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VISION

American Biological Safety Association (ABSA) will be the global leader in providing professional and scientific expertise in the practice of biological safety.

MISSION STATEMENT

ABSA is dedicated to expanding the biological safety awareness to reduce the potential for occupational illness and adverse environmental impact from infectious agents or biological derived materials.

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 professional standards for biological safety professionals.
PRESIDENT’S PAGE

Success can be measured in various ways—winning a gold medal, completing a college degree or landing that “perfect job.” For those of us in biological safety, success is personified in the healthy scientists, health care workers and support personnel who safely handle hazardous biological agents at work. Throughout my career, I’ve had the opportunity to support numerous organizations and realize that the key to success in safety is embodied in three concepts: vision, leadership, and innovation.

Creating an organizational vision for safety is the hub from which all other efforts flow. At Johnson & Johnson, CEO Ralph Larsen articulates his vision for safety by stating, “Johnson & Johnson will be the world leader in health and safety by creating an injury-free workplace.” The biological safety professional is a member of a multi-disciplinary team whose mission is to be a “partner in the prevention of injuries and illness.” Note that the vision is owned by the CEO, not the safety department. This results in full communication and deployment throughout the organization. Success is possible only when the vision is owned by the head of the organization.

Leadership is the vehicle that drives the organization toward its vision. Without it, the clarity of the vision fades and positive efforts are soon derailed. To be effective leaders, biological safety professionals must develop two critical skills—influence and salesmanship. Influence becomes possible when we take the time to understand the businesses we support and their underlying values. Then, we can tie biological safety to business goals and make it an integral part of daily operations. Salesmanship is required to convince others of the value of our mission. The work we do is extremely important and its contribution to institutional success cannot be overemphasized. Our simple, yet powerful message should be: when biological safety programs are successful, people are healthy.

Innovation in biological safety will allow us to eliminate many sources of exposure. Consider the following problem: What can we do to prevent lacerations with contaminated sharps? One could answer with a fairly predictable response, such as: “Use a syringe with a retractable needle to deliver medication.” An innovative approach will challenge the drug delivery process to eliminate the sharp entirely. Can we challenge our institutions to eliminate breakable labware and pipettes? How about automating repetitive lab operations? If we’re successful, we not only eliminate an aerosol risk but also significantly reduce the potential for repetitive motion injuries. When the Class II biological safety cabinet was introduced, it was an innovative solution to the problem of simultaneous protection of personnel and product. Innovations will continue to be born if we constantly challenge the status quo and continuously strive for improvement.

So how does this sound—I see a workplace where all biological hazards have been identified, sufficient controls have been implemented, and injuries and illness are unheard of? Seem impossible? Its not! Just formulate a vision for success, lead your organization to it and innovate along the way. You’ll be surprised at how quickly vision becomes reality.

[Signature]

Joseph Van Horne
EDITOR'S PAGE

Contributions concerned with the history of the profession of biological safety and the origins of the American Biological Safety Association continue in this issue with an article entitled, “A History of the American Biological Safety Association Part I: The First Ten Biological Safety Conferences 1955-1965” by Manny Barbeito and Dick Kruse. It follows three previously unpublished papers by Arnold G. Wedum, the “father of biosafety” and the legendary director of safety for Camp Detrick from its earliest days. Each of Dr. Wedum’s papers was preceded by an introduction by Emmett Barkley that placed it in its historic context and highlighted its current significance. This striving to gather together historical facts and recollections is an important effort intended to record the roots of biological safety while many of the pioneers are still accessible. We are delighted to learn that the team of Manny and Dick is committed to continuing the history up to current times and look forward to additional installments. This is no small undertaking and contributions of important documents and reminiscences concerning past meeting and significant events will be welcomed and acknowledged by them. Reading through the history of the first ten years of biological safety meetings one is immediately struck by a realization that the concerns of the first meeting, 42 years ago, safety cabinets, containment problems, and environmental monitoring, have not become historic curiosities but are still very much with us, even as we continue to apply similar solutions. Fortunately, we no longer need to be concerned with security clearances as we meet to exchange research findings and experiences.

Perhaps we are now in the process of initiating a new historic trend in the designation of biosafety level laboratories. We observe with increasing frequency references to BL-2* and BL-3* laboratories, although such designations are seldom defined and are not found in the current edition of the CDC-NIH manual on “Biosafety in Microbiological and Biomedical Laboratories”. What do these new designations signify? A BL-2* laboratory is not sanctioned for BL-3 organisms, or even for large quantities of aerosolized BL-2 organisms. Similar comments apply to BL-3* laboratories. Up to the present we have not encountered a reference to a BL-4* or BL-3 laboratory. Perhaps it is significant that the trend is in the direction of greater, rather than lesser, safety. Nevertheless, if there is a genuine need for a larger number of gradations of BL-designated laboratories, it would be prudent to convene an expert committee to carefully define what facilities these intermediate types should contain and what kinds of work will be authorized in each. One suggestion would be to require a “plus” facility when procedures indicate an above normal potential for aerosolization or splashing. However, until such a time as a “plus” facility can be described explicitly and the degree of permissible additional hazard can be defined in clear terms, it might be prudent to avoid use of this term.

Melvin W. First, Editor
LETTER TO THE EDITOR

From Richard H. Kruse

Dr. Emmett Barkley has done a masterful job submitting three papers (Volume 1, Number 1, 1996; Volume 2, Number 1, 1997; and Volume 2, Number 2, 1997) that were in Dr. Wedum’s files that he never published and I would like to thank him for the introductory commentary he provided for each. As an “old timer” of 20 years in Industrial Health and Safety at Fort Detrick, and 42 years attending Biological Safety Conferences, I had the honor and privilege of working for and with Dr. Arnold G. Wedum. Many newcomers to ABSA never had the privilege of knowing Dr. Wedum and his remarkable wisdom. At Safety Conferences he never criticized, but in his indomitable way, praised and encouraged the speaker. He was proud of the Biological Safety Conferences as he enjoyed the fellowship and learning what other individuals were doing. He did not believe in cliques, he constantly credited subordinates, he stressed education, and his willingness to impart safety served as a strong foundation to ABSA. At the 14th Conference when participants presented an engraved plaque to the “Father of Microbiological Safety” he was speechless. The plaque hung in his office until his death. When closing the 18th Biological Safety Conference, I stated I was fortunate to study with Drs. Norman Conant, Howard Larsh, and Arnold Wedum. Dr. Wedum said he was honored to be included in this distinguished company.

In many friendly conversations after working hours or at social events, I knew Dr. Wedum as well as anyone not part of his family. We had a camaraderie that existed even after I left Fort Detrick. He was a perfectionist and many can relate to his cognizance of safety situations.

As a co-author with Dr. Wedum, I learned from his expertise. Compilation of data for “Assessment of Risk” took many long hours resulting in additional information that was used in reports. His concern, not only of respiratory infection and aerobiological challenges, resulted in the completion of “Cross Infection with Eighteen Pathogens in Animals.” At the 18 Safety Conference, Dr. Wedum presented “History of Microbiological Safety.” Emmett has a copy and I believe it should be published in JABSA for it is a classic.
LESSONS LEARNED

The power of safety!

There once was a laboratory, like many laboratories, where good science was done by good people in an overcrowded, messy laboratory. These workers were so busy that they never had time to clean up, particularly the hallways which were filled with carts, boxes, and miscellaneous “good stuff.” The lab chief was always reminding the workers that all of the “stuff” was supposed to be on one side of the hallway so there would be a clear path, but nothing ever seemed to change. One day at a lab meeting (which took place in the hallway) the lights were turned off, a worker was blindfolded and told that there was a fire in the building and the hallway was filled with smoke. All the worker had to do to get to safety was to crawl down the hallway to the exit door. Unfortunately the worker could not quickly navigate the hallway and “died.”

Nothing else was said, but the hallway was cleared in less than one hour!

Lessons learned:
1. Safety has to be personal and important to the individual.
2. There are clever ways to remind workers that their rules which they may think are unnecessary.

Linda Martin, Associate Editor

The power of group persuasion!

Once there was a laboratory that handled quite a lot of human blood. Although the institution offered hepatitis B vaccination every year as part of the adult immunization program, few of the workers in the lab had received the vaccine. The lab director, who was concerned about the lack of immunization, decided to try something new. He asked one of the more energetic lab workers to schedule everyone for immunization and to personally escort them to the clinic. The workers who agreed to receive the vaccine drew straws to determine who would go first for the “shot.” The coordinator also reminded everyone when it was time for the booster shots. The percentage immunized workers increased from about 30% to 90%.

Lessons learned: Non-compliance and compliancy may be changed by experimenting with new approaches to solve the problem. Involvement of the workers in the solution is key to success.

Linda Martin, Associate Editor
A HISTORY OF THE AMERICAN BIOLOGICAL SAFETY ASSOCIATION PART I: 
THE FIRST TEN BIOLOGICAL SAFETY CONFERENCES 1955-1965

Manuel S. Barbeito* and Richard H. Kruse**

*USDA, Retired, Past President of ABSA, Frederick, Maryland
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On April 18, 1955 fourteen representatives from Camp Detrick, Pine Bluff Arsenal, Arkansas (PBA), and Dugway Proving Grounds, Utah (DPG) met together at Camp Detrick in Frederick, Maryland. The purpose of the meeting was to share knowledge and experiences regarding biosafety, chemical, radiological, and industrial safety issues that were common to the operations at the three principal biological warfare (BW) laboratories of the U.S. Army: the Biological Research Laboratories at Camp Detrick; the Biological Production and Development Laboratories at PBA; and the Biological Assessment Laboratories at DPG. This meeting was the First Biological Safety Conference.

The catalyst for a conference was the synergism that was typically present in all weekly staff meetings conducted by Dr. Arnold G. Wedum, Director of Industrial Health and Safety, U.S. Army Biological Research Laboratories, Camp Detrick. At one such meeting in 1954, while staff were discussing how to manage better the extensive correspondence and telephone conversations on safety issues among the three BW laboratories, the idea of a conference emerged as a collective thought. The idea was quickly ratified by the safety directors at the three BW installations as the perfect vehicle for sharing safety information. Due to the nature of the work conducted at the BW laboratories, papers presented at the conference would have to be cleared in advance by security officers and attendance would be restricted to persons with secret clearances. These restrictions were considered at the time a small burden in contrast to the enormous benefit that would be gained from sharing common experiences and collaboratively resolving important safety problems.

IN THE BEGINNING: 1955-1956

1st Biological Safety Conference

Dr. Wedum opened the First Biological Safety Conference with the keynote address “The Role of Safety in the Biological Warfare Effort.” Several papers were presented by Camp Detrick safety personnel. Everett Hanel, Jr. described the work and organization of the Agent Control Branch, one of two Branches that constituted the Industrial Health and Safety Division. The Branch was divided into sections which were organized to support the six principal functions at the Biological Research Laboratories: decontamination, pilot plant, aerobiology, engineering, research and training. There were 25 to 30 individuals on the staff of the Industrial Health and Safety Division, making it the largest biological safety organization ever assembled.
Orin T. Miller discussed bacteriological cabinet systems with special reference to Class III cabinetry. The Blickman Company had installed a Class III cabinet system in Building 550 which was to be the "Cabinet of the Future." This innovative design was developed jointly by safety and engineering personnel at Camp Detrick and equipment engineers at the Blickman Company. The Class III cabinet system had two levels. The first level contained a bottom-mounted freezer, a back-mounted refrigerator, back-and bottom-mounted incubators, animal holding space, two double-door autoclaves, and an area where two people could work facing each other. The second level provided additional animal holding space. The first and second levels were connected with service elevators. Air entering the Class III cabinet system was filtered and the exhaust air was both filtered and incinerated. Panels, windows, and adjacent cabinets were joined with interstitial neoprene gaskets and secured with stainless steel bolts and nuts. The Class III system had to meet a standard of leak tightness which was determined by demonstrating no leakage greater than $1 \times 10^{-8}$ cc per second using a calibrated halide detector when the system was pressurized to 3 in wg using 1% dichlorodifluoromethane gas. Problems with the cabinet system included difficulty in obtaining leak tightness, formation of moisture in the bottom of the incubators, and the long time required to pass materials and animals between the two animal levels.

G. Briggs Phillips made a presentation on aerobiological safety where he discussed containment problems associated with the million-liter test tank, known locally as the "8-Ball." He emphasized the particularly troublesome problems caused by leaking valves and pinhole air leaks in the welds of the 1.5 in mild steel panels. He also spoke about the methods used to aerosolize test microorganisms and the importance of complete decontamination. One of us (R.H.K.) discussed safety problems in a production area. The production steps were described by following the growth of a BW agent from a beginning in a test tube, through sequential transfers to larger flasks, and ultimately to a 10,000 liter vessel where the microorganism was then harvested and concentrated by centrifugation. Before work with infectious microorganisms could begin, the complete procedure was thoroughly reviewed, the systems were pressurized and tested for gas leaks, and the procedure was performed numerous times with non-virulent simulants like *Serratia marcescens* and *Bacillus subtilis* subsp. *niger* (BG). Environmental monitoring was conducted using sieve and slit samplers. Other papers presented by Camp Detrick personnel were: "Occupational Illnesses at Camp Detrick" by Dr. Wedum; "Operation of Sewer Sterilization Plants" by Gardner G. Gremillion; "Recent Safety Developments and Problems" by G. Briggs Phillips; "Laboratory Hazards" and "The BW Safety Orientation Program" by Dr. Morton Reitman.

Dr. Howard Moorman, who was the safety director at PBA, discussed the safety organization at PBA and described its functions at the Biological Production and Development Laboratories. Mr. Roger Lerwell, the safety director at DPG, discussed test vehicle decontamination. A presentation titled, "Off-Post Safety Considerations for BW" was made by CDR Robert Holdenreid, the U.S. Public Health Service liaison officer at DPG. In addition, there were informal round table discussions concerning ethylene oxide and formaldehyde decontamination, air sampling techniques, and the use of rubber gloves with Class I and Class III safety cabinets. It was noted that these gloves had a propensity to leak around the area adjacent to the hand rolled
bead. There was much concern about this problem because the leaks were difficult to locate and repair in spite of the dielectric test performed on each glove during fabrication.

Back then...

There was no formal program for the first biological safety conference, but an agenda had been prepared. In typical Army style, a "happy hour" and dinner was held at the Officer’s Club. The meeting participants enjoyed the camaraderie of visiting with colleagues and the informal setting that was so suitable for continuing discussions of important issues. Thus traditions were set at this first biological safety conference for a conference with keynote address, a reception, a banquet and a style that encouraged informal discussions among participants.

2nd Biological Safety Conference

The second biological safety conference was held November 14-17, 1955 at Pine Bluff Arsenal. Personnel from the U.S. Army Biological Production and Development Laboratories (PDL) introduced the conference participants to the scope of safety activities they performed at the PBA laboratories. Dr. George Connell discussed the purpose of the laboratories and then reviewed the ecology in the Pine Bluff area. Victor Jones reviewed surface and air sampling methods and results. Charles Kambar discussed the history of biological safety at the laboratories and gave a technical presentation on the neutralization of quaternary ammonium compounds. Thomas McNeilly reviewed industrial safety issues at PBA.

Camp Detrick personnel presented the following papers: “Camp Detrick’s Experiences with Agents Introduced at PDL” by Everett Hanel, Jr.; “Development of Bacterial Production Facilities, from 1943 Black Maria* to 1955 at Camp Detrick” by Charles Glick; “Detection of Organisms Recovered During Production of a Biological Warfare Agent at Camp Detrick” by one of us (R.H.K.) Everett Hanel, Jr., Charles Glick, and R.H.K. also presented an exhibit and roundtable discussion on recently developed vinyl plastic ventilated suits and plastic safety items. From DPG, Roger Lerwell reviewed the occupational illnesses that had occurred at the Biological Assessment Laboratories, and CDR Holdenreid showed a movie made under contract by the University of Utah on the ecology and epidemiology of wildlife of the area.

PBA staff lead extensive tours of the production facilities and development laboratories. The tours focused on general safety problems in operating production facilities.

3rd Biological Safety Conference

The third biological safety conference was held June 18-20, 1956 at Dugway Proving Grounds. The majority of papers were given by staff from DPG and emphasized the types of

*Black Maria was the first BW research laboratory facility built at Camp Detrick. It was a wooden rectangular structure covered in tar paper. Access was controlled by a security perimeter fence with attached tower where soldiers equipped with Thompson submachine guns maintained constant surveillance.
studies carried out at the Biological Assessment Laboratories. Roger Lerwell reviewed the safety program at DPG. L. T. Christian and P. W. Williams gave a presentation on BW aspects of field tests and range controls. Dr. J. Osborne gave a talk on epidemiological aspects of BW safety. He reported that coyotes (Canis latrans) were very resistant to anthrax, but would harbor Francisella tularensis, the etiological agent of tularemia, for at least 80 days without apparent illness. The common rabbit tick (Haemaphysalis leporispalustris) could carry and transmit tularemia, and Fr. Tularensis could pass through at least one molt. The majority of rodents were susceptible to tularemia and were carriers; one exception was the kangaroo rat (Dipodomys merriami). Sheep in the Dugway area had antibodies to brucellosis and tularemia. Q fever was uncommon among sheep but found in cattle. Anthrax was found on rare occasions in cattle of Utah. A. E. Western presented data that B. anthracis spores would not kill animals after 21 days in dry soil. However, in moist soil, such as the salt flats, survival persisted beyond three years. He postulated that salts and other chemicals in the soil enhanced the infectivity of Bacillus anthracis spores.

Dr. A. Andersen, also from DPG, described his first attempt to make an air sampler that could be used to determine the number of microbial particles and the particle size of recovered microorganisms. He combined the Fort Detrick sieve sampler into a stacked array. Each stage had a perforated plate with holes of constant size. The hole diameter varied with each stage making it possible to both count and size microbial particles. The Andersen Sampler is used worldwide to this day as a reference sampler.

Dr. J. C. Spendlove reported on the outbreak of psittacosis in employees at a rendering plant in Oregon which resulted in two deaths and 25 persons with elevated titters for psittacosis among the 30 exposed employees. S. marcescens and BG were used as tracer organisms to evaluate whether routine work practices were generating infectious aerosols in the rendering plant. A large number of microorganisms were recovered on agar plates placed 100 feet downwind from the rendering building.

Presentations by Camp Detrick safety personnel included: “Rubber Glove Specifications and Procurement” and “Disinfectant Studies, Ethylene Oxide and Formaldehyde” by Everett Hanel, Jr.; “Ultraviolet Airlocks” and “Ventilated Animal Cage Tops” by Elliott Purlson; and “Animal Cross Infection Studies” by George Bodmer.

Back then...

The tours were very interesting and to many beyond belief. To the first time visitor Dugway was like the end of the world. In the east distance was measured in blocks, but at Dugway it was miles.

THE TRANSITION YEARS: 1957-1963

The biological safety conferences during this period continued to be sponsored by and held at the three collaborating U.S. Army BW laboratories. The conference agendas, however, were planned to include non-classified sessions to enable the broader sharing of biological safety
information with safety personnel not associated with the U.S. Army BW programs. Dr. James F. Sullivan, Safety Director at the USDA National Animal Disease Laboratory (NADL) at Ames, Iowa, who attended the fourth biological safety conference, was the first person to participate in a non-classified session. Safety representatives from USDA laboratories attended all conferences held during these transition years.

4th Biological Safety Conference

The fourth biological safety conference was held April 24-26, 1957 at Fort Detrick. Camp Detrick had been renamed Fort Detrick on February 3, 1956. Dr. Waldemar Kirchheimer, Assistant Director of Industrial Health and Safety at Fort Detrick, discussed a collaborative study between New York University and Fort Detrick to use statistical methods to elucidate the causes of laboratory-acquired illnesses and accidents. The results corroborated prior Fort Detrick analyses; there were insufficient data to identify specific causes. The inability to determine the main causes, in spite of excellent cooperation among the staff and the full knowledge that adverse action would never be taken against individuals who would report exposures, was puzzling. Dr. Kirchheimer also informed the participants that negotiations between NYU and Fort Detrick were underway to develop a three week graduate level safety program for all military and civilian supervisory safety personnel. It was expected to start in a year.

Robert Alg reported on a glass Petri plate dropping accident that resulted in two clinical and three subclinical infections with tularemia. A study of this incident changed the type of laboratory glassware used at Fort Detrick. The accident was reenacted (Alg and on of us [M.S.B.J]) by dropping 20 glass Petri dishes containing agar inoculated with S. marcescens. Air samplers recovered the test organism 70 feet away in a hallway downstream. Secondary aerosols were produced when the accident area containing the broken glass plates with agar colonies was sprayed with a quaternary ammonium compound. Comparison studies of glass and plastic plates demonstrated that dropped plastic plates produced less aerosol. Fort Detrick adopted the use of plastic laboratory items and used disinfectant-soaked toweling and flooding action to clean up microbial accidents to reduce the potential for laboratory-acquired illnesses.

5th Biological Safety Conference

The fifth biological safety conference was held at Pine Bluff Arsenal April 21-23, 1958. This was the first year the USDA, Plum Island Animal Disease Laboratory was represented at the conference. It was also the first conference where Charles Glick discussed shipment of biological agents and diagnostic samples.

One of us (R.H.K.) reviewed a project to isolate Mycobacterium tuberculosis during autopsies at the University of Tennessee Medical Center. Pictures that were taken during the autopsies graphically showed the methods used in the attempted isolation. Because several conference participants were absent during the scheduled presentation, Dr. Wedum asked R.H.K. to repeat the presentation after dinner. The content was lost by half the audience because they left the room before the presentation was over.
Back then…

Travel by air was one class. The American Airlines flight to Little Rock (for the PBA conference) with intermediate stops at Nashville and Memphis was full. Fortunately one of us (R.H.K.) had acquired a flare for southern hospitality through marriage. Can you imagine Dr. Wedum’s surprise to see R.H.K. and sidekick Charlie Glick wearing AA flight caps and serving drinks and meals to the passengers. Add tolerance to Dr. Wedum’s list of qualities.

6th Biological Safety Conference

The sixth biological safety conference was held September 13-16, 1960 at Fort Detrick. For the first time, brief abstracts were assembled and distributed to all attendees. Thirty-one papers were presented, and there were tours of laboratories to illustrate the latest improvements in personnel protection. The conference also closed with a “wrap-up” session to plan the site, dates and subjects for the next conference. This started the tradition for having a business meeting.

Robert Alg, now Safety Director at DPG, discussed radiological field operations. Zeniff Cox briefed the attendees on safe field test procedures for chemical and biological warfare operations, a challenging task in the absence of accepted operational standards. Morris Levins reviewed biological operations at DPG’s Baker Laboratory in support of field operations. Dr. Jack Palmer discussed wildlife studies and general ecology at Dugway, and raised concern about the impact of an accidental release from the laboratory.

Dr. Moorman reviewed agent illnesses, the complexity of safety operations at PBA and the use of quaternary ammonium compounds for inactivating production agents. Victor Jones reviewed agent surveillance and monitoring techniques, isolation of low levels of agents from blood samples; detection of Fr. tularensis from laboratory surfaces, and airflow requirements for Class III safety cabinets. Charles Kambar described the development of media for propagation of agents in production quantities and methods used to decontaminate containers after filling with BW agents. He evaluated detection media use for rapid identification of BW agents by medical microbiologists.

Safety personnel at Fort Detrick covered a wide range of subject matter in 13 presentations. Everett Hanel reviewed activities to improve operations by: (1) increasing emphasis on the supervisor’s role and responsibility; (2) increasing cooperation with outside agencies and individuals; and (3) diversifying and sharing biological safety data with new Department of Defense organizations, universities, research organizations and private companies; and (4) increasing use of biological safety cabinets. A major breakthrough was reported. The significant reduction in laboratory-acquired illnesses at the Biological Research Laboratories was attributed to improved experimental vaccines.

Briggs Phillips reported on his visits to 111 laboratories in 60 cities in 18 countries in Asia, Australia, Europe, and the United States. Numerous slides of the laboratories and equipment showed that microbiological safety practices in foreign countries were below American standards. Elliott Purlson reviewed the development and use of ventilated personnel suits for
handling experimental animals exposed to aerosolized pathogenic microbes. The suit system was intended to reduce operating costs while offering maximum protection for operators compared to the use of Class III cabinet systems. Dr. Wedum analyzed rules, regulations, and basic investigations by the Fort Detrick safety staff on shipment of infectious and toxic materials. These data were very important to all BW organizations as they constantly shipped etiologic agents to each other.

Kenneth Hindman, Industrial Safety Officer, reviewed and illustrated the explosive properties of dried organisms in production operations. One of us (M.S.B.) reported on the use of Beta-propiolactone (BPL) as a gaseous chemical sterilant for aerosol chambers and rooms. Charles Glick reviewed the development of airlocks, dunk tanks, and gas-tight doors for Class III Cabinets. He demonstrated methods of removing infectious microbes from Class III Cabinets. During the 1950s, Fort Detrick safety staff evaluated the aerosol hazard associated with common laboratory procedures using S. marcescens and S. indica vegetative microorganisms, and BG spores. Aerosol hazards of common mycological procedures were evaluated using the highly infectious Coccidiodes immitis. One of us (R.H.K.) was the first to demonstrate that more arthroconidia from C. immitis were liberated than the vegetative bacteria. This was an important observation for assessing fungal hazards in the laboratory.

Back then...

One of us (M.S.B.), while investigating the use of beta-propiolactone (BPL) as a gaseous chemical sterilant for aerosol chambers and rooms, found that experiments do not always work as planned. Sometimes, something very different is found. There was to be a visit of the test-tank facilities by U.S. Army top military staff. Because outside personnel would be entering the area, a one-million liter test tank and building, recently painted white, had to be decontaminated. To validate effectiveness, BG spores were placed throughout the area. BPL was aerosolized into the area using a large smoke generator. When M.S.B. and his staff entered the room the next day, much to their surprise and grief, they saw sheets of paint hanging from the test tank and air lines, and strewn across the floor. The metal was “clean as a whistle” as all the paint had been removed. And, yes, the decontamination was 100% effective.

7th Biological Safety Conference

The seventh biological safety conference was held September 12-14, 1961 at Dugway Proving Grounds. Henry Schol evaluated bacterial aerosols produced by breakage of inoculated agar plates and reported isolating production agents from blood specimens. Charles Kambar reported that improvements in Class III cabinets could be obtained by: (1) sealing the autoclave flanges to a safety cabinet with silicone rubber; (2) designing a new seal for shaker shafts exiting penetrations in the cabinet; (3) using 9-in. I.D. glove ports for 8-in. gloves; and (4) using a key stock door design. One of us (M.S.B.) described the development, installation, and use of a biological alarm system in laboratory buildings at Fort Detrick and shared the results of a study to determine when fiberglass filters required replacement. Dr. Wedum reviewed the status of: (1) packaging requirements for transportation of etiologic agents; (2) use of a live tularemia vaccine strain as a stimulant; and (3) trends of accident rates at Fort Detrick during the past decade.
Robert Alg discussed the development of immunization cards for Chemical Corps personnel, and modifications of a building adjacent to Baker laboratory for decontamination of test vehicles with steam and formaldehyde. Dr. Paul Nichols from the University of Utah described the development of a fiberglass filter mask for respiratory protection. Zeniff Cox outlined the coordination required during field trials to protect the safety of the pilots, ground crews and the community.

*Back then...*

One night a loud and strange noise outside the barracks woke most of the attendees to the DPG Conference. A herd of wild mustangs was running and grazing in the field and scratching their backs on the barracks.

**8th Biological Safety Conference**

The eighth biological safety conference was held June 11-13, 1963 at Pine Bluff Arsenal. Briggs Phillips discussed an epidemiological approach to laboratory safety using data from surveys conducted at the three BW installations, PIADL and NADL. Theron Green presented data on hazards of pathogenic fungi and the first phase of a study of disinfecting various laboratory surfaces after exposure to the parasitic phase of four fungi. One of us (M.S.B.) spoke about developing gloves for handling monkeys and a one-handed syringe manipulator to reduce self-inoculations. Also discussed were various testing methods for respiratory protective masks. Henry Schol discussed antibacterial quaternary ammonium compounds and inactivation of a toxin with alkali solutions. Paul Williams reviewed the conversion of a section of Baker laboratory (DPG) into a ventilated-suit area.

**THE NEW ORDER: 1964-65**

The ninth and tenth biological safety conferences were the vanguard conferences for growth in the number of participating organizations and for openness in sharing biological safety information and experiences. More than twice the number of federal agencies were represented at these conferences than were represented in earlier years. 1964 was the first year that the federal government’s two major health and biomedical research agencies, the Communicable Disease Center (CDC) and the National Institutes of Health (NIH), were represented. Table 1 identifies the first year each participating organization was represented at one of the first ten biological safety conferences. The marked increase in participation in 1964 and 1965 was due in large part to two significant initiatives at that time. First, the conference presentations no longer contained classified material eliminating the previous restrictions on the sharing of information. Second, there was a genuine effort within the federal government to clear previously classified biological safety studies for open literature publication. It was known that this abundance of information would be available at the conferences.
9th Biological Safety Conference

The ninth biological safety conference was held August 18-20, 1964 at the National Animal Disease Laboratory, Ames, Iowa. It was the first time the conference was held at a government installation not associated with the BW program.

Presentations by NADL personnel included Joseph Songer's report on methods to test air filter systems, Donald Braymen's description of methods used to monitor sewage decontamination, and Dr. A. C. Pier's listing of animal dermatophytes transmissible to man. From Fort Detrick, Everett Hanel discussed laboratory-acquired mycotic infections, Gardner Gremillion described development and use of animal cages, and Theron Green talked about cross-infection studies using Macaca mulatta and various pathogenic agents, LJC James Yatso described monkey tuberculosis and chimpanzee hepatitis surveillance programs, and Charles Glick discussed the use of ventilated suits. Dr. Charles W. Beard, a former Fort Detrick employee, and later at the Department of Veterinary Science, University of Wisconsin, described modifications to the Henderson Apparatus used for aerosol exposure of large and small animals.

From the PIADL, Dr. G. E. Cottrial described the use of Newcastle disease virus and Escherichia Coli T3 bacteriophage to test laboratory exhaust air filters. A highlight of the conference was data presented by Dr. Jack Hyde, Safety Officer of PIADL, who demonstrated that foot and mouth disease virus could travel considerable distances by airflows and infect steers in another area of the building. Later, it was shown that the pressure differentials between zones had to be maintained to prevent transmission.

Back then...

G. Briggs Phillips received the first Ph.D. awarded for work in microbiological safety from New York University. The title of his thesis was, "Casual Factors in Microbiological Laboratory Accidents and Infections."

10th Biological Safety Conference

The tenth biological safety conference was held September 14-16, 1965 at Plum Island Animal Disease Laboratory, Greenport, New York. The safety conference had grown to approximately 25 safety officers from a number of government installations. James Johnson, CDC, described their new Arbovirus Research Containment Laboratory. J. A. Robertson, a consultant of the National Cancer Institute, outlined the Institute's proposed biohazards containment programs. George Bodmer, a former Safety employee at Fort Detrick, and later at Robert A. Taft Sanitary Engineering Center, Public Health Service, Cincinnati, Ohio, reviewed their program of environmental health. From Fort Detrick, Dr. Wedum updated shipping regulations; one of us (R.H.K.) described new experiments on animal cross infection; one of us (M.S.B.) reviewed the collection and disposal of refuse and salvage operations at the infectious disease laboratory; Kenneth Hindman talked about the effect of military accidents on the disabling injury rate; and Dr. Briggs Phillips outlined a contribution from microbiological safety to space research.
From NADL, Joseph Songer talked about the effect of relative humidity on the survival of some airborne viruses; Dr. Sullivan discussed temperature effects in ethylene oxide sterilization of animal pathogens; and Donald Braymen described chemical disinfection of soil following contamination with aerobic and anaerobic organisms. From PBA, O. S. Robinson discussed the many problems associated with dried agents including the monitoring the waste and its effect on the Arkansas River. Dr. D. J. Giron gave a presentation on decontamination problems related to the use of infectious agents in space cabin simulators at Brooks Air Force Base, San Antonio, Texas. At this conference, a formal Planning Committee was established for the first time to plan for the next conference.

CONCLUSIONS

What started as informal discussions among three BW installations to provide insight into the many problems faced by safety officers in a day’s work, grew over ten years to include representatives from all federal agencies that sponsored and conducted research with pathogenic microorganisms. These biosafety officers were the pioneers who tested programs and conducted innovative experiments that required the foresight and wisdom of program managers who sanctioned research endeavors without an assured outcome. To be adventurous was an asset. As one reviews the list of topics covered during the first decade of the biological safety conferences, it is evident that much progress in the emerging discipline of biosafety was made. Table II lists selected publications by these early pioneers on subjects that were first presented at one of the first ten biological safety conferences. These contributions have stood the test of time because they elucidated the basic principles of our profession.

During this entire period, Dr. Arnold G. Wedum, the “Father of Microbiological Safety,” provided the wisdom and energy on which to build a new safety discipline. He was an advocate for the biological safety conferences and encouraged all biosafety professionals from the interested organizations to attend and participate in them. He understood that this conference would broaden a participant’s knowledge and establish valuable avenues of communication with knowledgeable colleagues. It was a very exciting time for biological safety officers and we are proud to have been participants. Not least because these informal meetings were the training grounds for our future leaders (see Table III), and evolved into the American Biological Safety Association.
# TABLE 1

Year Organizations Were First Represented at The First Ten Biological Safety Conferences 1955 - 1965

<table>
<thead>
<tr>
<th>Organization</th>
<th>Year First Represented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camp Detrick</td>
<td>1955</td>
</tr>
<tr>
<td>Dugway Proving Ground</td>
<td>1955</td>
</tr>
<tr>
<td>Pine Bluff Arsenal</td>
<td>1955</td>
</tr>
<tr>
<td>U. S. Department of Agriculture</td>
<td>1955</td>
</tr>
<tr>
<td>U. S. Public Health Service</td>
<td>1955</td>
</tr>
<tr>
<td>National Animal Disease Laboratory</td>
<td>1957</td>
</tr>
<tr>
<td>Plum Island Animal Disease Laboratory</td>
<td>1958</td>
</tr>
<tr>
<td>University of Utah</td>
<td>1961</td>
</tr>
<tr>
<td>Communicable Disease Center</td>
<td>1964</td>
</tr>
<tr>
<td>National Institutes of Health</td>
<td>1964</td>
</tr>
<tr>
<td>Naval Biological Laboratory</td>
<td>1964</td>
</tr>
<tr>
<td>Naval Weapons Laboratory</td>
<td>1964</td>
</tr>
<tr>
<td>Robert A. Taft Sanitary Engineering Center</td>
<td>1964</td>
</tr>
<tr>
<td>U. S. Air Force School of Aerospace Medicine</td>
<td>1964</td>
</tr>
<tr>
<td>U. S. Army Desert Test Center</td>
<td>1964</td>
</tr>
<tr>
<td>U. S. Army Materiel Command</td>
<td>1964</td>
</tr>
<tr>
<td>U. S. Army Test and Evaluation Command</td>
<td>1964</td>
</tr>
<tr>
<td>University of Wisconsin</td>
<td>1964</td>
</tr>
<tr>
<td>National Cancer Institute</td>
<td>1965</td>
</tr>
</tbody>
</table>
TABLE 2

Selected Publications By Participants at
The First Ten Biological Safety Conferences
1955 - 1965


### TABLE 3

ABSA Presidents Who Participated in One or More of The First Ten Biological Safety Conferences 1955 - 1965

<table>
<thead>
<tr>
<th>ABSA President</th>
<th>Year President</th>
<th>Year First Participated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Everett Hanel</td>
<td>1983 - 1985</td>
<td>1955</td>
</tr>
<tr>
<td>Jerry Tulis</td>
<td>1985 - 1986</td>
<td>1960</td>
</tr>
<tr>
<td>Joseph Songer</td>
<td>1988 - 1989</td>
<td>1964</td>
</tr>
<tr>
<td>Manuel Barbeito</td>
<td>1994 - 1995</td>
<td>1957</td>
</tr>
</tbody>
</table>
AEROSOL INFECTION OF MICE WITH MYCOBACTERIA USING A NOSE-ONLY EXPOSURE DEVICE

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ABSTRACT

The effectiveness of an aerosol producing system (In-Tox Products, Albuquerque, NM) commonly used for toxicological studies of chemicals and radioisotopes was evaluated for experimental tuberculosis infection of mice. Previously, experimental tuberculosis of rodents has been accomplished by aerosol infection devices which exposed the entire animal to the aerosol within a chamber. We utilized the In-Tox system to infect animals via the respiratory route with Mycobacterium bovis strain Bacille Calmette-Guerin (BCG) as a model for experimental pulmonary tuberculosis. The advantage of the device tested is that exposure to the infectious aerosol is limited to the nose of the animal. The present instrument was found to yield good implantation of mycobacteria into the lungs. The infectious load was reproducible and, with the addition of some filtration, the infectious agent was well contained within the device. Our results showed that this device is easy to operate, produces an effective experimental aerosol infection and is safe for the investigator.

INTRODUCTION

Despite the decline of tuberculosis in the developed world throughout the 20th century, the disease remains an enormous global health problem. While the incidence of tuberculosis in the United States is approximately 9 cases per 100,000 per year, in Asia and Africa the rates approach 110-165 cases per 100,000 per year (Leonard 1990). Tuberculosis is now the world's foremost cause of death from a single infectious agent (Snider 1994). HIV infection, which renders individuals significantly more susceptible to mycobacterial infection, has caused a marked increase in the incidence of tuberculosis.

In order to study the course of tuberculosis infection and the host response it is important to develop experimental animal models of pulmonary tuberculosis. It has been shown that aerosol infection of rodents results in progressive granulomatous disease and persistence of mycobacterial replication (North 1995) indicating the usefulness of this model. Aerosol systems have been previously used to induce experimental respiratory infection in animals with Bordetella pertussis (Sato 1980; Oda 1983; Shahin 1990), influenza virus (Schulman 1963; Sullivan 1976; Johansson 1991), and Mycobacterium tuberculosis (Wiegeshaus 1970; North
1995). In all these systems, the animal is placed within a chamber and is exposed to aerosolized bacteria. Following exposure to the virulent organisms the animal must be irradiated with ultraviolet light for 30 minutes to disinfect the animal’s skin and hair. Snout only or nose only aerosol delivery systems have been previously used to deliver substances such as radioactive materials (Raabe 1973) or pesticides (Newhouse 1978; Ferguson 1982). It has been reported that *Legionella pneumophila* was induced in guinea pigs using an inhalation system that limited delivery of the aerosol to the snout (Davis 1982). However, to date, nose only delivery systems have not been used to induce experimental tuberculosis.

We report here the use of a modified device for aerosol infection of mice (In-Tox Products, Albuquerque, NM), which differs from other previously described devices (Wells 1940, Lurie 1950, Middlebrook 1952). The system tested here is a negative pressure system that exposes only the nose of the animal to the infectious aerosol ensuring a pulmonary infection and reducing the potential spread of infectious organisms.

A protocol for the use of the system was developed and tested by infecting mice via the respiratory route with avirulent *Mycobacterium bovis* strain Bacille Calmette-Guerin (BCG). Once a satisfactory protocol was established, *Mycobacterium tuberculosis* strain Erdman, an infectious virulent strain, was used as a model for experimental pulmonary tuberculosis.

**PROCEDURES**

**FACILITY**

The work was carried out at an animal biosafety level 3 (ABSL3) facility equipped with hoods in which animal cages were placed. Following the CDC recommendations for handling cultures with *M. tuberculosis* (CDC/NIH 1993), a Class II, Type A/B3 biological safety cabinet, Model SG 400 (Baker Co., Sanford, Maine) was used as a Type A cabinet in the ABSL3 facility. Ventilation studies of the animal room using a theatrical fog machine (Party 1996) showed that the fog was exhausted through the animal hoods. These hoods had face velocities of about 40 feet per minute (fpm).

**AEROSOL SYSTEM**

The aerosol producing system consisted of a compressed air nebulizer (Lovelace Nebulizer Model No. 01-100, Style L009, In-Tox Products, Albuquerque, NM), a nose-only exposure chamber, a control panel, a vacuum pump and vacuum surge tank with controls, and a clean compressed air surge tank with controls. In-line 0.2 μm hydrophobic membrane filters (Gelman, Acro 50) were added to the connecting tubes to protect the control box from microbial contamination (FIG 1). The acrylic nebulizer, operated by compressed air, generated a high concentration of aerosol droplets.
FIG 1 Nose only aerosol exposure system. Control box components: a) chamber flow rotameter; b) filter flow rotameter; c) impact flow rotameter; d) nebulizer air rotameter; e) dilution air rotameter; f) pressure chamber (magnehelic gauge); g) filter pressure (magnehelic gauge); h) impactor pressure (magnehelic gauge); i) nebulizer air connection; j) air pressure gauge; k) dilution air connection; l) nebulizer air connection; m) impactor connection (not in use); n) filter connection (not in use); o) chamber exhaust connection.
The nose-only exposure chamber can be customized to accommodate animals of different sizes. The chamber used here for mice was a 24-port single-sided chamber made of anodized aluminum with polycarbonate tubes. All inlets and outlets were made of brass outfitted with o-rings to provide tight seals. A polyvinylchloride (PVC) plunger was also used (FIGS 1 and 2) to ensure that the animals were maintained in the appropriate position. The chamber was designed to operate at a slightly negative pressure, drawing the aerosol past the animal’s nose. The aerosol was brought into the chamber by top entry and delivered in front of the animals through a small orifice in the nose cone. The aerosol was drawn past the animal’s nose and then pulled into the exhaust tube through a series of holes around the nose cone. The chamber has a rectangular distribution, top to bottom and left to right. It was not necessary to have all 24 animals in place for an experiment. If fewer animals were needed the unused ports were plugged with aerosol inlet caps and animal tube port plugs.

The control box allowed the adjustment of the nebulizer, dilution and chamber air flow. A tube connecting the chamber to a magnetohelic gauge on the control panel enabled monitoring of chamber conditions during exposure. The impactor pressure and the vacuum and air pressure could also be monitored (FIG 1).

The nebulizer and nose-only chamber, which are both relatively small and compact, were placed inside a biological safety cabinet. The control panel was placed under the biological safety cabinet, on a low platform on wheels. The air and vacuum tanks and pump were placed away from the biological safety cabinet. Sound-proofing, if feasible, is recommended to reduce the noise caused by the pump.

FIG 2 Cross section of aerosol chamber and air flow pattern.
WORKING PROTOCOL

A working protocol for the infection of mice via the respiratory route was designed and evaluated using *M. bovis* strain BCG. Personnel protective equipment was utilized for all work with the aerosol infection system. Tyvek™ coveralls and two pairs of latex gloves were always used. For face and respiratory protection either a positive air pressure respirator (PAPR) (Racal Breathe-Easy 12) or a N95 disposable respirator and safety glasses were used.

1. Preparation Nebulizer Suspension

*M. bovis* strain BCG, stored at -70 C was thawed, bath sonicated for 10 s to produce a single cell suspension, and diluted with Proskauer-Beck medium (Difco Laboratories, Detroit, MI) containing 0.01% Tween 80. The bacterial suspension contained 0.5-1.5x10⁷ colony forming units (CFU) per mL. Mycobacteria are rod shaped organisms 4-5 microns x 1 micron in size. The nebulizer was adjusted according to the manufacturer’s specifications to generate a droplet size of 5-10 microns. In this way, mycobacteria remained as single infectious particles and did not clump. Droplet size was kept constant so that there was 1-5 mycobacteria per droplet. According to the manufacturer’s specification, the nebulizer was thus providing 50-80 µL /min of very fine mist.

2. System Settings

System settings were used according the manufacturer’s specific guidelines to achieve desired aerosol droplet size and uniform exposure of mice. A 20-25 g mouse requires 25 mL/min airflow. For the 24 port chamber, therefore, 0.6 L/min are needed. As specified by the manufacturer, impactor air flow requires 1 L/min and filter flow requires 5 L/min. Since there are 2 filters, filter flow required for this system is 10 L/min. Thus, a minimum flow of 11.6 L/min is required. Nebulizer output is set to 1.6 L/min, so the total chamber flow should minimally be 13.2 L/min. The aerosol system was operated at a total chamber flow rate of 15-18 L/min, a slightly higher airflow than needed. Negative pressure in the system was maintained during the entire course of the experiment.

3. Placement of Animals

Each mouse was carefully inserted into an animal tube with the nose pointing to the aerosol outlet. The animal tubes are specifically designed to contain one mouse per tube. The tube are 86 mm long and have an inner diameter of 30 mm. Using the plunger in the tube the mouse was gently immobilized in the correct position. This step required experimentation to determine adequate immobilization. If the mouse was too closely confined in the holder tube it would die during the course of the experiment. A mouse that was too loose within the holder tube could turn around several times during the exposure, receiving a smaller infective dose of mycobacteria and potentially exposing the hair to bacterial contamination. Different strains of mice exhibited different behavior within the tube, necessitating careful adjustment of the plunger for immobilization.
4. Exposure to Aerosol

Exposure was carried out within the biological safety cabinet. The holder tubes with animals in place were inserted into the chamber while fresh air was circulating in the system. The nebulizer was turned on for 5 min to allow the bacterial aerosol to equilibrate in the chamber. The mice were then exposed to the aerosol for an additional 20 min. Next, dilution air was run for 5 min after exposure to flush the system. At the end of this 30 minute exposure period, the air system was turned off, holder tubes were removed from the chamber and the animals were placed back into their cages.

5. Disinfection of System

After removing the animal tubes, the chamber openings were plugged and a solution of 70% ethanol was run through the nebulizer for 10-15 min. The tubes and nebulizer were disinfected by dipping for 3 hr in a bath containing a solution of IODEX AR/18 (Quip Laboratories, Inc., Wilmington, DE) following manufacturer's instructions. The chamber side ports were opened, the internal chamber pipes were cleaned with the aid of very fine bottle brushes, and the chamber was rinsed with the IODEX disinfecting solution followed by 10% chlorine bleach (Clorox) internally and externally. The entire system was then rinsed extensively with water and allowed to air dry. After drying the system had to be carefully reassembled to prevent leaks.

RESULTS

AEROSOL INFECTION OF MICE

Separate experiments were performed to establish the working protocol and to evaluate the reliability of the system. To vary the initial infecting dose, bacterial suspensions were diluted to a concentration of $10^5 - 1.5 \times 10^7$ CFU/mL. The aerosol exposure time of each experiment was kept constant at 20 min. Three hours after the experiment, mice were sacrificed to evaluate the effectiveness of the aerosol infection. Lungs (without the large airways) were homogenized in phosphate buffered saline (PBS) containing 0.05% Tween 80 and plated on 7H10 agar (Difco Laboratories, Detroit, MI). Colonies were counted after 15 days incubation at 37 C.

All procedures including sacrifice of animals, dissection and homogenization of tissues, and plating were carried out within the biological safety cabinet. Plates were incubated in an incubator within the ABSL3 facility.

Table 1 shows the results of 9 such experiments carried out with aerosolized M. bovis BCG. For each experiment littermates (of the same sex, age and size) of either B6D2F1 or Balb/c mice (Charles River Laboratories, NJ), 8-10 wk of age were used. There was no significant difference in the number of CFU among the mice placed in different sections of the chamber. Mice from the same experiment had consistent numbers of mycobacteria implanted in the lungs.
TABLE 1
Aerosol Infection of Mice

<table>
<thead>
<tr>
<th>Experiment #</th>
<th>Mouse Strain</th>
<th>Number of Mice</th>
<th>Bacterial Suspension BCG (CFU/mL)</th>
<th>Mean CFU/Mouse (both lungs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B6D2F1</td>
<td>7</td>
<td>$1.5 \times 10^7$</td>
<td>260±140</td>
</tr>
<tr>
<td>2</td>
<td>B6D2F1</td>
<td>5</td>
<td>$1.5 \times 10^7$</td>
<td>120±80</td>
</tr>
<tr>
<td>3</td>
<td>Balb/c</td>
<td>3</td>
<td>$1.5 \times 10^7$</td>
<td>340±90</td>
</tr>
<tr>
<td>4</td>
<td>Balb/c</td>
<td>6</td>
<td>$1.5 \times 10^7$</td>
<td>110±40</td>
</tr>
<tr>
<td>5</td>
<td>B6D2F1</td>
<td>6</td>
<td>$1.5 \times 10^7$</td>
<td>300±80</td>
</tr>
<tr>
<td>6</td>
<td>B6D2F1</td>
<td>7</td>
<td>$1.5 \times 10^7$</td>
<td>640±160</td>
</tr>
<tr>
<td>7</td>
<td>Balb/c</td>
<td>8</td>
<td>$7 \times 10^6$</td>
<td>320±80</td>
</tr>
<tr>
<td>8</td>
<td>B6D2F1</td>
<td>6</td>
<td>$7 \times 10^6$</td>
<td>370±150</td>
</tr>
<tr>
<td>9</td>
<td>B6D2F1</td>
<td>5</td>
<td>$7 \times 10^6$</td>
<td>240±130</td>
</tr>
</tbody>
</table>

To determine the extent and uniformity of the pulmonary pathology induced by infection with the aerosol device, animals were sacrificed 40 or 50 days following the infection and lungs were fixed in 10% neutral buffered formalin (Fisher). Histological examination of the lungs revealed scattered granulomas composed of numerous T cells and macrophages containing intracellular acid fast bacilli (FIG 3).

FIG 3 Histological sections of the lung of a mouse infected by aerosol with 300 cfu of \textit{M. bovis} strain BCG. a) 40 days post infection; acid-fast stain of the sections revealed formation of a granuloma in the lung parenchyma (circle). The granuloma is surrounded by lung tissue which is partially infiltrated by inflammatory cells, occluding the air spaces (magnification x 10). b) 50 days post infection; acid-fast stain. Most of the lung parenchyma is occupied by a large granuloma with numerous lymphocytes (Ly) with dark staining nuclei and macrophages with lighter staining cytoplasm (magnification x 10). c) 50 days post infection; acid-fast stain. Acid fast bacilli are seen (arrows) within the cytoplasm of macrophages. A cuff of T lymphocytes with dark staining nuclei is seen at the bottom left of the micrograph (magnification x 60).
CONTAINMENT AND STERILITY TESTS

As provided by the manufacturer, the control box and the compressed air surge tank appeared vulnerable to microbiological contamination. Therefore inline filtration was added as described above. To function properly the animal exposure device must be leak-free. To verify that the instrument did not leak into the surrounding environment, the aerosol system was submitted to two tests: a pressure test and a microbiological test.

Pressure

Five empty animal tubes were placed in the upper row of the chamber. All other chamber openings were plugged using the aerosol inlet caps and port plugs. The chamber exhaust was connected to the vacuum source through a line with a needle valve. Vacuum was drawn into the system to -1.8 in. w.g. (-0.45 kPa) as indicated on the magnehelic gauge. The valve was closed and pressure was maintained for at least 20 min.

Since the system held a steady pressure, it was concluded that the system did not leak and the unit was properly sealed.

Microbiological Leak

All areas of the animal chamber were tested for microbial leaks using an aerosol prepared from a suspension of approximately 1.5x10⁷ CFU/mL of M. bovis strain BCG. While the instrument was in operation 7H10 agar plates (regular size, 100 mm in diameter) were placed under potentially leaky connections so that any leaking aerosol could impinge onto the surface of the agar (FIG 4). An unexposed plate was used as a negative control and 0.1 mL of the bacterial suspension was plated as a positive control. Plates were incubated for 15 days at 37 C.

FIG 4 Microbiological leak test indicating agar plate positions.
The test plates and the negative control were all bacilli free. The positive control had confluent bacillary growth. It was therefore concluded that the system did not leak.

**Secondary Containment**

To ensure that in case of a failure of the integrity of the aerosol system, the infectious microorganisms would be contained, the animal chamber and nebulizer were placed inside the Class II Type A/B3 biological safety cabinet that was used as a Type A cabinet. The performance of the cabinet with the aerosol system in place was evaluated according to the NSF 49 (NSF 1992) specifications. The smoke test was carefully observed for signs of any disturbance in the smoke pattern, changes in the split between the front and back air flow and in particular air losses out of the cabinet.

Downflow and inflow measurements were within acceptable NSF 49 ranges. Dioctylphthalate (DOP) test indicated that there were no leaks in the cabinet filters. A titanium tetrachloride smoke tube was used to test the airflow patterns. The smoke test indicated that the nose-only chamber and nebulizer units placed inside the cabinet did not produce airflow disturbances that affected the performance of the cabinet. All the smoke was contained within the cabinet and the airflow patterns were normal.

**Aerosol Distribution**

To determine whether the aerosol was spread equally to all 24 tubes, we evaluated the seeding of aerosolized mycobacteria onto 7H10 agar small petri dishes (35 mm in diameter). Each dish was placed inside the conic part of the animal tubes against the aerosol opening and exposed to the aerosol containing $1.5 \times 10^7$ mycobacteria, for 20 min. The same tubes were used for either animal exposure or exposure of the petri dishes. After 15 days of incubation at 37 C, all petri dishes were shown to have similar numbers of mycobacterial colonies (380 CFU ± 112/dish). These results indicate that there is an equal distribution of aerosolized mycobacteria within the chamber.

**External Contamination**

Animal. Following completion of the aerosol infection, those mice which had been turning in the tube during the experiment were withdrawn, and the fur and tail were touched to 7H10 plates. Following incubation, no colonies were observed indicating that despite their movement the mice were not contaminated by the aerosol.

Investigator. Similarly, at the end of each experiment the gloved fingers of the investigators that handled the mice were touched to 7H10 plates. No colonies developed after the incubation.
CONCLUSIONS

Previously aerosol infection has been carried out using a device which exposed the hair and skin of the animal and potentially the investigator to bacterial contamination. We therefore modified and evaluated the InTox aerosol producing device for use as a respiratory infection system for experimental animals.

Since the system is intended for use with infectious, virulent microorganisms it is important to establish the effective containment of the organisms within the biological safety cabinet. Even with the aerosol system in place, the cabinet performed according to NSF Standard 49 (NSF 1992) specifications and all smoke was contained within the cabinet. Within the cabinet, the system maintained a steady air pressure without any air leaks. During operation with an aerosolized bacterial suspension, the animal chamber was tested for leaks of microorganisms. No bacilli were detected. Moreover, the fur and tail of the mice were uncontaminated following exposure to the aerosol obviating the need for UV irradiation of the experimental animals which is necessary in some other systems.

We then established the protocol for aerosol infection of mice using a nonpathogenic strain of mycobacteria, M. bovis BCG. The aerosol generated by the nebulizer contained single microorganisms or small numbers of bacilli. Following infection, mice from the same experiment had similar numbers of mycobacteria implanted in the lungs (Table 1). The number of mycobacteria implanted in the lungs was independent of either the mouse strain or the number of mice infected per experiment. The absolute number of CFU in both lungs of infected mice differed from experiment to experiment within the acceptable variation for microbiological systems. Histologic examination of the lungs of infected mice revealed pathology similar to that obtained using conventional aerosol infection systems (North 1995). After 50 days, granulomas containing intracellular bacilli were observed in the lungs of all infected mice confirming the potential usefulness of this system in studies of pulmonary tuberculosis (FIG 3).

The aerosol infection system described here proved easy to operate, eliminated the need for irradiation of the experimental animal and provided an adequate safety barrier for the investigator. Our results indicate that this device can be successfully used for respiratory infection of mice with aerosols containing mycobacteria as an experimental model for human pulmonary tuberculosis.

ACKNOWLEDGMENTS

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REFERENCES


BACTERIAL SURVIVAL ON RESPIRATOR FILTERS AND SURGICAL MASKS

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ABSTRACT

The Centers for Disease Control and Prevention recently developed recommendations for respirators worn to minimize exposures to *Mycobacterium tuberculosis*. These recommendations include criteria for filters and facial fit, but do not address how respirators are worn or disposed of. Handling may increase the risk of infection if loaded organisms remain viable.

Respirator filters were challenged with aerosols containing three bacteria: *Mycobacterium abscessus, Staphylococcus epidermidis, and Bacillus subtilis* subsp. *niger*. Elution and surface contact sampling were used to recover organisms from filters after loading and following 5 days storage at 85% RH.

Almost all filters showed culturable organisms immediately after loading. After storage, filters showed the least culturability for *M. abscessus* (20% of all filters) followed by *S. epidermidis* (61%), while *B. subtilis* remained highly culturable (98%). A similar trend was seen for the number of organisms recovered before and after storage (*M. abscessus* demonstrated the least survival and *B. subtilis* the most).

INTRODUCTION

Current certification and use policies for respirators in environments containing non-biological aerosols do not address exposure to contaminants once captured by filters, because there is no evidence that they pose a further risk to the respirator user. However, this may not be true when the contaminant includes viable cells. The potential for captured pathogens to survive and replicate on filter media must be considered.

This research evaluated the long-term culturability of three species of bacteria following capture on respirator and surgical mask filter media. Culturable bacteria were recovered from filters at two separate times: 1) immediately following loading and 2) after five days of storage at 85% relative humidity. Two assessment methods were employed. In the first, bacteria were eluted from throughout the filter; the second involved agar-contact surface sampling of both the front and back of the filter. Organism release from filters was not evaluated in these experiments.

Surgical masks have traditionally been used in the health care setting to protect the patient from infectious particles expelled by the worker (Davis, 1991). However, these masks, which do not fit tightly to the face, will not protect the wearer from inhaling airborne particles. The growing interest in the protection of health care workers from infectious aerosols has resulted in the use of respirators in medical settings. Further impetus comes from the Centers for Disease
Control and Prevention (CDC) and the Occupational Safety and Health Administration (OSHA), both of which recommend that National Institute for Occupational Safety and Health (NIOSH)-approved respirators be used to minimize exposures to Mycobacterium tuberculosis (CDC, 1994; Clark, 1993). To control costs in these facilities disposable respirators are sometimes reused numerous times until the fit is compromised (Nettleman et al., 1994; Rivera et al., 1997; Adal et al., 1994; Jackson, 1994; Clark, 1993). The consequences of storage conditions and respirator reuse have not been evaluated and the risk of exposure to organisms released from filter media has not been thoroughly addressed by CDC or OSHA.

One investigator found that storage of respirator filters in high humidity conditions resulted in heavy microbial contamination, particularly when the filter material was biodegradable by microorganisms (Pasanen et al., 1993). Two filter types were loaded in an agricultural barn and in a wastewater treatment plant and the culturable organism load was evaluated immediately following loading and after storage at 98% relative humidity (RH) for up to 35 days. Following incubation the actinomycete and other bacterial and spore concentrations were one to three orders of magnitude higher in both filters; the fungal spore concentration increased in one filter but decreased in the other. Another study investigated growth of a fungus (Stachybotrys atra corda) on respirator filters and found strong evidence of visible growth on filters consisting of 85% cellulose under conditions of 100% RH (Pasanen et al., 1994).

Infection from exposure to particles released from respirator filters has not been investigated, but the risk has been documented for surgical masks and other equipment used on infectious patients (Castle, 1980; JAMA, 1937). In one instance, anesthetic face masks worn by patients with active pulmonary tuberculosis were found to contain viable M. tuberculosis bacilli, which were shown to be infective in guinea pigs (Livingstone, 1941).

Before captured organisms can cause infection it must be assumed that there is detachment from the filter and continuing culturability. Particle detachment can occur if the adhesion forces (e.g., van der Waals, electrostatic and surface tension forces) are disrupted. This may occur due to changes in air conditions, changes in the particle, or physical agitation of the filter, resulting in the reintroduction of the particle into the airstream (Brown, 1993).

Once released from the filter, cells must remain viable to cause infection. Environmental conditions such as temperature, relative humidity and nutrient availability may either increase or decrease organism viability. With the proper environmental conditions (i.e., moisture, nutrients), viable cells may replicate and create a new reservoir. Filters are typically considered nutrient-free environments, which may prevent replication but will not lessen microbial viability. Fungi, actinomycetes, and other bacteria can utilize components of the filter media (such as cellulose) or dust and organic material captured by the filter as nutrients (Pasanen et al., 1993; Gravesen, 1979). Ventilation system filters have been suggested as potential bioaerosol sources, particularly for fungal spores (ACGIH, 1989; Darlow, 1966; Macher et al., 1995) and microbial amplification on filters has been documented in several cases (Morey, 1980).
BACTERIAL SURVIVAL ON RESPIRATOR FILTERS AND SURGICAL MASKS

This study evaluated the culturability of organisms after capture on filters. Experiments were designed to determine if bacteria could survive on filter media for a period of five days when stored at 85% RH. These conditions were selected to simulate a respirator sealed in a bag immediately following use or stored in a high humidity area such as a locker room. Since lack of moisture may result in desiccation, which can cause significant losses in bacterial viability (Cox, 1995), high relative humidity was chosen to represent a supportive storage condition.

Bacteria were recovered from filter media immediately following loading and after five days of storage using two different methods (McCullough et al., (1997a)). Both qualitative and quantitative endpoints were used to evaluate differences among filters, methods and loading conditions. The presence of viable organisms on the filter was evaluated qualitatively. The presence of even one colony forming unit (CFU) following storage indicated that there was the possibility that a cell remained viable throughout storage and therefore could potentially lead to infection. The likelihood that organisms would remain viable throughout storage was estimated by a quantitative measure of “survival,” which compared the CFUs recovered from the filter before and after storage.

METHODS

Organisms were loaded onto respirator filter media during efficiency tests which are described briefly here and in detail elsewhere (Brosseau et al., 1997; McCullough et al., (1997a); McCullough et al., (1997b)). Five types of surgical masks and eighteen types of respirator filters were challenged with three aerosolized bacteria. Filters from both disposable and reusable respirators were tested. Respirator filters were from the three NIOSH-approval categories: 1) dust/mist (DM), the least protective filters, 2) dust/fume/mist (DFM) and 3) high efficiency particulate air (HEPA), the most protective filters (Respiratory Protective Devices, 30 CFR Part 11, 1972). Surgical masks are not certified by NIOSH and were considered “not-approved” (NA) for this study.

Three bacteria (*Mycobacterium abscessus*, *Staphylococcus epidermidis* and *Bacillus subtilis* subsp. *Niger* (in spore form)) (described in Table 1) were utilized in these tests. *M. abscessus* and *S. epidermidis* were removed from agar plates and the *B. subtilis* were used as dried spores. All were added directly to filtered, deionized water and placed in a Collison nebulizer (BGI,) operated at 3 psi (McCullough et al., (1997a)). Bacteria were aerosolized to a target concentration of 1 particle/cm³ for NA, DM, and DFM filters and 2 particles/cm³ for HEPA filters. The aerosolization method was refined so that clumps were minimized; mean aerodynamic diameter indicated the majority of particles were single cells.

Organisms were loaded on respirator filters during aerosol challenge tests at two flows (45 and 85 L/min) and two conditions of relative humidity (30 and 70% RH). Three replicates were conducted on each filter for each organism at each test condition. Testing took an average of 30 minutes for the NA, DM and DFM filters and an average of 50 minutes for the HEPA filters. Greater loading occurred at the higher flow and the higher aerosol concentration. Tests were not
controlled for the number of bacteria loaded on a filter. Rather, the primary purpose of the aerosol challenge tests was to measure filter efficiency.

The efficiency of each filter was determined by measuring the concentration of particles and viable bacteria upstream and downstream of the filter. Those bacteria which did not penetrate the filter were collected by the filter media and their culturability and survival were evaluated during this research.

Two methods, elution and agar-contact surface sampling, were developed for recovering organisms from filters (McCullough et al., [1997a]). Immediately following loading the filter was cut in half, if necessary. In some cases, two separate filter cartridges were tested simultaneously. One cartridge or filter-halve was put immediately into storage while the other was kept for sampling. A 6.45 cm$^2$ piece was cut from the center of a filter-halve; a similar-sized square was cut from one quadrant of a paired filter. Elution, which involves the removal of particles attached to a substrate by transfer to a liquid, was accomplished by mixing the piece with 10 or 20 mL of a 0.05% Tween 80 solution (FischerBiotech, Pittsburgh, PA) in a 50 mL polypropylene centrifuge tube (Corning, Inc., Corning, NY). The tube was then manually shaken 200 times, after which 100 μL of liquid was plated on appropriate agar (described in Table 1). Plates were incubated and counted; results were reported as CFUs / 6.45 cm$^2$ (1 in$^2$) of filter.

Surface sampling was accomplished with the use of replicate organism detection and counting (RODAC) plates (Becton and Dickinson, Lincoln Park, NJ) which contain a 25.8 cm$^2$ (4 in$^2$) raised agar meniscus that contacts surfaces without interference from the rim of the plate (Pavero et al, 1968). Plates were used to collect organisms from the remaining half of the filter surface. (The side of the filter which is toward the ambient air will be referred to as the front of the filter and the sampling location as “RODAC-front”; conversely, the side of the filter which is toward the wearer’s face will be referred to as the back of the filter and the sampling location as “RODAC-back”.) Following sampling, plates were incubated and enumerated; results were reported as CFUs / 6.45 cm$^2$ (1 in$^2$) of filter. For RODAC plates which were overloaded a maximum value of 5000 CFUs was assigned.

The organism recovery data were evaluated with respect to two variables: “culturability” and “survival.” To qualitatively evaluate the presence of viable organisms on the filter (“culturability”) each sample was first assigned a categorical variable based upon the recovery of viable organisms (i.e. 1 = viable organisms present, 2 = no viable organisms present). The recovery of any viable organisms resulted in the assignment of “1”, regardless of the number of organisms present. A total of 4551 samples were collected. Culturability before ($C_1$) and after ($C_4$) the 5-day storage period was determined as

$$C_1 \text{ or } C_4 = (F/F_{\text{tot}}) \times 100 \tag{1}$$

where $F_1$ = number of samples with viable organisms and $F_{\text{tot}}$ = total number of samples. Additionally, “culturability” after storage ($C_4$) was statistically evaluated using categorical analysis of variance (ANOVA); predictor variables included in the statistical model are shown in Table 2. Multiple comparison tests were used to detect differences between levels of significant predictor variables ($\alpha \leq 0.05$) (Kleinbaum et al., 1988).
BACTERIAL SURVIVAL ON RESPIRATOR FILTERS AND SURGICAL MASKS

| TABLE 1  
Organism Characteristics |
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mycobacterium abscessus</strong></td>
</tr>
<tr>
<td><strong>Source</strong></td>
</tr>
<tr>
<td>CDC</td>
</tr>
<tr>
<td><strong>Size, μm</strong></td>
</tr>
<tr>
<td>length 1 - 4 width 0.3 - 0.5</td>
</tr>
<tr>
<td><strong>Stain</strong></td>
</tr>
<tr>
<td>acid-fast</td>
</tr>
<tr>
<td><strong>Biological State</strong></td>
</tr>
<tr>
<td>vegetative</td>
</tr>
<tr>
<td><strong>Resistance to Desiccation</strong></td>
</tr>
<tr>
<td>medium</td>
</tr>
<tr>
<td><strong>Agar Used in Recovery Tests</strong></td>
</tr>
<tr>
<td><strong>Incubation Temperature, C</strong></td>
</tr>
<tr>
<td><strong>Incubation Time, days</strong></td>
</tr>
</tbody>
</table>

*Runyon et al., 1974; †Baider-Parker, 1974; ‡Gibson and Gordon, 1974; §Chen et al., 1994; ¶Johnson et al., 1994; ‖Favero and Bond, 1991

The second endpoint, "survival" (S), was determined for the 1119 samples in which viable organisms were detected following storage. "Survival" is the ratio of CFUs measured before and after storage:

\[ S = \left( \frac{R_s}{R_a} \right) \times 100 \]  
(2)

where \( R_s \) = CFUs recovered after storage and \( R_a \) = CFUs recovered before storage. For those filters for which \( R_s \) was greater than zero but \( R_a \) was zero, a value of 1 CFU was assigned for \( R_a \). Logarithmic transformation was used to normalize the data. "Survival" was evaluated using ANOVA with the same initial model and statistical procedures as for C, (shown in Table 2).

| TABLE 2  
Factors Included in the Analysis of Variance |
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Factors</strong></td>
</tr>
<tr>
<td>Organism</td>
</tr>
<tr>
<td>Loading Flow, L/min</td>
</tr>
<tr>
<td>Loading Relative Humidity, %</td>
</tr>
<tr>
<td>Filter Approval</td>
</tr>
<tr>
<td>Recovery Method</td>
</tr>
<tr>
<td>Filter Model</td>
</tr>
</tbody>
</table>

*Individual manufacturers and models were nested within filter approval; the specific models tested are listed in Brosseau et al., 1997.

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RESULTS AND DISCUSSION

For pre-storage conditions, culturability (percent of filters with culturable organisms) ranged from 35 to 100% for *M. abscessus*, 50 to 100% for *S. epidermidis* and 88 to 100% for *B. subtilis*. After storage, culturability ranged from 1 to 60% for *M. abscessus*, 0 to 100% for *S. epidermidis* and 87 to 100% for *B. subtilis*. Recovery (numbers of organisms cultured from the filter) ranged from 0 to 5000 CFUs for all filters. Mean recovery before and after storage (R₀ and R₄) is shown in Table 3 for each organism and recovery method.

<table>
<thead>
<tr>
<th>TABLE 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Numbers of CFUs Recovered From Filters Before Storage (R₀) and After Storage (R₄) for All Filters at All Loading Conditions (mean and standard deviation)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td><em>M. abscessus</em></td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>SD</td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>SD</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>SD</td>
</tr>
</tbody>
</table>

Analysis of variance was used to determine those factors (Table 2) of greatest influence on culturability before and after storage (Cᵢ and C₄) and survival (S). Mean values for Cᵢ and C₄ and adjusted least squares means for S are shown in Table 4. Those factors which were significant are shown in Table 5.

<table>
<thead>
<tr>
<th>TABLE 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Culturability Before Storage (Cᵢ), Culturability After Storage (C₄) and Survival (S) for All Factors in the Statistical Model</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Factors and Levels</td>
</tr>
<tr>
<td>Organism</td>
</tr>
<tr>
<td><em>M. abscessus</em></td>
</tr>
<tr>
<td><em>S. epidermidis</em></td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
</tr>
<tr>
<td>Loading Flow</td>
</tr>
<tr>
<td>45 L/min</td>
</tr>
<tr>
<td>85 L/min</td>
</tr>
<tr>
<td>Loading RH</td>
</tr>
<tr>
<td>30% RH</td>
</tr>
<tr>
<td>70% RH</td>
</tr>
<tr>
<td>Recovery Method</td>
</tr>
<tr>
<td>Elution</td>
</tr>
<tr>
<td>RODAC-front</td>
</tr>
<tr>
<td>RODAC-back</td>
</tr>
</tbody>
</table>

*Adjusted least squares means re-transformed into the original scale, *Adjusted least squares means, *Standard errors in the least squares means scale.
Multiple comparison tests indicated that culturability after storage (Cₜ) and survival (S) were both significantly different among the three organisms. As expected, both culturability and survival were highest for filters loaded with spores of *B. subtilis*. This bacteria is the least susceptible of the three test organisms to stress and desiccation. We initially expected that Cₜ and S would be least for filters loaded with *S. epidermidis* (because this organism was thought to be the most susceptible of the three to stress and desiccation), instead, filters loaded with *M. abscessus* showed the least culturability and survival (Table 4).

<table>
<thead>
<tr>
<th>Independent Variable</th>
<th>Cₜ p-value</th>
<th>S p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>organism</td>
<td>&lt;0.0009</td>
<td>&lt;0.0009</td>
</tr>
<tr>
<td>loading flow</td>
<td>&lt;0.0009</td>
<td>0.029</td>
</tr>
<tr>
<td>loading relative humidity</td>
<td>&lt;0.0009</td>
<td>0.608</td>
</tr>
<tr>
<td>approval category</td>
<td>0.084</td>
<td>0.087</td>
</tr>
<tr>
<td>recovery method</td>
<td>&lt;0.0009</td>
<td>&lt;0.0009</td>
</tr>
<tr>
<td>model (nested in approval category)</td>
<td>NA</td>
<td>0.003</td>
</tr>
<tr>
<td>flow * loading relative humidity</td>
<td>0.690</td>
<td>0.900</td>
</tr>
<tr>
<td>organism * recovery method</td>
<td>&lt;0.0009</td>
<td>&lt;0.0009</td>
</tr>
<tr>
<td>approval category * recovery method</td>
<td>NA</td>
<td>0.009</td>
</tr>
<tr>
<td>organism*approval category</td>
<td>NA</td>
<td>0.032</td>
</tr>
<tr>
<td>organism * approval category * recovery method</td>
<td>NA</td>
<td>&lt;0.0009</td>
</tr>
<tr>
<td>organism*loading flow</td>
<td>0.002</td>
<td>NA</td>
</tr>
<tr>
<td>organism*loading relative humidity</td>
<td>0.001</td>
<td>NA</td>
</tr>
<tr>
<td>organism<em>loading flow</em>loading relative humidity</td>
<td>0.423</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA = not applicable; this variable was not included in the analysis of variance model.

There are several possible explanations for the poor recovery of *M. abscessus* cells: 1) they did not survive generation, transport to and deposition on the filter as well as the other organisms; 2) they were not recovered from the filter media as efficiently as *S. epidermidis* or *B. subtilis* (perhaps due to their hydrophobic cell wall); 3) they did not survive as well on filter media as the other two test organisms or 4) the culturing conditions (i.e., media) inhibited growth in some manner.

The ability of organisms to survive aerosolization, transport to, and deposition on the filter may have influenced the number of viable organisms loaded on the filter, and therefore the number available for survival on filter media. *B. subtilis*, in spore form, is the most likely to survive the forces associated with aerosol challenge tests, while *M. abscessus* may have lost initial culturability due to desiccation and other physical factors. As shown in Table 4, culturability of *M. abscessus* prior to storage (Cₜ) was approximately 18% less than that of *B. subtilis*, and 10% less than for *S. epidermidis*.

Survival of *B. subtilis* was much higher than expected (523%). Possible reasons for this may be: 1) more efficient recovery after storage than before or 2) organism replication during storage.
At this point, there is no evidence to support or refute either explanation; further research is needed.

Higher relative humidity during loading resulted in more filters with culturable organisms after storage. This may have been due to less organism desiccation during aerosol challenge. Higher flow during loading resulted in higher culturability after storage for all organisms, which was expected. More organisms will be captured at higher than at lower flow, if particle concentration and loading time are similar.

Culturability after storage and survival were greatest when organisms were recovered using RODAC sampling on the back of the filter (Table 4). Viable organisms on the back of the filter may be cause for concern because there may be increased potential for exposure to captured organisms. We suspect that fewer organisms were detected on the front of many filters because many of the disposable models had a relatively inefficient layer of material protecting the actual filter medium. Future experiments are needed to measure the presence and viability of organisms captured beneath this protective layer.

For all recovery methods, culturability after storage was highest for *B. subtilis*, followed by *S. epidermidis* and *M. abscessus* (Figure 1). Survival of *B. subtilis* was much higher than for either of the other organisms, using any of the recovery methods. There were no clear trends for the two recovery methods among the organisms, however. The effectiveness of each recovery method appears to differ among organisms, suggesting that preliminary evaluation of the methods used to recover a particular organism will be necessary in future experiments.

![Survival and Culturability Graph](image)

**FIG 1.** Mean Culturability (C<sub>Ca</sub>, C<sub>Cb</sub>) and Survival (S) for Three Recovery Methods (Elution, RODAC-front, RODAC-back) and Three Organisms (All Filters at All Loading Conditions)
The results shown here exhibit a great deal of variability, largely due to lack of control over important test parameters. For example, because recovery was not expected to vary among organisms, recovery methods were initially developed using only \textit{M. abscessus} at one challenge condition (45 L/min and 70% RH) on two filter types (McCullough et al., 1997a). During method development, filter type was found to influence recovery. The results shown here indicate that organism and challenge condition (in particular, relative humidity) are also significant factors in the degree of recovery from a filter.

Recovery measurements immediately followed filter efficiency tests. Organism loading was not controlled in these tests. Rather, the number of organisms loaded on a filter depended on efficiency of particle capture and the time it took to assess this efficiency. Very efficient filters (e.g., HEPA) capture more organisms than inefficient filters (e.g., NA) in the same period of time; the former require longer sampling times to assure adequate particle counts downstream of the filter. Better control of the number of organisms loaded on the filter, resulting in a similar \( R_s \) for all filters, would have reduced the variability in recovery and prevented cases where \( R_s = 0 \).

While these experiments provide information concerning organism culturability and survival over a five-day storage period at high relative humidity, they should be viewed as preliminary. Although few data are available on the nature and concentration of infectious aerosols in workplaces, the concentrations used in these experiments were probably higher than found in typical occupational settings.

**SUMMARY**

The objectives of these experiments were to determine 1) if cells are viable on filters following loading and 2) if organisms can be recovered from filters following storage for 5 days at 85% relative humidity. The results should be useful when designing future recovery and survival filter tests as well as when considering respirator handling, use and disposal procedures or policies.

Information obtained during this research can be utilized to improve future test protocols and reduce experimental variability. The experiments described here were initially designed to evaluate filter efficiency and did not control for organism loading. If tests had been designed to assure a uniform number of viable organisms on filters before storage (\( R_s > 0, C_s = 100 \)) the data would have exhibited less variability. Design of future tests can take advantage of these results when selecting conditions which assure high culturability and survival. Further experiments are needed to evaluate additional factors which enhance or hinder survival, including environmental conditions and filter type. Tests are also needed to identify recovery methods which are most effective for specific filter types and organisms.

For organisms captured by filters to cause health effects the cells must remain viable and be released from the filter. These experiments did not address the release of organisms from filters during typical use situations. Anecdotally, we found that organisms were released from filters by handling and air movement. Culturable cells were found to be present on the outside surfaces of
gloves worn while handling loaded filters. Additionally, when clean air was passed through loaded filters (reversed in the test set-up), organisms could be sampled downstream of the filter. Further research is needed to evaluate whether organisms are released, both to surfaces and into the air, under typical handling and use conditions.

We have shown that culturable cells can be recovered from filters following a five-day storage period. This suggests that policies regarding reuse, handling and disposal of respirators and surgical masks should be carefully considered. If, as these results imply, bacteria can remain viable on filters for several days, wearers should be informed of the risks of reusing respirators and surgical masks. While concentrations used in these experiments were probably higher than those found in occupational settings, wearers should be trained to recognize when exposures might require immediate disposable of respirators. For example, respirators used in the isolation room of a potentially infected patient might be worn all day, while those used during respiratory therapy or autopsy procedures on an infected patient might be discarded immediately. Additionally, used respirators should be disposed of in accordance with policies for other potentially infectious waste.

ACKNOWLEDGMENTS

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REFERENCES


AUTOCLAVE EMISSIONS—HAZARDOUS OR NOT

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The Hebrew University of Jerusalem, Israel

ABSTRACT

Autoclaves used for sterilization and decontamination of biological materials operate at temperatures of 121°C or higher. Substances placed in the autoclave produce emissions that may have unpleasant odors, and in some instances may be hazardous. Agar and media cause odors when autoclaved but are not hazardous. However certain chemicals cause irritation of the eyes, nose and throat and may also be hazardous when released during autoclaving. Plastics such as polystyrene used in the manufacture of disposables, are unstable when autoclaved and may release gases such as styrene and ethyl benzene. Adequate ventilation systems are needed in areas where autoclaves are located.

INTRODUCTION

Autoclaves that operate under steam pressure at high temperature are widely used in biological laboratories for sterilization and decontamination. The combination of steam and heat under pressure provide an excellent means of destroying microorganisms. A wide variety of materials introduced into the autoclave include various utensils, glassware, plastics, agar, and media. Heating the products placed in the autoclave results in the release of odors that are not always pleasant, and upon opening the autoclave, the emissions are detected by everyone in close proximity and may even be detected at some distance if carried by air currents. Odors from autoclaves often give rise to the question—are they in any way connected to the release of hazardous substances?

MEDIA AND AGAR

Culture media and agar are routinely sterilized and decontaminated in the autoclave. Agar is a phycocolloid (water-soluble polysaccharide) obtained as an aqueous extract of certain seaweeds (Armsen 1991). It is a stable inert gel with a melting temperature of about 87°C and a gelling temperature of about 36°C. When sterilized in the autoclave, agar releases typical odors due to decomposition of the ingredients.

With regard to media in autoclaves, chemical compounds will release odors as bonds are broken by heating. Compounds that will cause odors when heated include bile salts, sulfur, meat peptones and nitrogen compounds (Difco Laboratories, Detroit). There is no evidence to suggest that these chemical decompositions may be hazardous. Thus agar, media and other biological substances such as blood, will release odors from the autoclave that are not always pleasant, but are not hazardous.
HAZARDOUS CHEMICALS IN THE AUTOCLAVE

Placing dangerous chemicals in the autoclave when decontaminating biological materials should be avoided. Apart from damage to the autoclave, toxic gases are liable to be released and there may be health hazards.

Suggested Procedures

Special procedures need to be followed to inactivate the agents that cause mad cow’s disease or bovine spongiform encephalopathy (BSE), Creutzfeldt-Jakob disease, scrapie and kuru. These diseases are caused by agents called prions that are stable protein molecules (CDC 1993, DeArmond 1995). In some cases, NaOH is added to prion solutions which are then autoclaved. This causes the emission of strong odors and leads to erosion of the autoclave itself.

To avoid the problems associated with placing NaOH in the autoclave, the following procedures are recommended:

a. Dry waste (mainly from tissue culture), should be autoclaved for at least 30 min at 134°C before removal as regular waste.

b. Liquid waste is collected into a special container with NaOH to a final concentration of 1N. Before autoclaving for 30 min at 134°C, the NaOH is neutralized with phosphoric acid to around pH 8. To avoid the emission of unpleasant odors, the waste should be left to cool in the autoclave before disposal into the sewage system or according to institutional practices.

When autoclaving hazardous biological agents, if it is considered necessary to decontaminate the evacuated air, unsaturated steam, condensates or any airborne microorganisms that may be emitted during the initial warm-up, autoclaves should have a special recycling mechanism or attachment that will ensure heating of the initial emissions to high temperature before release into the sewage system. Autoclaves with such attachments are available.

Sodium hypochlorite (or bleach) which is widely used for inactivation of microorganisms, may be partially neutralized by organic material in the waste treated. However any remaining hypochlorite may release hazardous chlorine gas when autoclaved. For this reason, bleach solutions must be neutralized with sodium thiosulfate before being placed in the autoclave (1 ml of 5% sodium thiosulfate per ml of 5% hypochlorite ion). This will also prevent the corrosive action of chlorine on autoclave parts (Fleming 1995).

Further examples of hazardous chemicals in the autoclave, include materials such as agar, treated with formaldehyde in aqueous solutions during staining procedures. If placed in an autoclave load, there will be an unpleasant release of formaldehyde gas. Formaldehyde, which is a suspected human carcinogen (ACGIH 1996, Blair 1990, Roe 1992), and gluteraldehyde, are known to be irritants of the eyes and the respiratory passages (Cottone 1996, Leslie 1996). The
same applies to phenol used in DNA extraction or for other purposes. Any of these agents placed in an autoclave load, inadvertently or otherwise, will cause enormous discomfort and may be hazardous to those in the surrounding area. It is to be avoided at all cost.

**RADIOACTIVE MATERIALS**

With regard to radioactive materials in the autoclave, media containing isotopes such as H-3 and C-14 are routinely autoclaved before disposal. In such cases there should be a radioactive warning sign on the autoclave and smear tests for radioactivity should be regularly performed. Problems arise when compounds with radioisotopes that are volatile are autoclaved. For example, substances containing I-125 at active levels should not be autoclaved due to the potential release of radiolabelled iodine. By the same token, materials containing I-125 should not be treated with chlorine-containing solutions, since the resulting chemical reaction may release iodine. In such cases, formaldehyde or glutaraldehyde should be used for chemical decontamination instead of autoclaving (Fleming 1995).

**PLASTICS**

In everyday use of the autoclave plastics may comprise 50% or more of the load that undergoes decontamination. Materials placed in the autoclave include many kinds of plastic that range from high- and low-density polyethylene to polypropylene to polystyrene. Many lab workers have had the experience of polyethylene bags melting in the autoclave and sticking to everything, and polystyrene plates crumpling during autoclaving.

<table>
<thead>
<tr>
<th>TABLE 1</th>
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<tr>
<td>Autoclavability of certain plastics and possible emission of gases during thermal decomposition.</td>
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<tr>
<td>PC - polycarbonate</td>
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<td>PP - polypropylene</td>
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<td>HDPE - high-density polyethylene</td>
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<td>LDPE - low-density PE</td>
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<tr>
<td>PS - polystyrene</td>
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<tr>
<td>ACL - acetal (polyoxymethylene)</td>
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Source: MSDS and Nalgene® catalog.
The Nalgene® catalog (Nalge Company, Rochester), clearly specifies whether or not their plastic products are autoclavable. The catalog also lists a number of plastics (e.g., polystyrene, polyvinyl chloride, nylon, acrylic) that are not autoclavable under any conditions.

Polystyrene products such as petri dishes, culture flasks, roller bottles, and microwell plates, constitute most of the disposables used in the lab. The Becton-Dickinson catalog of Falcon® products states that virgin polystyrene is specially treated to allow improved cell attachments and spreading for tissue culture. Thus in many laboratories, polystyrene utensils are used in tissue culture, since the cells appear to have a better affinity for the polystyrene matrix than for other plastics. In most cases, the polystyrene containers are autoclaved before disposal.

A list of plastic products is presented (Table 1) that shows their maximum use temperatures and whether they may be autoclaved or not, as well as gases that may be released during thermal decomposition.

It should be noted that acetal releases formaldehyde in the autoclave. The Nalgene® catalog recommends autoclaving acetal test tube racks at 121°C maximum for 20 minutes at 15 psig. Proper ventilation is required for autoclaving of acetal. Autoclaving acetal racks for longer periods or at higher temperatures results in the release of dangerous levels of formaldehyde.

Bottles and other products containing polyvinyl chloride (PVC) should not be autoclaved unless otherwise stated by the manufacturer. Vinyl chloride, the monomer used to produce PVC, is a human carcinogen (Amdu 1991, ACGIH 1996).

Polystyrene which is widely used in the manufacture of disposable laboratory equipment is one of the least stable plastics when autoclaved and may release styrene and ethyl benzene. Polystyrene resins are obtained by polymerization of styrene which is an aromatic hydrocarbon. Styrene liquid and vapors are an irritant to skin, eyes and mucous membranes and narcotic at high concentrations. Exposure to its vapors may cause drowsiness, nausea, headache, fatigue and dizziness in humans. Inhalation is the major path of absorption into the body. Inhalation of 10,000 ppm for 30-60 minutes may be fatal to humans (Patnaik 1992). The short-term exposure limit (STEL) for the styrene monomer is 100 ppm (426 mg/m³) (ACGIH 1996). There are inadequate data on which to classify styrene in terms of its carcinogenicity in humans and/or animals.

Ethyl benzene is a flammable liquid used as an intermediate to produce styrene monomer. Exposure to high concentrations produces narcotic effects. It is an irritant to the skin, eyes and nose (Patnaik 1992). The STEL is 125 ppm (543 mg/m³) (ACGIH 1996).

EXPERIMENTAL

Attempts were made to measure styrene levels under actual working conditions in laboratories, where polystyrene plates are included in biohazard bags containing biological waste for decontamination. Measurements were performed using a Foxboro Miran® 1B Portable
Ambient Air Analyzer which is a single-beam infrared spectrometer. The gas analyzer is used to monitor the air in workplace environments for the presence of specific gases. The Miran was calibrated for styrene detection, and measurements were made next to the autoclave during a 20 min cycle at 121°C. On opening the autoclave, the probe was held close to the load and readings were made. Measurements were made of loads consisting of several polypropylene autoclave bags containing polystyrene plates with agar, disposable pipettes and tissue culture flasks. Loads of glassware and metal pipette canisters for sterilization were also checked. The autoclaves were located in standard laboratories, without local exhaust ventilation.

Decontamination of biological waste is usually done on a wet cycle. Steam from the chamber is released into the sewage system at the end of the cycle, but the load is wet and steam is still present when the autoclave is opened, even after sterilization of glassware. Because of interference due to steam absorption of the infrared beam, styrene was not detected by the Miran® Analyzer. However, instead of styrene, readings of steam vapors that were obtained, showed that after opening the autoclave in an area ventilated with air exchanges provided by the building HVAC (heating, ventilation and air-conditioning) system, periods of up to 10 minutes were required for dispersal of vapors for wet loads. With dry loads of glassware and pipettes, the steam dispersed within 2-3 minutes.

According to Nunc InterMed, Denmark, polystyrene (PS) may contain up to 0.5% styrene, which theoretically can be liberated during autoclaving, i.e., the air in the autoclave contains styrene after having autoclaved polystyrene articles. On this basis we calculated the amount of styrene that could be released from twenty 90 x 15 mm PS dishes weighing a total of about 300 gm, in an autoclave chamber of 250 liters. The concentration of styrene in the autoclave chamber, could be as high as 6000 mg/m³. This is equivalent to 1400 ppm which is much higher than the STEL value of 100 ppm. Uniform dispersal of such a concentration of styrene in a room of 27 cubic meters for instance, would result in a level of 50 mg/m³ or 12 ppm. However, although it is possible that styrene is released from autoclave loads containing polystyrene, it should be noted that a large portion of emissions are vented to the sewage system during steam evacuation at the end of the sterilization cycle. Thus the actual amounts of volatile gases left when opening the chamber door, are probably very low, with minimal health hazards.

Analyses of emissions from autoclaves have been performed using methods that are not hampered by steam, such as gas chromatography/mass spectrometry (GC/MS). According to information we obtained from a large medical waste autoclave manufacturer, measurements have been made of emissions from a hospital waste autoclave using GC/MS analysis. The average weight of the load of regulated medical waste (RMW) per cycle was 180 pounds. The autoclave cycle was done at a temperature of 138°C (282°F) for 31 minutes and pressure of 30 psi (sterilization phase). During this cycle, both steam and non-steam exposed test spores of B. stearothermophilus and B. subtilis were inactivated. The major volatile organic compounds detected from air exhaust vents during cooling periods, included xylenes, ethyl benzene, toluene, carbon disulfide, acetone and to a lesser extent, styrene. Although the individual compound concentrations were low, up to 30 mg/m² of total volatile organic compounds were measured in autoclave emissions from a typical hospital waste load.
DISCUSSION

Biological materials such as agar and media cause unpleasant odors in the autoclave but they are not hazardous. Volatile radioactive substances like I-125 should not be autoclaved, nor should hazardous chemicals. The immediate effects of placing certain common laboratory chemicals in the autoclave will be irritation of the eyes, nose and throat causing discomfort to those people in close proximity to the autoclave. The long-term effects of these exposures are not known at present.

Plastics such as polypropylene and polycarbonate are stable at autoclave temperatures and are recommended for this purpose. Acetal used for test-tube racks is known to release formaldehyde in the autoclave, and may be detected by its characteristic odor. Polystyrene used in the manufacture of a large variety of laboratory disposables, may release styrene and ethyl benzene during thermal decomposition in the autoclave, but the actual amounts present when opening the chamber door are probably very low.

Analyses of emissions from autoclave loads of regulated medical waste in a hospital setting indicated that a number of volatile organic compounds are released at low levels during sterilization. For most volatile organic compounds, these concentrations do not constitute an acute health hazard. However, they may be higher than acceptable indoor air quality (IAQ) limits (Bearing 1993), and may cause discomfort, headaches or other symptoms when workers are exposed to autoclave emissions.

Dioxins that may be formed during incineration in the low-temperature region of the incinerator, do not pose a problem in autoclaves. Dioxins produce a wide range of metabolic dysfunctions in humans (Patnaik 1992), but they are released at temperatures well above those used for autoclaving medical and biological waste.

It is clear however, that certain volatile compounds will be released during the use of the autoclave. For this reason, areas where autoclaves are situated require adequate general ventilation. Ventilation in labs should provide for at least 6-8 air changes per hour (DiBerardinis 1993). These are the minimal requirements for the area where the autoclave is situated. For improved ventilation for a room containing an autoclave, 10-12 air changes per hour are recommended. Supplementary local exhaust ventilation, closed systems, or respiratory protection may be needed if the autoclave is situated in a poorly ventilated space and cannot be relocated.

It should be noted that after opening the autoclave, we found that it takes up to 10 minutes or more for the vapors to disperse. Thus we recommend that in the absence of a local exhaust system, personnel leave the area for 10-15 minutes before dismantling the load, in order to avoid exposure to emissions from the autoclave.

Deodorizers are available to overpower unpleasant odors from autoclaves but there have been anecdotal reports of headaches caused by the use of these deodorizers.
AUToclave EMISSIONS—HAZARDous OR NOT

Autoclaves play a major role in the decontamination of biological and medical waste, but there needs to be an awareness of the fact that if substances containing volatile organic compounds are placed in the autoclave, they will be emitted on opening the autoclave door, and in some cases, these emissions may be hazardous.

RECOMMENDATIONS

1. Appropriate ventilation systems should be operating in areas where autoclaves are located: either a well-designed local exhaust system, or a system assuring 10–12 air changes per minute in the autoclave area.

2. If there is inadequate ventilation, after opening the autoclave, leave the area for a suitable period (as a rule of thumb, at least 10 minutes) to allow for dispersion of emissions before dismantling the load.

3. Routine cleaning of the autoclave with a detergent is recommended to help eliminate odors. Citric acid (2 tablespoons to half a liter of water) is a good agent for cleaning stainless steel autoclaves. Commercial oven cleaners can be used for obstinate stain removal.

4. Development and use of Standard Operating Procedures (SOP's) for the Laboratory Autoclave. This is to ensure that emissions of potentially hazardous substances are minimized or eliminated.

ACKNOWLEDGMENTS

The constant support and encouragement of Mr. Micha Bar-On, Head of the Department of Safety and Occupational Health, is most appreciated. Difco Laboratories, Detroit, were very helpful in replying to queries about smells of media and agar when heated, as well as the Nalge Company, Rochester NY and Nunc InterMed, Denmark regarding queries about plastics. We are grateful to San-I-Pak, Inc., California, for sharing with us their information on emissions from a hospital waste autoclave.

REFERENCES


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