored by the Salk Institute for Biological Studies, was held October 20-23, 1985 at the La Jolla Village Inn, La Jolla, California.

Debra Hunt, Duke University Medical Center, continuing the aerobiological monitoring previously reported at the 27th biological safety conference said effective control of Aspergillus spp. depends on appropriate filtration of source air, airflow barriers, restrictive access, and modified work practices.

Dr. David Stuart stated that raising the window height on a Class II biosafety cabinet to 10 in. affected the performance envelope. Long discussions ensued as safety cabinet manufacturers stated that the work access opening in their biosafety cabinets was 10 in. and they met NSF Standard Number 49, including the performance envelope tests.

Jolanda Janczewski, Smithsonian Institution, discussed parasitic infections in animal keepers in zoological parks. Five of seven animal keepers at the National Zoological Park's primate area had intestinal parasites: two had Giardia lamblia and three had Entamoeba histolytica. The medical history of the animals revealed that several monkeys were infected with either Giardia or Entamoeba. Animal parks and zoos must be cognizant of the dangers of parasitic infections.

Raymond Hackney, UNC, reported a laboratory-acquired Neisseria gonorrhoeae infection. There was no documented accident.

Dr. Jonathan Richmond discussed eight possible Bordetella pertussis infections. A throat culture isolate confirmed presence of B. pertussis. Serum antibody levels were negative.

Deborah Wilson discussed the high number of diseases among pathologists and mortuary workers.

N.J. Brace, Bootle, Merseyside, England, stated that H.M. Factory Inspectorate had been responsible for occupational health and safety in the United Kingdom for 150 years. In 1975, the Inspectorate was incorporated into the Health and Safety Executive Agency. E.A. Meyrick, Central Public Health Laboratory, London, described the relocation of the Public Health Laboratory to a newly designed complex.
Back then...

We boarded buses and traveled to the San Diego Wild Animal Park. After observing African dancers, we took a train ride and observed the animals at dusk. The evening culminated in a delightful buffet at the park, but the guide had an acerbic wit when he questioned where the meat came from.

Dr. Colin Ludford discussed the Australian National Animal Health Laboratory, a maximum containment laboratory with a multiple barrier system to control infectious material.

The 10th Arnold G. Wedum Memorial Lecture entitled, "Risks of Containment of Viruses" was delivered by Nobel Laureate Dr. Renato Dulbecco of the Salk Institute. Dr. Dulbecco pioneered research in virology and oncogenic virology, and his presentation corroborated the intensive research he performed that earned him the Nobel Prize.

Manuel Barbeito, in Development of Evaluation Standard for Animal Containment Room Which Functions as the Primary Barrier, described USDA's Agricultural Research Service Evaluation Standard for assessing an animal containment room. Research with infected livestock and poultry was conducted in which the animal room served as the primary barrier. This primary barrier (animal room) prevented horizontal transmission between large research animals in adjacent rooms when test animals were infected with a virus with an infectious dose of a single particle.

Dr. Eric Sansone discussed decontamination and destruction of antineoplastic agents in laboratory wastes.

At the 27th biological safety conference, the American Biological Safety Association, henceforth known as ABSA, became a reality. Elected to office were: Everett Hanel, Jr., President; Dr. Jerome Schmidt, Secretary-Treasurer; and an Executive Council. However, the election procedures stated in ABSA's constitution were not followed and the elected officials became pro tem. A new election was held and elected were: Jerry Tulis, President; Jerry Schmidt, Secretary-Treasurer; and as members of the Executive Council: Judith Gordon, Kathlene Peterson, John Keene, and Manuel Barbeito.

29th Biological Safety Conference

The twenty-ninth biological safety conference, sponsored by MEDI, Inc., was held October 5-9, 1986 at the Radisson Plaza Hotel in Lexington, Kentucky.

Back then...

Richard Kruse drew the wrath of some Council members when the letter announcing the conference was in "Southern Drawl." He was prepared to scrap the second letter and apologize, but Emmett Barkley said, "No! You do not owe anyone an apology and besides the world needs a little humor." While attending a Canadian safety conference, Vince Oviatt and his wife offered congratulations for the letters; they had made copies for many member countries of WHO. Sunday night at the cocktail party, along with the usual cheeses, cold cuts, and dips were steamship rounds of beef, country hams, and beaten biscuits. Who can ever forget Cynde's Bourbon fountain and the aroma?

The conference started with two Keynote Speakers. Dr. Peter Gerone discussed leprosy in animals, and Dr. Ward Bullock, University of Cincinnati Medical Center, discussed leprosy in humans.

Dr. Eric Sansone evaluated the potential occupational hazard to laboratory and maintenance personnel who work in chemical fume hoods. Mutagenic activity of the organic fraction was assessed by the Ames Salmonella mammalian assay method.

Dr. Clarence Styron, Monsanto Company, described the facilities at St. Louis which consisted of laboratories, fermentation pilot plants, growth chambers, green houses, and farms. Dr. Melvin First described a commercial version of the old balanced laminar airflow cabinet. The unit is similar to a Class 100 clean work station except it has a view screen and slotted air intake grill extending across the entire width of the work opening. The unit was evaluated by test protocols of NSF Standard Number 49, and when tested microbiologically failed to provide personnel and product protection.

At the banquet, President Tulis announced the formation of the President's Award. The recipient was Joseph Songer from the National Animal Disease Center, Ames, Iowa.

Dr. Howard W. Lash, Emeritus Professor of Microbiology, University of Oklahoma, delivered the 10th Arnold G. Wedum Memorial Lecture entitled, "Fungi: Interesting, Remarkable and Ex-
tremely Complicated. He stated that steak is enhanced by mushrooms and penicillin has saved many lives, yet fungi cause dangerous pulmonary infections. He discussed his long association with Dr. Wedum and emphasized the assistance received. After his speech, Dr. Joyce Scott presented the Wedum Memorial Plaque to Dr. Larsh. The audience was surprised when they learned Joyce was Dr. Wedum’s daughter. She too was surprised when, like her father at the 18th biological safety conference, she was inducted into the Honorable Order of Kentucky Colonels.

Dr. Kurel Styblo, International Union Against Tuberculosis, The Hague, Netherlands, presented Tuberculosis in Europe and the World. Tuberculosis was, and continues to be, a major problem in developing countries. It has been estimated that the disease will be virtually eliminated in developed countries in a few decades. Nevertheless, approximately four million cases develop in the world annually. Poor results of nationwide case-findings and chemotherapy programs were the main reasons for this deplorable situation. Dr. Hans Rieder, CDC, stated that cases of tuberculosis in America had decreased 34.5% from 1975 to 1984, but data indicate tuberculosis remains a problem as the case rate decreased only 0.2% in 1985.

Dr. George Kubica, CDC, stated that infection could result by inhaling a single tubercle bacillus. The risk of tuberculosis infection is 3- to 5-times greater for mycobacteriology laboratory workers than for other hospital personnel. Problems of safety in the tuberculosis laboratory were compounded by world-wide distribution, the speed and extent of international travel, and the mass movement of immigrant populations.

Dr. Mac Vandiviere, University of Kentucky Medical Center, discussed skin testing in his presentation entitled, PPD: A Witches Brew - Interpretation: An Enigma Too. PPD is a mixture of several tuberculo-proteins, each capable of eliciting a delayed hypersensitivity reaction. Cross reactions and “false positives” were possible. Approximately 8% of bacteriologically confirmed cases gave “false negatives.” A positive PPD meant there was reasonable probability of an infection or disease due to M. tuberculosis, but it might indicate a non-M. tuberculosis etiology. These factors make eradication of tuberculosis (that was predicted in the 1950s) unrealistic.

Dr. Donald Ahern, Georgia State University, warned individuals wearing mascara and contact lenses. The applicator for applying mascara could cause accidental trauma to the corneal epithelium. Many mascaras support the growth of Pseudomonas aeruginosa. Serious infection may result when the eye’s integrity is compromised by a scratch or chemical irritation. Contact lenses may cause infections by improper hygienic practices and long periods of contact on the eyes.

Robert O’Leary, American Sterilizer Company, described AMSCO’s vapor phase hydrogen peroxide (VHP) sterilant for special medical applications.

Manuel Barbeito summarized USDA’s Guidelines for Biotechnology Research that followed closely NIH’s Guidelines for Research Involving Recombinant DNA Molecules.

Drs. David Henderson, NIH, and Albert Balows, CDC, discussed occupational risks of AIDS. Dr. Robert McKinney, NIH, moderated a panel discussion on biosafety in AIDS virus production operations.

30th Biological Safety Conference

The thirtieth biological safety conference, sponsored by Memorial Sloan Kettering, was held October 18-21, 1987 at the Meadowlands Hilton Hotel in Secaucus, New Jersey.

The opening session repeated a subject of intense interest at the 29th biological safety conference, AIDS. P. O’Donnell discussed the mechanism of action of retroviruses; B. Polsky reviewed the impact nosocomial infections have on AIDS patients; and D. Armstrong described information on the increase and distribution of AIDS.

Dr. Jonathan Richmond, NIH, discussed the challenges and successes of an interdisciplinary task force that designed a large centralized biomedical research animal facility.

Several presentations focused on biosafety cabinets. James Edwards, Charcoal Services Corporation, discussed the nuclear industry’s HEPA filter test; Shigeto Hino stated that a decrease of electrical voltage drastically reduced airflow in a biosafety cabinet. From The Baker Company, Theodore Greenier reviewed the discrepancies that occur when inflow velocity is calculated or measured in Class II, Type A biosafety cabinets, and Robert Jones
described the effects that low ceilings have on the Class II biological safety cabinet’s performance.

Ashraf El Dessouky described biosafety and human behavior in developing countries. He attempted to avoid hazardous problems that might evolve from the behavior of personnel working in a laboratory. Biosafety rules, regulations, personal hygiene standards, and the tasks and risks of their jobs were explained to 14 employees. They were not told they would be observed for six months. For six to eight weeks they followed rules. Thereafter, there was a gradual decrease with the following mistakes: (1) eating or drinking in restricted areas; (2) not washing hands properly; (3) going three to four days without shaving; (4) hands brought to mouth, eyes, nose, and hair while working; and (5) not wearing coats, gloves, or mask.

Alfredo Tavares described Fort Detrick’s continuous flow sterilization system that is capable of treating 280,000 gal/day of infectious liquid waste at 270°F for a minimum retention time of 28 min. The system has a heat recovery exchanger for energy conservation.

Dr. Donald Vesley, University of Minnesota, discussed a 1986 survey of laboratory-acquired infections and injuries in clinical laboratories.

Dr. Jerry Tulis environmentally analyzed specific habitats of an elderly male who was diagnosed as having hypersensitivity pneumonitis. Fungi such as *Aspergillus spp.* and *Penicillium spp.*, which are classified as aeroallergenic fungi, were isolated.

James Otten, Oak Ridge National Laboratory, reviewed air sampling methods to identify sources of bioaerosols in the indoor environment.

The 11th Arnold G. Wedem Memorial Lecture was presented by Dr. Robert E. Shope, Yale University Arbovirus Research Unit. He described his twenty years experience with biosafety level 3/4 arboviruses. The early equipment afforded protection, but did not compare with today’s equipment. His research with arboviruses included his interest in finding vaccines and prophylaxes.

**CONCLUSIONS**

We did not intend this to be a history of the authors, but one was present at every biological safety conference.

The conferences we chaired and the commit-
a database for the first 30 conferences. Many organizations have a designated historian who maintains these records, and we believe the Council should consider this suggestion.

Everyone agrees that the safety conference had an auspicious beginning with the Keynote Address by Dr. Arnold G. Wedum. Dr. Wedum was a person one respected, his counsel was timely, and of the utmost importance. The preponderance of his influence was the embodiment of the safety conference as his name was and still is synonymous with safety. We were very fortunate as we worked for and with him.

ACKNOWLEDGMENTS

We thank W. Emmett Barkley and Melvin W. First for their critical review of the manuscripts, and for many valuable suggestions; Joseph R. Songer and David G. Stuart for locating many old biological safety programs that provided data for the manuscripts.

REFERENCES


National Sanitation Foundation. 1976. Class II (laminar flow) biohazard cabinet. National Sanitation Foundation standard no. 49. National Sanitation Foundation, Ann Arbor, MI.


TABLE 1

Selected Publications by Participants at the Ten Biological Safety Conferences 1978-1987


Barbeito, M.S. 1979. Special design features for maintenance requirements of a biomedical facility, p. 177-193. In D.G. Fox (ed.), Design of biomedical research facilities; proceedings of a cancer research symposium 1979 October 18-19, Frederick Cancer Research Center, Frederick, MD. NIH publ. no. 81-2305. National Institutes of Health, Bethesda, MD.


Harvard University School of Public Health. 1987. Testing class II laminar airflow cabinets (course). Harvard University School of Public Health, Boston, MA.


Johns Hopkins University School of Hygiene and Public Health. 1980. Control of biohazards in research laboratories (course). Sponsored by Division of Safety, National Cancer Institute, Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD.


TABLE 1
(continued)


Table 2
ABSA Presidents Who Participated in One or More of the Ten Biological Safety Conferences 1978-1987

<table>
<thead>
<tr>
<th>ABSA President</th>
<th>Year President</th>
<th>Year First Participated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mary Cipriano</td>
<td>1993 - 1994</td>
<td>1986</td>
</tr>
</tbody>
</table>
### TABLE 3
Date, Location and Chairperson(s) of the First Thirty Biological Safety Conferences

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Chairperson(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apr 18-20, 1955</td>
<td>Camp Detrick, MD</td>
<td>A. Wedum, E. Hanel, Jr.</td>
</tr>
<tr>
<td>Nov 14-17, 1955</td>
<td>Pine Bluff, AR</td>
<td>G. Connell</td>
</tr>
<tr>
<td>Jun 18-20, 1956</td>
<td>Dugway, UT</td>
<td>R. Lerwell</td>
</tr>
<tr>
<td>Apr 24-26, 1956</td>
<td>Fort Detrick, MD</td>
<td>W. Kirchheimer, R. Kruse</td>
</tr>
<tr>
<td>Apr 21-23, 1958</td>
<td>Pine Bluff, AR</td>
<td>C. Kambar</td>
</tr>
<tr>
<td>Sep 13-16, 1960</td>
<td>Fort Detrick, MD</td>
<td>E. Hanel, Jr., R. Kruse</td>
</tr>
<tr>
<td>Sep 12-14, 1961</td>
<td>Dugway, UT</td>
<td>R. Lerwell</td>
</tr>
<tr>
<td>Jun 11-13, 1963</td>
<td>Pine Bluff, AR</td>
<td>E. Lazear, C. Kambar</td>
</tr>
<tr>
<td>Sep 14-16, 1965</td>
<td>Plum Island, NY</td>
<td>J. Hyde</td>
</tr>
<tr>
<td>Aug 16-18, 1966</td>
<td>Fort Detrick, MD</td>
<td>E. Hanel, Jr.</td>
</tr>
<tr>
<td>May 16-18, 1968</td>
<td>Cincinnati, OH</td>
<td>G. Bodmer</td>
</tr>
<tr>
<td>Oct 14-16, 1969</td>
<td>Brooks AFB, TX</td>
<td>J. Schmidt, D. Giron</td>
</tr>
<tr>
<td>Apr 13-15, 1971</td>
<td>Atlanta, GA</td>
<td>J. Johnson, R. Huffaker</td>
</tr>
<tr>
<td>Oct 10-12, 1972</td>
<td>Bethesda, MD</td>
<td>W.E. Barkley, W. Powell</td>
</tr>
<tr>
<td>Oct 15-17, 1974</td>
<td>Raleigh, NC</td>
<td>L. Taylor</td>
</tr>
<tr>
<td>Oct 14-17, 1975</td>
<td>Lexington, KY</td>
<td>R. Kruse</td>
</tr>
<tr>
<td>Oct 18-20, 1976</td>
<td>Fort Detrick, MD</td>
<td>E. Hanel, Jr., M. Barbeito</td>
</tr>
<tr>
<td>Oct 12-14, 1977</td>
<td>San Antonio, TX</td>
<td>J. Schmidt</td>
</tr>
<tr>
<td>Nov 6-8, 1978</td>
<td>Scottsdale, AZ</td>
<td>N. Petersen</td>
</tr>
<tr>
<td>Oct 15-17, 1979</td>
<td>Bethesda, MD</td>
<td>M. Barbeito</td>
</tr>
<tr>
<td>Oct 5-7, 1981</td>
<td>Athens, GA</td>
<td>J. Richardson</td>
</tr>
<tr>
<td>Nov 4-6, 1982</td>
<td>Boston, MA</td>
<td>L. Harding, M. First, D. Liberman</td>
</tr>
<tr>
<td>Oct 17-19, 1983</td>
<td>Houston, TX</td>
<td>L. Bilowich</td>
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<tr>
<td>Oct 14-17, 1984</td>
<td>Raleigh, NC</td>
<td>J. Tulis</td>
</tr>
<tr>
<td>Oct 21-23, 1985</td>
<td>LaJolla, CA</td>
<td>G. Spahn</td>
</tr>
<tr>
<td>Oct 5-9, 1986</td>
<td>Lexington, KY</td>
<td>R. Kruse</td>
</tr>
<tr>
<td>Oct 18-21, 1987</td>
<td>Secaucus, NJ</td>
<td>F. Pearce</td>
</tr>
</tbody>
</table>
NEBULIZER CHARACTERISTICS FOR CERTIFICATION TESTS OF BIOSAFETY CABINETS WITH BACTERIA AND SIMULANTS

Melvin W. First, Janet Macher, Robert Gussman, David Stuart, and Terence Webb
1Harvard School of Public Health, Boston, Massachusetts, 2California Department of Health Services, Berkeley, California, 3BGI Inc., Waltham, Massachusetts, 4The Baker Company, Sanford, Maine, and 5Microzone Corporation, Ontario

ABSTRACT

NSF International Standard 49-1992, that covers certification of biological safety cabinets, makes “special note” that a “stainless steel 6-jet Collison refluxing nebulizer will deliver the [required] bacterial spore aerosol” when certain stated conditions are met and “need not be retested for performance before use” (Appendix-C, page C1) (1). The basis on which this nebulizer was vetted was presented at the XXV Biological Safety Conference (Boston, MA, 1984) but never published in the open literature. In view of the importance of this device for the procedures used to certify the performance of biological safety cabinets, the authors are of the opinion that the test protocols and test data on which the selection was based should be made a matter of record.

Collison nozzle studies were conducted to determine (a) whether all 6 Collison nozzles manufactured by BGI give the same spore output when operated at 140 kPa (20 psig) with an equal number of spore suspension in the flask (b) whether spore delivery by the 6-jet Collison nozzle equals or exceeds the minimum number specified by NSF 49 when charged with the recommended spore suspension (c) whether performance of Collison nozzles with a bacterial spore aerosol can be predicted accurately with a monodisperse 1.1 μm polystyrene latex spherical simulant, and (d) the optimum nebulizer flask geometry.

INTRODUCTION

The Collison Nebulizer

K.R. May published a definitive paper on this instrument in 1973 (2) that should be consulted for a thorough knowledge of the device and all its performance factors. May worked only with 1-jet and 3-jet nozzles (“nozzle” refers to the active tip of the nebulizer that contains the spray-making jets) whereas all the devices used in the present study contained 6-jet nozzles obtained from BGI, Inc. (3). In all respects, (other than the presence of 6 jets rather than 3) the BGI nozzle conforms exactly to the Collison nozzle described by May. For the BGI 6-jet nozzle, air-flow rates and liquid consumption rates at equal air pressure correspond to double the values reported by May for the 3-jet nozzle. At 140 kPa (20 psig) air pressure, free air consumption for the 6-jet nozzle is 14.2 L/min and liquid loss with dry compressed air is 0.3 mL/min.

For the experiments reported here, a variety of flasks was employed (a) a small screwtop jar having an ID of 3.8 cm (1.5 in.), a 1.3 cm (0.5 in.) diameter spout, and containing 15 mL of suspension (b) a larger screwtop jar having an ID of 5 cm (2 in.), a 1.3 cm (0.5 in.) diameter spout, and containing 25 mL of suspension (c) a graduated series of straight-sided metal flasks having inside diameters of 2.0 cm (0.79 in.), 2.5 cm (1.02 in.), 3.9 cm (1.54 in.), 5 cm (2.00 in.), and 7.5 cm (2.94 in.), all with an outlet spout of 2.1 cm (13/16 in.) diameter, and containing an AGI-type sampling flask used routinely at the Baker Co. (4) having a spout diameter of 2.1 cm (13/16 in.) and containing 55 mL of suspension. All tests were conducted with an air pressure of 140 kPa (20 psig). Seven different 6-jet nozzles were used in various combinations with the several flasks in the experimental series that was conducted. The nozzles were designated A, B, C, D, E, F, and Baker, and the identity of each nozzle preserved throughout. The Baker nozzle was one that had been carefully calibrated by D. Stuart (4) for satisfactory spore delivery and one that he had been using successfully in biosafety cabinet tests for several months. Data from 32 trials using the Baker nozzle
showed that with a starting suspension of between 5 and $8 \times 10^8$ *Bacillus subtilis, var. niger* (BG) (formerly *Bacillus globigii*) spores per mL this nebulizer consistently delivered $1-4 \times 10^8$ spores during 5 min. of continuous operation, as called for in NSF 49. Its performance characteristics were considered the standard against which the characteristics of the other nebulizers were compared.

**TEST PROTOCOLS**

**BG Tests**

As the preparation of the spore suspension and the methods used for generation, sampling, plating, and counting the BG aerosols followed standard NSF 49 procedures, they are adequately identified by reference (1).

**PSL Tests**

Monodisperse polystyrene latex spheres (PSL) of 1.1 μm diameter closely match BG suspensions in size, shape, and specific gravity and therefore were assumed to be a reliable simulant when comparing the number output of similar nebulizers. The manufacturer’s label showed that the PSL suspension contained 10% spheres by volume in water. As one drop of suspension is 1/25 mL, each drop of the stock suspension contained approximately $6 \times 10^9$ particles. For all PSL tests, 3 drops of stock suspension were added to each 10 mL of dust-free water plus antifoam to prepare the suspension placed in the nebulizer flask. The PSL suspension contained $17 \times 10^9$ PSL particles per mL. Aerosolized PSL particle counts were performed with a laser spectrometer after dilution with 0.0243 m$^3$/s (50 cfm) of HEPA-filtered air using pooled data within the following size intervals.

- 0.76 - 1.24 μm (singlets)
- 1.24 - 1.88 μm (doublets and triplets)

As the counts were intended only to compare various combinations of nozzles and flasks under identical operating conditions, no attempt was made to translate the count data taken from the spectrometer readout device into particles generated per 5 min. intervals, as is customarily done for the BG tests. For reference and comparison purposes, the 5 min. output was approximately $5 \times 10^8$ particles.

**RESULTS**

**Effect of Varying the Flask Diameter of the Nebulizer**

Tests were conducted with Nozzle C in metal flasks of graduated diameter. The distance between the bottom of the nozzle and the bottom of the flask was held constant and the immersion of the nozzle was maintained at 1.9 cm (0.75 in.) by varying the volume of the suspension placed in the flask. Comparative counts are shown in Table 1. A flask diameter of 5 cm (2 in.) appeared to be optimum and coincides with the dimensions of the M unit (2). A larger flask diameter of 7.5 cm (2.94 in.) gave almost as high delivery rates. The disadvantage of a larger flask is that it requires a larger volume of suspension, but it could also be considered an advantage because each spore passes through the nozzle fewer times during a run and the kill rate from trauma may

<table>
<thead>
<tr>
<th>Particle size - (μm)</th>
<th>No. of Particles Counted in 100 s.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flask Inside Diameter - cm (in.)</td>
</tr>
<tr>
<td></td>
<td>2 cm (0.79) 2.5 cm (1.02) 3.9 cm (1.54) 5 cm (2.0) 7.5 cm (2.94)</td>
</tr>
<tr>
<td>0.76 - 1.24</td>
<td>540 1,100 1,500 2,400 2,100</td>
</tr>
<tr>
<td>1.24 - 1.88</td>
<td>27 77 95 220 190</td>
</tr>
<tr>
<td>% doublets and triplets</td>
<td>4.8 6.7 6.0 8.6 8.2</td>
</tr>
</tbody>
</table>
be less. However, the same effect could be achieved in a smaller diameter flask by raising the height of the nozzle to accommodate a larger liquid volume without changing the nozzle-to-liquid geometry. There does not seem to be any question that flask diameters less than 5 cm (2 in.) resulted in progressively fewer particles in the discharge. The conclusion reached is that a flask of 5 cm (2 in.) diameter is about the optimum dimension. Two factors interact in the selection of flask diameter: (a) as the diameter increases, the upward air velocity declines for the same airflow rate of 18 L/min and, as a consequence, larger drops settle back into the pool and (b) as the flask diameter decreases, the horizontal liquid jets strike the walls of the flask at higher velocity, and this tends to decrease the number of droplets that become airborne, perhaps deceasing the larger droplets at a greater rate because of their greater inertia. It is reasonable to conclude, therefore, that there should be a diameter that delivers a maximum number of droplets to the aerosol discharge spout.

**PSL as a Simulant for BG**

Four nozzles were tested with both PSL and BG. Some of the nozzles had metal chips in some of the jets when they were first tested so that the output was not uniform; but this made an analysis of comparative numbers between the two types much more valuable as it provided an opportunity to compare the BG/PSL ratio over a range of nozzle deliveries. Table 2 summarizes BG and PSL data for nozzles that originally contained chips and repeat tests after the chips were removed. It may be seen from Table 2 that the trend of both BG and PSL counts was the same before the nozzles were cleaned. After the nozzles were cleaned the counts were indistinguishable taking into account experimental variability. On the basis of the two series of tests, it was concluded that when appropriate quality control measures are observed during manufacture, 6-jet Collison nebulizers can be depended upon to deliver essentially identical numbers of spores when operated under standardized conditions of air pressure and liquid suspension numbers.

**DISCUSSION**

An open question was what the discharge velocity from the spout should be because the “throw” of a discharge nozzle depends not only on nozzle diameter but also on droplet size and discharge

<table>
<thead>
<tr>
<th>Nebulizer Combination</th>
<th>5 min. BG spore delivery no.s</th>
<th>PSL laser spectrometer counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baker Nozzle in Baker Flask</td>
<td>3.3 x 10^8</td>
<td>2.4 x 10^4</td>
</tr>
<tr>
<td>Baker Nozzle in 3.9 cm (1.5-in.) diameter screwtop jar with 1.25 cm (0.5-in.) diameter spout</td>
<td>-</td>
<td>4.6 x 10^4</td>
</tr>
<tr>
<td>Nozzle A in screwtop jar</td>
<td>-</td>
<td>3.7 x 10^4</td>
</tr>
<tr>
<td>Nozzle B in screwtop jar</td>
<td>-</td>
<td>3.3 x 10^4</td>
</tr>
<tr>
<td>Nozzle C in screwtop jar</td>
<td>0.46 x 10^8</td>
<td>2.1 x 10^4</td>
</tr>
<tr>
<td>Nozzle D in screwtop jar</td>
<td>0.60 x 10^8</td>
<td>2.4 x 10^4</td>
</tr>
<tr>
<td>Nozzle E in screwtop jar</td>
<td>0.70 x 10^8</td>
<td>3.6 x 10^4</td>
</tr>
<tr>
<td>Nozzle F in Baker Flask</td>
<td>3 x 10^8</td>
<td>-</td>
</tr>
</tbody>
</table>

*BG numbers are 5 min. delivery counts. PSL numbers are counting machine output figures that have not been translated into 5 min. delivery values.
velocity. The discharge velocity from the DeVilbis 40 nebulizer is 1.5 m/s (300 fpm). From Collison's 3-jet nebulizer, the discharge velocity is 1-m/s (200 fpm), and from the 6-jet nebulizer at 140 kPa (20 psig), using a 1.25 cm (0.5 in.) diameter spout, the discharge velocity is about 2 m/s (400 fpm). What made this an issue was that it was feared that a discharge velocity of 2 m/s (400 fpm) would prove to be excessive for cabinet testing, i.e., give a false indication of failure, and that the discharge spout of the 6-jet Collison nebulizer assembly should be made to give a discharge velocity identical with that of the DeVilbis 40 nebulizer that has been used for prior testing. Fortunately, the discharge velocity from the 6-jet nebulizer could be modified simply by using a spout of a different diameter and it was engeraged to give a discharge velocity of 1.5 m/s (300 fpm).

At the time the experiments reported here were conducted, NSF Standard No. 49 had only one requirement for nebulizers used to certify biosafety cabinets, namely, spore delivery numbers. The standard called upon the certifier to verify that the delivery from the chosen nebulizer met the spore delivery standard prior to conducting cabinet certification procedures. A number of different nebulizers were in use at that time that met the delivery requirement (some are reported to be still in use). The difficulties that were being experienced with these nebulizers were that the glass units have thin glass jets that are subject to erosion, chipping, and breakage, and the plastic units often experience distortion as a result of sterilization and ageing. This means that these nebulizers must be retested frequently for the verification of spore delivery numbers, a time consuming and onerous task. The advantage of using a standard, machined, stainless steel nebulizer is that it does not have to be verified initial or retested periodically for spore delivery. It does, however, have to be cleaned carefully after each use as solid deposits left in the capillary-scale passages will alter the delivery characteristics of the nebulizer.

The discharge air volume and discharge velocity from the nebulizer were not specified in NSF Standard No. 49 but it was considered prudent to duplicate the discharge characteristics of nebulizers in current use for biosafety cabinet certification lest cabinets already certified might not meet recertifi-

cation requirements, or unsafe cabinets might be able to qualify for certification. This consideration was the motivation for the extended search for a stainless steel nozzle, glass containment jar, and discharge spout combination that would duplicate all the operating characteristics of glass and plastic units in then-current use for biosafety cabinet certification with BG spores.

**SUMMARY**

The successful search for and validation of a nebulizer that can meet all requirements for conducting biological testing of biosafety cabinets for safety certification and that requires neither initial nor repeated verification of its performance characteristics is described. On the basis of these test results the BGI 6-jet Collison nebulizer is now authorized by NSF Standard 49-1992 for use when conducting biosafety cabinet certification tests with microorganisms. A picture of the unit is shown in Figure 1.

**REFERENCES**


Dow Chemical Co., Midland, MI.
MARS SAMPLE RETURN AND BIOCONTAINMENT

Margaret S. Race
SETI Institute, Mountain View, California

ABSTRACT

In its continuing exploration of the solar system, NASA currently has plans to launch a sample return mission to Mars as early as 2005. The design of such a mission will utilize a variety of contamination control measures, both on the outbound flight to Mars and during the return to Earth of the spacecraft and sample return canister. Biocontainment and quarantine will most certainly be required at a receiving facility where a comprehensive battery of tests will be done to determine if any living, replicating entities are included in the samples and whether the returned materials are harmful in any way to Earth's biota or ecosystems. The task of developing hardware, facilities, laboratory protocols, operations plans and certification standards for extraterrestrial materials will require input from many disciplines, including biosafety and public health experts. By combining basic principles of biocontainment with information about the nature and capabilities of microorganisms, a preliminary protocol has been developed for handling, containing and testing extraterrestrial samples. Plans for biocontainment facilities, quarantine and testing methods for Mars sample return missions will also be important in planning future extraterrestrial sample returns from comets, moons and asteroids which also have the potential for harboring life.

INTRODUCTION

NASA's ambitious plans for solar system exploration in the coming decade include a series of robotic missions to Mars to explore the planet's geochemical, geophysical and atmospheric features; to seek evidence for water, either past or present; and eventually to return samples of soil and rock to Earth for further study. As planners and engineers design the hardware and software for particular missions, it is also necessary to consider planetary cross contamination. According to the Outer Space Treaty of 1967 (U.N., 1967), all space exploration must be done in a way that avoids harmful contamination to celestial bodies or adverse changes in the environment of Earth from the introduction of extraterrestrial matter. For Mars exploration, planetary protection controls of various types are used to avoid both forward contamination of Mars by terrestrial microbes on the outbound spacecraft, and back contamination of Earth by the introduction of organisms or contaminants potentially present in returned Martian materials. These same concerns applied to the return of lunar samples during the Apollo program, the only other time that extraterrestrial samples were deliberately returned to Earth.

For a long time, Mars has been a destination of exobiological interest. It is a planet with the necessary ingredients for the origin, evolution and possibly the persistence of primitive life. Although the surface of Mars is cold, dry, and apparently lifeless today, its earlier geological history had a global environment that was warmer and moister. In fact, Mars and Earth both had similar climatic, geological and water conditions about 3.5 billion years ago, when convincing fossil evidence of primitive life on Earth can been found. Planned robotic missions to Mars are designed to look for rocks that might contain similar fossil evidence of primitive life on Mars. If liquid water persists in some rare subsurface refugia or geothermal oases on the planet, it might also be possible that extant primitive life forms could be found, just as they are in thermal springs and subsurface environments on Earth. Ultimately, returning actual Martian materials to Earth will help resolve questions about whether life ever existed on Mars, whether it might persist today, and if so, whether it is the same or different than life on Earth.

Currently, NASA plans a series of one-way robotic exploration missions to Mars with landers and orbiters taking off every 26 months when Earth and Mars are suitably aligned. By the year 2005, NASA hopes to launch the first round-trip mission that will return Martian samples to Earth. In prepara-
tion for these missions, researchers and engineers have begun serious discussion of how to avoid both forward and back contamination. They are guided in their deliberations by the findings of a recent study by the National Academy of Sciences’ Space Studies Board, which recommended that sample materials returned from Mars should be considered hazardous until proven otherwise (SSB, 1997). In practical terms the planetary protection controls will include a variety of measures including sterilization of the outbound spacecraft, special design of instrumentation and experiments to avoid taking terrestrial microbes on the outbound trip, strict containment of all returned Martian materials and avoidance of “hitchhiker” contaminants on external surfaces or spacecraft parts, and rigorous analyses of returned materials at a special receiving facility with biocontainment and strict quarantine upon return to Earth.

Containment and Testing of Sample Materials from Mars

Much can be learned about how to handle extraterrestrial materials by analyzing containment approaches used by the biomedical and genetic engineering sectors. In addition, the conceptual and operational approaches used during the Apollo program are still applicable, albeit with considerable updating in technology, science, and legal requirements. In retrospect, while there were admittedly some problems experienced in handling early samples from the moon, the quarantine facilities and testing protocols that were used ultimately accomplished their objective of safely containing and screening incoming materials to determine whether they could eventually be released (Allton, 1998). Considering that only 500 grams or less of Martian materials will be returned to Earth during the first sample return mission, the sample receiving facilities for Martian materials can be far less elaborate than the first Lunar Receiving Facilities, which provided quarantine and containment of all returning astronauts, spacecraft, and lunar materials during its operation from 1969-72.

As a step towards addressing sample handling needs and planetary protection measures for Mars sample return, a special Protocol Development Workshop was convened in June, 1997 at NASA Ames Research Center (DeVincenzi, 1998). Experts with diverse backgrounds were invited to participate, including representatives from CDC, USDA, EPA, NASA, university researchers, and the aerospace industry. In their deliberations they addressed how to implement the recommendations of the SSB report (1997) by focusing on three particular areas: biocontainment, life detection, and biohazard testing. Their preliminary findings are summarized briefly below:

Biocontainment: Containment of Martian materials must be designed to protect those working with the samples, as well the Earth’s biota and ecosystems. In addition, containment must ensure the integrity of the samples themselves and preserve them for scientific investigation. Containment will require addressing two very different aspects of the sample return: 1) containment in transit, both in space during the return flight from Mars and on Earth during transport to the sample receiving facility, and 2) containment and handling of extraterrestrial materials in the sample receiving laboratory itself.

Strict en route containment should be maintained from the time of sample collection on Mars through reentry to Earth and during transfer to the sample receiving lab. A sealable transport container should incorporate a fail-safe passive monitoring system to detect any breaches prior to reentering the Earth’s atmosphere. Sterilization en route should be possible in the event of such a breach. In addition, contingency and cleanup plans should be developed for any in-transit accidents that could occur between re-entry and delivery to the sample receiving facility. The sealable transport container should only be opened after it has been securely delivered into the containment laboratory at the receiving facility. At the sample receiving facility, suitably strict containment can be accomplished by a combination of primary and secondary containment. Primary containment should utilize a BSC III cabinet or glovebox line with negative pressure. Secondary containment should be high-end BSL-3, with HEPA filtered air, personal showers and waste water sterilization.

Life Detection and Biohazard Testing: The exact protocols for studying and analyzing returned samples have not yet been developed, although a conceptual approach and recommended types of tests have been identified. Analytical and testing
requirements have implications for biosafety and containment because they will effect the size of laboratory and type of equipment required within the quarantine area, as well as the types and numbers of personnel who will work on the samples. Findings from the comprehensive sample analyses will ultimately be used to determine whether materials can be released from containment for distribution to researchers elsewhere, or whether they warrant continued containment. Multiple lines of investigation will be required to determine whether any living entities or parts of putative Martian organisms are contained in returned materials. Test protocols should include a wide variety of chemical analyses, geochemical characterizations, microscopy and biohazard challenge tests. Tissue culture and cell lines, rather than whole organisms challenge tests, were recommended as a way of effectively scanning for potential toxic effects, infectivity, and ecological disruptions, while minimizing the need for animal containment space and equipment. Research will also be needed to identify effective sterilization method(s) that can be used on Martian materials with the least impact on the samples or their scientific interpretation.

CONCLUSION

A Mars sample return mission will no doubt generate considerable public interest because of excitement about potential extraterrestrial life and questions about possible adverse effects on Earth, however unlikely they may be. Through the environmental impact statement process, the public will be able to scrutinize information about mission plans, risk assessments, biocontainment decisions, laboratory operations, testing procedures, worst case scenarios and contingency plans. Ultimately, the overall success of a Mars sample return mission may depend, in part, on how confident the public is that biosafety concerns have been addressed. Clearly, it will be important to utilize a rational and effective approach to biocontainment as mission plans are developed.

REFERENCES


HEPA FILTERS

Melvin W. First
Harvard School of Public Health, Boston, Massachusetts

ABSTRACT

The high efficiency particulate air (HEPA) filter has become an indispensable item in the maintenance of biological safety and is also used as a means of preserving cultures from contamination originating in the surrounding air. HEPA filters originated with military requirements for protection against chemical, biological, and radiological warfare agents and to avoid emissions from nuclear weapons production facilities. Everything about these filters was classified “secret” during WWII and for a number of years after the end of the war. When they were declassified and commercial production commenced, many new uses were found in medicine, microelectronics manufacturing, and pharmaceutical production. A thorough understanding of these filters proved to be so important that they stimulated research and development activities that established the science of air filtration on a firmer theoretical basis and promoted rapid advances in materials of construction and production methods. The history of their origin and development is an interesting story in itself and helps our understanding of their capabilities and limitations.

INTRODUCTION

The 1952 Handbook on Air Cleaning, published by the U.S. Atomic Energy Commission (USAEC), contained one of the earliest descriptions of the newly declassified high-efficiency filter. It was “made of CC-6 paper which was originally developed by the Chemical Corps for use in gas masks. It consisted of fine asbestos fibers mixed with coarse cellulose fibers to give mechanical strength and act as a support for the asbestos. The asbestos mesh does most of the filtering...cellulose-asbestos paper is expensive and not available in large quantities” [1]. The origin of these filters goes back to the early days of WWII, when the U.S. Army Chemical Corps received from the British army a piece of paper that had been removed from a captured German gas mask canister. Its remarkably high capture efficiency for chemical smoke caused the Army Chemical Corps and the Naval Research Laboratory to duplicate it and manufacture it in large quantities on conventional paper making machinery for use in service gas masks. The navy paper contained Bolivian crocidolite asbestos with cellulose pulp, the Army version (CWS) contained African crocidolite asbestos with esparto grass pulp. Crocidolite asbestos has long flexible fibers that can be cleaved to less than 0.25 mm diameter by mechanical beating.

Protection against warfare agents was also required for operational headquarters, where the wearing of an individual gas mask is impractical. For these situations, the Army Chemical Corps developed a combination mechanical blower and air purifier unit known as a “collective protector.” As relatively large air flows were required, the filter, incorporating the same cellulose-asbestos paper used in the service gas mask, was fabricated into a deeply-pleated form with spacers between the pleats to keep them apart and serve as air passages. It was the precursor of the air filter we now know as the high efficiency particulate air (HEPA) filter (Figure 1). It was referred to then as an “absolute” filter. The nuclear version of the absolute filter was designated AEC No. 1. Its design efficiency was 99.9% for all particles down to 0.1 mm diameter.

To reduce dependence on imported materials, domestic fibers, such as Kraft paper, viscose, and even coarse glass, were found to be acceptable substitutes for esparto, but it was not until the Naval Research Laboratory found ways to make glass fibers as small as 0.25 mm in diameter, that a domestic substitute for crocidolite asbestos was available. It then became possible to make an all-glass filter paper with filtration characteristics superior to cellulose-asbestos composites. Elimination of the cellulose component made it possible to make noncombustible filters, an urgent safety concern, by incorporating: (a) high chlorine content, self-
extinguishing, flexible organic adhesives to bond the filter pack to the filter frame, (b) fire retardant plywood filter frames, and (c) aluminum corrugated separators.

**Development of HEPA Filter Test Methods**

Filter performance standards and test methods also had their origin during WWII by the U.S. Army Chemical Corps with the advice of the National Defense Research Committee. The Army Chemical Corps asked Nobel Laureate Irving Langmuir to examine the physical basis for the capture of small particles by fibrous media and to recommend filter test methods. Langmuir concluded that the principal mechanisms involved were interception, which affected suspended particles substantially greater than 0.1 mm diameter when moving through a devious flow path in a bed of porous material, and diffusion, which affected suspended particles substantially less than 0.1 mm [2]. His analysis, later modified by Ramskill and Anderson to include inertia [3], indicated that the combined effects of these forces on a particle would be at a minimum when the particle was 0.3 mm in diameter; and he advised the Army Chemical Corps to test their gas mask filters with a smoke of this size. He indicated that, when particles were present during field use of the gas mask that were either greater or smaller than 0.3 mm, they would be removed at higher efficiency than the 0.3 mm test particles. Later investigations confirmed the existence of a minimum filterable particle size, but it has been found to be closer to 0.1 mm for the flow rates and paper compositions in use for currently manufactured nuclear grade HEPA filters [4].

Langmuir’s theory affected U.S. filter technology profoundly and led directly to the development of a filter test by LaMer and Sinclair during 1942-1945 which used an aerosol containing dioctyl phthalate (DOP) droplets [5]. It has become the U.S. standard method for bench testing unused ultrahigh efficiency, or absolute, filters.

It was discovered as early as the initial installation of HEPA filters at the Oak Ridge National Laboratory’s (ORNL) graphite reactor in 1950 that the full capabilities of HEPA filter performance were not being achieved because of damage during shipment and faulty installation. As a consequence,
in-place testing of all filters by methods initiated and developed at ORNL has become routine [6]. These tests take place before initial start-up of new facilities and periodically thereafter.

Specifications for HEPA filters, HEPA filter media, and installation and field test consensus standards are published by the American Society of Mechanical Engineers as ASME N509 [7], ASME N510 [8], and Nuclear Air Cleaning Equipment Code AG-1 [9], respectively. All utilize monodisperse DOP aerosols for bench testing and polydisperse DOP aerosols for field testing HEPA filters in conjunction with a forward light scattering photometer for aerosol concentration measurements.

**Air Filtration Theory**

**Filters.** Although all manner of air and gas cleaning devices are often called “filters,” in this paper the word filter will only be used to refer to “a porous mass through which a gas is passed to separate particulate matter in suspension” [10]. The filter structure exhibits considerable porosity for easy passage of the carrier gas. This means that the spaces between adjacent fibers will be larger than the particles the filter is designed to collect and that sieving, that is, retention of particles by openings too small to permit their passage, is not a primary mechanism for filtering fine particles. Instead, inertia and diffusion plus interception are the primary mechanisms. Particle size is the most important property of an aerosol because the smaller the particles the more stable the aerosol and hence, the greater the difficulty in separating particles from the gas phase in which they are suspended. It is common practice to characterize a particle’s diameter in terms of a dynamic parameter, the aerodynamic equivalent diameter, defined as the diameter of a homogeneous sphere of unit density that has identical terminal settling velocity in still air as the particle under consideration. For example, a 1.0 mm sphere of UO₂₂, density 11 g/cm³, has an aerodynamic equivalent diameter of 3.3 mm. In all cases in this paper, aerodynamic equivalent diameter will be the size designation.

**Filtration.** Figure 2 shows the streamlines around a single filter fiber lying normal to the flow direction. A particle entering the flow field surrounding the fiber must follow the curved path of the streamlines if it is to pass around the fiber.

![Figure 2: Streamlines around a filter fiber.](image)

- Particle caught by interception.
- The effect of inertial forces.
- Particle caught by diffusion.

When particles possess sufficient inertia, because of their higher momentum relative to that of the conveying gas molecules, they resist following the curvature of the air stream and come in contact with the fiber. The effect becomes greater as aerodynamic equivalent diameter increases and as the velocity of the air approaching the fiber increases.
When suspended particles are very small, however, they tend to follow the curved streamlines closely, that is, they have little inertia, but they will be in vigorous Brownian motion. Therefore, when a streamline passes close to the fiber surface, the random movements around the streamline may result in some of the particles contacting the fiber and adhering there by Van der Waals force. This sets up a concentration gradient between the zone close to the fiber and the bulk of the aerosol which, in turn, results in particle diffusion in the direction of the fiber surface. The smaller the particles, the more vigorous will be their Brownian motion, and the more effective will be filtration by diffusion. Because the rate at which particles cross streamlines under the influence of diffusional force is slow relative to the effects of inertial force on large particles, diffusional separation of small particles is enhanced by slower velocities through a filter.

Particle collection by interception occurs when a particle traveling in a streamline that approaches a fiber within one particle radius touches the fiber and adheres. Interception is enhanced when the diameter of the collecting fiber or granule approaches the geometric diameter of the particle. Interception is independent of flow velocity.

The several filtration mechanisms of importance are shown together in Figure 3 where penetration (equal to 100 minus collection efficiency) is plotted against particle size. The penetration curves are not additive, inasmuch as particles can be collected but once, but the net effect can be approximated by the solid summation curve. Figure 3 makes it clear that there is a particle size for which both inertial and diffusional forces are minimal and only interception is unaffected. This explains the concept of a minimum filterable particle size. The exact minimum size depends on the fiber diameter, filter construction, and flow velocity. As noted earlier, the minimum filterable particle size for HEPA filter papers is close to 0.1 mm when operated at the design flow rate of 2.5 cm/s.

**FIGURE 3**
The effects of inertia, diffusion, and interception on the penetration-particle-size curve.
(Courtesy International Atomic Energy Agency)
The effect of flow velocity on particle penetration for HEPA filter paper is shown in Figure 4. In this diagram, there is also a minimum efficiency point. In practice, the usual test velocity for HEPA filter paper occurs about midway on the steep ascending sector of the curve. This means that penetration should decrease when flow velocity decreases to the design rate of the full-scale filter, and, in fact, that does happen.

**Particle Retention.** After an airborne particle contacts a filter element, retention forces come into play to prevent reentrainment under the influence of the drag of the air. For small particles, the principal retentive force is a surface phenomenon referred to as the Van der Walls force, which is proportional to the total area of contact. For small particles, the fraction of the total surface area in contact with a filter fiber will be relatively large, whereas the projected area subject to air drag will be relatively small, resulting in a retention force that will exceed the reentrainment force. Seepage of particles collected on HEPA filters never occurs unless the filter paper becomes thoroughly wet. For this condition, different entrainment mechanisms are involved.

**FIGURE 4**
The effects of inertia, diffusion, and interception on the penetration-velocity curve for HEPA filter papers.
(Courtesy International Atomic Energy Agency)

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**Filter Resistance Characteristics.** As particles collect on the surfaces of fibers and in the interstices between them, the collected particles tend to form a coherent dust layer known as a filter cake. When this occurs, particle collection gradually shifts from media filtration (i.e., by the individual filter fibers) to cake filtration. This transformation produces two important changes: (1) efficiency increases in proportion to the increase in thickness of the cake and (2) after the formation of a coherent filter cake, the resistance of the filter to airflow, that initially increased at a slow and steady rate as particles accumulated, now increases at an accelerating rate in response to additional particle deposition and narrowing of pathways. Once this begins, the filter rapidly reaches its terminal design airflow resistance. Figure 5 shows a typical pressure rise curve for a HEPA filter exposed to atmospheric dust [11]. The long, slow pressure rise followed by a rapidly accelerating increase is clearly evident. The reason for the abrupt change is the onset of sieving. It takes over when the collected particles form a structure containing less space between particles than the characteristic diameter of the particles being collected. When HEPA filters reach this stage, they must be replaced by new ones.
Construction and Service Characteristics of HEPA Filters

Filter Paper. Although originally composed of a mixture of asbestos and cellulose fibers, all high-efficiency filters are now made from a mixture of glass fibers of carefully graduated diameters that give the required particle retention efficiency without exceeding the maximum airflow resistance criterion at the design airflow rate. Consequently, there are innumerable combinations of fiber sizes that are capable of satisfying both requirements and each filter manufacturer has a proprietary formula that qualifies the product. The paper may incorporate up to 7% by weight of organic matter, divided between (1) a binder addition, such as latex, to give the paper strength and resistance to cracking at the bends and (2) a water repellent addition to protect the paper against wetting from deposition of liquid droplets, should they be present (9). Additional qualification criteria include:
1. Not less than 99.97% retention of 0.3 mm DOP particles at a flow rate of 32 L/min. through a paper area of 100 cm²;
2. Clean airflow resistance not in excess of 40 mm of water (0.4 kPa) at a filtration velocity of 320 cm/min. (0.053 m/sec);
3. Average tensile strength after exposure to 6.0-6.5 x 10⁷ rads (0.6-0.65 MGY) of not less than 179 g/cm of width in either direction; (not required for biosafety applications)
4. Resistance to excessive strength degradation after exposure to high temperature (370 ± 28 C) for 5 min. and to wetting by immersion for 15 min. (9)
HEPA filter papers acceptable for biological safety installations routinely give collection efficiencies greater than 99.99% when tested with 0.3 mm diameter DOP. By increasing the fraction of fine glass fibers that are less than 0.25 mm in diameter in the paper, it is possible to obtain efficiencies greatly in excess of 99.999% for 0.1-0.3 mm particles with a modest increase in filter resistance, typically about 25%. These papers are often referred to as ultra low penetration aerosol filters (ULPA) and are of special interest to manufacturers of microelectronic chips. These filters do not appear to have an advantage over HEPA filters manufactured to military, nuclear, or biological safety (NSF Standard 49) standards inasmuch as the sizes of bacteria and fungal spores are well above the minimum filterable size (where inertia provides increasing collection efficiency) and virus particles are well below, (where vigorous Brownian motion provides for high collection efficiency by the diffusional mechanism).

Deep Pleat HEPA Filters with Corrugated Separators. Filters constructed with paper pleated the full depth of the rigid outer frame and with adjacent pleats held apart by full-depth corrugated separators are widely used for biological safety applications. Construction requirements and acceptance criteria for filter units are provided by ASME (reference 9) that calls for the exclusive use of filter paper qualified in accordance with the criteria contained therein. The Institute for Environmental Standards has alternative consensus standards that have been adopted for “clean-room” use. It provides for three grades of high efficiency filter: The ASME or the IES “C” grade filters should be specified for biological safety applications. Both standards provide for: (1) standard rectangular filter sizes, (2) a variety of filter frame materials, (3) face gaskets constructed from flat strips of expanded closed cell neoprene sponge rubber having cut surfaces on both faces and notched or dovetailed corners, and (4) corrugated separators made of 0.4 mm thick, hardtempered aluminum foil. Often, the inside edge of the corrugated aluminum separator, that may be in contact with the paper is turned back on itself to form a less sharp edge to avoid paper cuts from this source during shipment.

Filter Mounting. Filters with gaskets should be mounted against a smooth, flat, continuously-welded structural-steel flange. Gasket compression is achieved with screw-down clamps. Single filter housings, referred to as caissons, can also be used for mounting individual filters. They are usually used when remote handling or “bag-in, bag-out” methods of filter changing are required. Whichever filter mounting method is selected, it has been found necessary to compress the gasket at least 80% to maintain a leak-tight seal for the life of the filter. Eighty percent compression of closed cell neoprene sponge requires a loading of approximately 1.4 kg/m² of gasket area, or a total clamping load of about 635 kg for a 61 x 61 cm filter unit. This is the reason that rigid structural shapes, rather than bent sheet metal structures, are needed for mounting frames, i.e., to avoid distortion under load.

Filter Operation and Maintenance. For biosafety installations, it is usual to design for enough filter capacity to give a clean filter resistance no greater than 0.25 kPa. New biosafety cabinet filters are likely to be even less, perhaps 0.15 kPa. Although HEPA filters are qualified to maintain their integrity and filtering efficiency up to a minimum resistance of 2.5 kPa, they are seldom operated up to that resistance level because of fan and fan motor limitations. A doubling of clean filter resistance before change is common for biosafety installations. Dust holding capacity is greatly influenced by the properties of the aerosol, with smaller particle sizes and greater size uniformity producing a more rapid increase in filter resistance for the same weight of deposited dust. For this reason it is not possible to give more than a general idea of filter service life. It is possible to monitor filter pressure drop and to change filters whenever the fan can no longer deliver the required air volume rate because of back pressure increase. It is important to keep in mind that airflow rate through the filter must be measured simultaneously with filter resistance, lest an acceptable pressure drop reading merely reflects a shift along the fan performance curve that responds to increased loading of the filters by a reduction in airflow capacity.

In a clean environment, HEPA filters may run for more than a decade before reaching their maximum design dirty filter resistance. This raises serious concerns regarding the detrimental effects of filter aging on loss of strength, paper embrittlement, loss of water repellency, shrinkage of adhesives, and a general lowering of safety factors to assure continu-
ing design service performance until the next annual in-place filter test. These concerns have been expressed in a prior publication (12).

**Filter Testing**

**Bench Testing of New Filters.** Bench testing of new HEPA filters certified for biosafety service according to NSF Standard 49 [13] are conducted with a penetrometer, called a Q107, that was designed by the U.S. Army Chemical Corps during the 1950s [14]. The complete penetrometer consists of a monodisperse DOP aerosol generator, an instrument that measures the size and uniformity of the particles formed, a clamping device to seal the filter under test into the test rig, a forward light scattering photometer to measure DOP penetration, and a manometer to measure filter resistance at rated airflow rate. The size of the DOP aerosol is 0.3 mm. HEPA filters certified for biosafety applications will have test efficiency and filter resistance values marked on a side of the filter frame.

**In-Place Testing of Filter Installations.** Unlike bench tests for new filters that are designed to determine filter quality by means of an efficiency test utilizing an aerosol containing a substantial fraction of particles in the minimum filterable size range, in-place tests are designed to reveal the presence of defects in the filter unit that resulted from such things as rough handling during transportation, paper and gasket damage during installation, inadequate pressure against intact gaskets, and penetrations through the housing to which the filter units are attached. Inasmuch as each filter unit is assumed to have been satisfactory when it was certified by the manufacturer, aerosol penetration during an in-place test in excess of established limits is taken as a sign of a defective filter or installation, and standardized procedures are conducted to locate and correct the defects: e.g., gasket compression will be increased; gaskets will be examined for breaks and tears; and penetrations, cracks, and open seams in the filter house and mounting frames will be closed by welding. Each time, after repairs are made, the system must be retested until it meets established criteria for leak-tightness.

The standard method for in-place testing of filters for biosafety service is described in NSF Standard 49 [13]. It utilizes a polydisperse aerosol generated by compressed-gas nebulization of cold or heated liquid DOP. It is characterized by a light scattering median size of 0.7 mm, and a geometric standard deviation of 1.4 [13, sect. 2.8]. This aerosol has very different size characteristics from the one produced by the monodisperse DOP aerosol generator. The filter penetration values obtained by using a compressed gas-generated DOP should be, and are, different than when using the bench-test aerosol. This means that the in-place penetration criterion for acceptance cited in NSF Standard 49 is unrelated to the acceptance criterion for filter efficiency cited in reference 9. This should not be viewed as a defect in the procedure for assuring the quality of installed filter systems, but it must be recognized that the aerosol penetration numbers obtained from bench and in-place filter tests are not interchangeable.

**Searching for Defects.** The detailed examination that is undertaken to meet in-place acceptance criteria for biosafety cabinets is called filter scanning. It is conducted by generating an aerosol challenge in the usual way and then passing the probe of a direct-reading light-scattering photometer in overlapping strokes over the entire face of the filter (being careful to include all gasket edges and housing joints). This search for defects in installed systems is made easy by the use of direct reading aerosol detection instruments because, when the probe is located exactly in front of a leak, it draws in unfiltered aerosol and the indicator goes offscale. It is a tedious, but very sensitive, method for finding even small leaks, such as thin spots in the filter paper.

**Filter Performance in Service**

The wide diversity of aerosols generated in biosafety cabinets raises an important question regarding the relevance of the test procedures that are conducted with prescribed DOP aerosols, none of which will be likely to be encountered under normal service conditions. In fact, the standard qualification tests tell us very little about the performance of HEPA filters under realistic use conditions. Certainly, the aerosols present from microbiological operations are very different from the monodisperse DOP aerosols used to qualify filters for nuclear service, so that the efficiencies observed during the standard qualification tests are not necessarily the results that will be obtained in practice; they may
be better or worse depending on the characteristics of the aerosol challenge.

Why perform the standardized tests if they do not tell us exactly how the filters will perform when called upon during normal service? The answer is that standard qualification test results should be looked upon as an index of merit, an indication of quality, rather than as a quantitative description of filter efficiency under unknown or ill-defined operating conditions. Passing a standardized qualification test gives reasonable assurance that the filters have been produced from high quality components and carefully assembled to exacting standards. Lacking test results with the precise aerosols that will be encountered during actual service conditions, this is probably the best that can be accomplished, and it is comforting to know that overall efficiency will not be lower than the value for the least filterable size. It is relevant to note that the qualifying penetration value was selected originally on the basis of what commercial suppliers of filters were reasonably expected to provide at that time. Today, filter efficiency usually exceeds the requirement by a substantial margin when the filters are manufactured in full compliance with the nuclear or applicable IES standard.

SUMMARY

The needs for high efficiency air cleaning systems have resulted in the development of very low penetration filters for submicrometer particles that represent the least filterable sizes (0.1-0.3 mm). The introduction of very low penetration filters made it necessary to develop testing methods capable of assessing a penetration fraction of $10^{-5}$, or less. In-place testing of newly installed very low penetration filter systems has been incorporated into a number of national standards in recognition of the need for quantitative assurance that completed installations do not contain defects that reduce microbiological safety. For the same reason, periodic in-service retesting of filter systems is required. The current status of high efficiency air cleaning technology for aerosols is generally satisfactory, but improvements in materials for greater reliability, higher efficiency, improved capacity, and greater resistance to the detrimental effects of aging are possible and desirable.

REFERENCES


W.L. Anderson (La Plata, MD), Personal communication to M.W. First, 1988.

V.K. LaMer and D. Sinclair, A Portable Optical Instrument for the Measurement of Particle Size in Smokes, (The OWL); and, An Improved Homogeneous Aerosol Generator, OSRD 1668 (OSRD, Office of Technical Services, Washington, DC, 1943).


COMMITTEE POSITION PAPER OF ABSA ON OSHA’S PROPOSED RULE ON OCCUPATIONAL EXPOSURE TO TUBERCULOSIS

On February 17, 1998, the American Biological Safety Association (ABSA) filed a Notice of Intent to Appear in the matter of the Occupational Safety and Health Administration (OSHA) proposed rule concerning the *Occupational Exposure to Tuberculosis* (Federal Register 62:54159-54308, Docket Number H-371 (proposed rule). The testimony to be delivered by an ABSA representative follows:

**General Comments**

ABSA believes tuberculosis (TB) is a serious public health concern and appreciates the active interest the OSHA has in controlling the impact of this disease in the workplace.

ABSA commends OSHA for incorporating sound biosafety principles currently in use and advocated by biosafety professionals into the proposed rule. Adopting principles from primary biosafety resource documents such as the Centers for Disease Control and Prevention (CDC)/National Institutes of Health (NIH) *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) reinforces proven practices already in use. BMBL is re-published approximately every five years to include updated information on engineering controls, administrative procedures, and specific work practices for laboratories that work with infectious agents. ABSA commends OSHA for incorporating resources such as BMBL by reference, where practical, as that allows for future flexibility in applying recommendations published by these organizations.

ABSA believes performance-based regulations provide greater benefits for all, and applauds OSHA’s effort to make this standard performance-based. ABSA believes that performance-based standards provide the flexibility needed by biological safety professionals to address workplace hazards based on the risks posed in the different work environments.

OSHA requested input regarding the need for laboratory specimens to be labeled. ABSA feels there is no need for special labeling practices for specimens from TB patients. *M. tuberculosis* will be present overwhelmingly in sputum specimens from untreated TB patients and less frequently in other body fluids and tissues. The standard practice at most laboratories is to open any sputum specimen in a biological safety cabinet. Therefore, worker safety would not be compromised without the label. Patient confidentiality considerations should be considered if additional labeling is required.

OSHA is to be commended for not requiring the use of a respirator for employees who are using a biological safety cabinet. If the biological safety cabinet has a current certification record and the employee has been trained in the proper safe work practices, additional protection is not needed. If work is done outside the biological safety cabinet, the proposed rule requires respiratory protection for those workers. OSHA has handled this issue particularly well.

In the proposed rule preamble, OSHA recognizes that ultraviolet (UV) light is not an acceptable means of primary engineering controls for controlling aerosolized *M. tuberculosis*. The comprehensive appendix (Appendix D) on how to select and maintain UV light systems, could be misconstrued as an endorsement of UV light as a primary engineering control. In order to clarify OSHA’s position, it should be stated in the proposal’s text that UV light is not an acceptable primary containment system for aerosolized *M. Tuberculosis*.

**Section (a) Scope**

ABSA seeks clarification as to whether the scope of the proposed regulation includes industry-based first aid squads and occupational medical offices under the category of emergency medical services. Industry-based first aid squads and occupational medical offices often provide services to an employee population with a TB risk lower than that of the general population. If these groups are included under the regulation, ABSA would appreciate a further reduction in their responsibilities. ABSA recommends their responsibilities be limited to a documented risk assessment that identifies the lower risk of this employee population to demonstrate that no additional measures (e.g., exposure control plan) need be taken.
Section (b) Application

ABSA appreciates OSHA’s effort to provide employers with reduced responsibilities under circumstances in which the probability of exposure to TB is reduced. However, the proposed exemption from certain provisions is currently too limited to be of real value or to really assist employers whose employees have a very low or no risk of TB exposure. In addition, the remaining requirements do not necessarily address the real risk of exposure to TB.

The current exemption still requires the employer to establish a complex written Exposure Control Plan. Such a plan is facility specific in its intent and therefore, needs to be established within a facility. However, the proposed standard includes agencies and organizations that provide external services (e.g., social work, legal services) to facilities like hospitals, long term care facilities, and so forth... (as listed in 1910.1035 Scope). How can social services, police departments, attorney offices, schools and universities establish an Exposure Control Plan for facilities for which they have no control or jurisdiction? How can these agencies determine or document the number of confirmed TB cases in such facilities when they don’t operate, manage or control the hospitals or nursing homes they occasionally visit?

To require the same complex Exposure Control Plan of employers performing high risk procedures in a hospital setting as well as the attorney office providing legal services to somebody in a nursing home does nothing to address or manage the real risk of TB in the work place for the attorney.

In addition, OSHA fails to provide any information on occupational exposure rates for these groups. Rather OSHA states “Thus, although the data on employee conversion rates in other work settings cannot be used to directly quantify the occupational risk of infection for those work settings, there is strong evidence that employees in various work settings other than hospitals can reasonably be anticipated to have exposure to aerosolized M. tuberculosis and that TB can be transmitted in these workplaces when appropriate TB infections control programs are not implemented.”

This statement not only reemphasizes the difficulty of assessing the real risk to social workers, attorneys, police officers and others, it also clearly identifies the responsibility as being facility specific. For example, the hospital has the responsibility for providing a safe work environment, the nursing home has the responsibility for ensuring the appropriate management, etc. This also includes providing the necessary information to outside agencies and services who may be performing work in those facilities.

ABSA recommends that OSHA establish a clearly defined exemption category with its own unique minimal requirements which addresses the real risk of exposure for employees from social services agencies, police departments and others. If a written plan is deemed necessary, it should be limited to post-exposure follow-up and initial training/information without annual retraining requirements. One challenge for the protection of these individuals is how to protect the police officer, or social worker that has to question a patient with suspected TB sitting in an acid fast bacilli (AFB) isolation room. The primary protection for these individuals in this situation is the proper signage of and the procedures in place for the AFB isolation room which address personal protective equipment needs prior to entry.

Exemption should be granted to all employers whose employees do not perform or are not involved in high hazard procedures and do not admit or provide medical services to individuals with suspected or confirmed infectious TB. All additional requirements such as no confirmed TB cases and certain county TB rates are unnecessary, since they do not take the real risk of exposure into consideration. What difference does it make for the attorney if the county had 0 or 1 confirmed infectious TB case in a certain year? What difference does it make to a social worker visiting a certain nursing home if another social worker from the same agency acquired TB at home and is on medical leave?

The key to protecting these workers is to establish appropriate procedures at the facilities they are visiting.

Section (c) (2) Exposure Control Plan

It is not clear in whether the Tuberculosis Exposure Control Plan may be combined with a facility’s Bloodborne Pathogen Exposure Control Plan or whether it must be a separate Plan. ABSA recommends that employers be allowed to establish and
maintain a single Exposure Control Plan. This would reduce unnecessary duplication of information for multiple Exposure Control Plans.

**Section (e) Clinical and Research Laboratories**

This section identifies requirements that pertain to both clinical and research laboratories and establishes additional requirements for research laboratories. The requirements are a subset of the guidance provided in BMBL for Biosafety Level 2 and Biosafety Level 3. BMBL offers the following guidance for determining the appropriate biosafety level:

The recommended biosafety level(s) for the organisms in Section VII (Agent Summary Statements) represent those conditions under which the agent can ordinarily be safely handled. The laboratory director is specifically and primarily responsible for assessing risks and for appropriately applying the recommended biosafety levels. Generally, work with known agents should be conducted at the biosafety level recommended in Section VII. When specific information is available to suggest that virulence, pathogenicity, antibiotic resistance patterns, vaccine and treatment availability, or other factors are significantly altered, more (or less) stringent practices may be specified.

Following that guidance, ABSA recommends that section (e) of the proposed rule be modified as follows in order to ensure laboratories adopt the complete, proper biosafety level(s) for their specific activities:

(e) (1): No change.

(e) (2): No change.

(e) (2) (i) - (e) (2) (ii) (E): Replace with the following:

(i) The laboratory director is specifically and primarily responsible for assessing risks and for implementing the biosafety level(s) identified in the Exposure Control Plan. The laboratory director shall prepare or adopt a biosafety manual which meets the following criteria:

(A) Provides the identification and assessment of the special hazards posed by the laboratory activities involving *M. tuberculosis*.

(B) Adopts a combination of standard and special practices, safety equipment and facility requirements that are specifically appropriate for the operations performed, the routes of infection, and the laboratory function or activity per the biosafety level identified in the Exposure Control Plan.

(e) (2) (iii) (A): Change to allow the use of a certified Class 2 or Class 3 biological safety cabinet.

(e) (2) (iii) (B): ABSA supports the certification requirements of this section but recommends the adoption of the following statement based on the CDC document *Primary Containment for Biohazards: Selection, Installation, and Use of Biological Safety Cabinets*:

The operational integrity of a BSC shall be validated by certification before it is put into service and after a cabinet has been repaired or relocated. Relocating a BSC may break the HEPA filter seals or otherwise damage the filters or the cabinet. Each BSC shall be tested and certified at least annually to ensure continued proper operation.

(e) (2) (iv): As written, there is some question as to how "as near as feasible" may be interpreted. BMBL provides packaging recommendations that allows either decontamination outside the immediate laboratory or decontamination off-site. This provides flexibility in the development of waste management procedures at a facility. ABSA recommends that this strategy be adopted by OSHA. The fact that the U.S. Department of Transportation, in granting its 1996 packaging exemption, recognized that current medical waste packaging and transportation practices for *untreated* discarded cultures and stocks of Biological Safety Level 3 agents such as *M. tuberculosis* pose no adverse risk to human health or the environment supports this strategy.

(e) (3): This section would not be necessary under the ABSA proposed (e) (2) (i) (B) above.

(e) (3) (i) (A): As a point of information
ABSA recommends that all laboratory doors be kept closed when work involving *M. tuberculosis* is in progress, and not just limit this requirement to research laboratories. Voluntary standards (National Fire Protection Association and the American Society of Heating, Refrigerating and Conditioning Engineers) indicate biological and chemical laboratories should be negative to the corridor and have single-pass air. In the event of a laboratory mishap, occupants in areas surrounding the laboratories will be protected if the doors remain closed. An expansion of OSHA’s proposed requirements to include all clinical and research laboratories will provide greater employee protection.

Section (f) Respiratory Protection

(3) (i): This requires employers to “select and provide properly fitted negative pressure or more protective respirators.” ABSA believes there is an opportunity for misinterpretation of “more protective respirators.” Alternative wording that would provide clarification would be “N95 respirators which have met the NIOSH certification criteria detailed in 42 CFR Part 84 or powered, air-purifying (positive pressure) respirator.”

Section (g) Medical Surveillance

The proposed rule indicates that medical surveillance is to be done in accordance with CDC recommendations. OSHA recognizes in the preamble that medical knowledge of TB disease is dynamic. OSHA states that it believes that it is the employer’s responsibility to inform health care professionals of the medical surveillance requirements of the proposed rule. However, TB is a disease with which many health care professionals may lack real experience or education on its management. The OSHA bloodborne pathogen standard (29 CFR 1910.1030) requires employers to provide copies of CDC recommendations to health care professionals performing medical surveillance under the provisions of that standard. This proposed rule should have a similar provision. Such a provision would increase the level of awareness of the employer and it would help to improve the level of medical surveillance provided. Provision of only the proposed rule would not provide enough technical detail to ensure the desired quality of the medical surveillance would be achieved.

Section (g) (3) (i) (E)

ABSA appreciates OSHA’s intent behind the requirement for a TB skin test within 30 days of termination of employment. However, this is unenforceable on the part of the employer once an employee has resigned or been terminated. ABSA recommends that OSHA require an employer to provide TB testing within 30 days of employment only if the former employee requests or agrees to be tested.

Section (h) Communications of Hazards and Training

(1) (ii): The use of the universal biohazard symbol is noted as needed to address labeling requirements of the proposed rule. However, a graphic of this symbol is not provided. A graphic or reference to 29 CFR 1910.1030 (g) (1) (B) should be provided for anyone unfamiliar with the universal biohazard symbol.

(2) (iii): The “STOP” sign noted in the proposal will get the attention of people about to enter a patient’s room. Trained employees at risk of TB infection should recognize what a respirator is and for what it is used. However, the signage provides no indication as to the type and nature of the hazard within the room. Some employers may have employees on staff who do not go into TB patient isolation rooms. Employers may train only their workers at risk of TB infection. Untrained workers may not recognize the hazards within the room posted in this manner. A posting such as “Airborne Infection Hazard” (or an equivalent) would enhance hazard communication and could prevent exposure events.

(3) (ii) (C): ABSA appreciates OSHA’s flexibility in accepting a demonstration of employee knowledge and skill in lieu of an automatic annual re-training requirement. This allowance will enable employers to utilize newer, more efficient and more flexible technologies to demonstrate employee understanding.
(3) (vii): This section is missing the requirements for spill clean-up and decontamination training for an accidental spill of *M. tuberculosis*. This is a critical oversight. Spill training is mentioned in Section (e)(2)(ii)(D) and states "All spills shall be contained and cleaned up by employees who are properly trained and equipped to work with potentially concentrated *M. tuberculosis.*" Since it is required, it should be identified in this section.

Appendix G to Sec. 1910.1035—Smoke-trail Testing Method for Negative Pressure Isolation Rooms or Areas

OSHA has not indicated whether or not this appendix is a mandatory or a non-mandatory appendix. This needs to be clarified.

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