Applied Occupational and Environmental Hygiene

ISSN: 1047-322X (Print) 1521-0898 (Online) Journal homepage: https://www.tandfonline.com/loi/uaoh20

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To cite this article: Thomas F. Bloom & Ana Maria Osorio (1991) Biological Safety Cabinets: Considerations for the Industrial Hygienist, Applied Occupational and Environmental Hygiene, 6:2, 119-124, DOI: 10.1080/1047322X.1991.10387845

To link to this article: https://doi.org/10.1080/1047322X.1991.10387845

Biological Safety Cabinets: Considerations for the Industrial Hygienist

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Researchers from the National Institute for Occupational Safety and Health (NIOSH) investigated the use of a biological safety cabinet (BSC) in connection with an exposure to *Legionella pneumophila* at a research facility laboratory. The exposure occurred during the handling of bacteria-containing solutions associated with the analysis of water samples. Inhalation of the bacteria was thought to be the most likely exposure route. Bacteria escaping from the BSC via inadequate filtration of cabinet exhaust air or via leaks in the cabinet structure were considered to be two possible exposure mechanisms. Our investigation included consideration of the following issues related to BSCs: 1) the types of BSCs available for specific applications, 2) certification of the cabinets, 3) information available to users regarding cabinet uses and limitations, and 4) recordkeeping. This article briefly discusses the aforementioned issues and summarizes recommendations as they relate to an industrial hygiene program or investigation dealing with BSCs. The article is intended to provide basic information to industrial hygiene managers or investigators who may have limited familiarity with the subject of BSCs. Relevant references for more detailed information regarding BSCs are also noted. **Bloom, T.F.; Osorio, A.M.: Biological Safety Cabinets: Considerations for the Industrial Hygienist. Appl. Occup. Environ. Hyg. 6:119–124; 1991.**

Introduction

Researchers from the National Institute for Occupational Safety and Health (NIOSH) investigated the use of a biological safety cabinet (BSC) in connection with an exposure to *Legionella pneumophila* at a research facility laboratory. *L. pneumophila* has been identified as the agent responsible for Legionnaires' disease.[1] The BSC was used by a microbiologist during the testing of potable and cooling tower water samples for the presence of *L. pneumophila*. The BSC underwent a concurrent performance evaluation to determine its containment effectiveness. The report of the evaluation suggested that the cabinet did not provide effective containment for manipulation of this bacterium. The index individual subsequently underwent a series of immunofluorescent titer evaluations. An initial serum titer evaluation (polyvalent, serogroup 1–6) was negative at 1:16. However, a second evaluation conducted one month later indicated an eightfold rise to 1:128. This finding indicated an immunologic response to the bacterium. The individual remained asymptomatic throughout the time period.

Based on information obtained during this investigation, three possible exposure scenarios were suspected. One possible scenario involved inhalation exposure to the bacterium due to inadequate filtration of exhaust air. A second possible scenario involved leakage of contaminated air under positive pressure through defects in the cabinet structure. A third possible scenario involved exposure during the manipulation of bacterium-containing samples on a laboratory bench outside of the BSC. Thus, an inhalation exposure could have occurred as a result of sample aerosolization during any one of several mixing, pipetting, or decanting procedures, or ingestion exposure could have occurred as a result of hand contact with the sample. However, the investigators were unable to clearly identify the actual exposure mechanism.

The investigation of the cabinet's primary containment capability illuminated several technical and programmatic issues that should be considered by practicing industrial hygiene managers or investigators regarding BSCs. These issues form the main theme of this article and include: 1) cabinet selection, 2) cabinet certification, 3) cabinet user information, and 4) cabinet documents. To illustrate these issues, the relevant details concerning this investigation are discussed along with major conclusions. Based on these findings, a more detailed discussion of BSCs is presented.

Report of Investigation

To determine past as well as present containment effectiveness provided by the cabinet, the investigators first performed a visual check of the external cabinet structure and of the cabinet workspace. Information regarding prescribed cabinet uses and limitations was obtained from a representative of the cabinet's manufacturer. Following

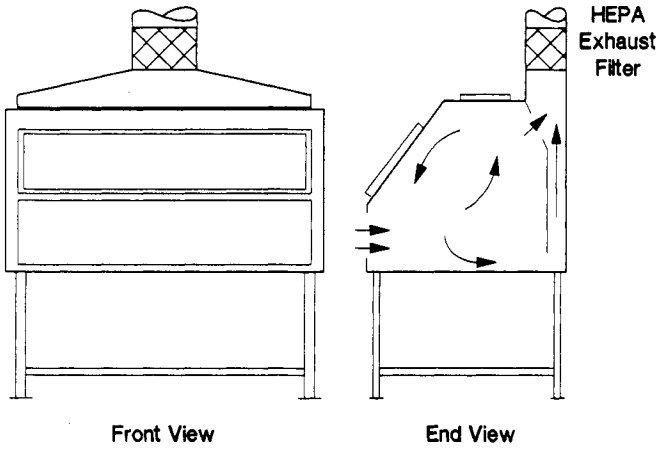

FIGURE 1. Section view of Class I cabinet workspace and air flow patterns.

cabinet decontamination with paraformaldehyde, several structural panels were removed, and the cabinet's internal air flow design was inspected. Once the panels were reattached, the cabinet's exhaust filter and external panel structures (primarily at the joints) were tested for leaks. Finally, a report of the tests conducted on the cabinet just prior to this investigation was reviewed.

Figure 1 shows a section view of the cabinet workspace, internal air flow patterns, and associated workspace. In general, the cabinet appeared to be similar in construction and air flow pattern to a predecessor of the currently designated Class II, Type A cabinet. External inspection revealed the cabinet's exhaust port was not connected to a duct exhausting to the outside atmosphere, but instead, cabinet air was exhausted back into the workroom. Air flow into the cabinet workspace came from two sources: 1) supply air descending vertically from a high-efficiency particulate air (HEPA) filter and 2) air drawn in through the front opening. User protection is achieved, in part, by a proper velocity balance between air descending vertically and air entering horizontally. Such a balance would prevent biologically active aerosols from escaping through the front of the cabinet. Conversely, a lack of balance might allow bacteria to escape through the front of the cabinet.

The cabinet manufacturer provided the following information. The cabinet was a vertical flow type cabinet designed for gross contamination situations where work being performed in the cabinet may cause discomfort to the operator. Furthermore, this cabinet model was not structurally leak tight. Because of these specifications, the cabinet should not be used for work involving low to moderate risk agents. Finally, it was noted that these limitations were included in the equipment literature that accompanied the sale of the cabinet to the facility.

In inspecting the internal air flow pattern, two main observations were made: 1) the cabinet air flow is such that the inner cabinet workspace is under negative pressure and the outer interior of the cabinet is under positive pressure and 2) the cabinet had only one HEPA filter. This sole filter was used for filtering both air supplied to the work area and air exhausted from the cabinet. Furthermore, inspection of the filter showed a pronounced yellowish discoloration of the portion that filters air exhausted to the outside. The first observation indicated that contaminated air from the workspace under positive pressure near the outer cabinet walls could leak to the workplace environment at improperly sealed structural interfaces or at inadequately tightened fasteners. The second point dealing with the yellow discoloration could be suggestive of some type of corrosive action of the contaminant-laden air stream on the filter. Such corrosive action, if indeed that is what occurred, may have compromised the integrity of the filter. Both of these functional characteristics, acting individually or collectively, could have resulted in contaminated aerosol escaping from the cabinet.

The exhaust port air was evaluated to determine the degree (if any) of penetration of a test aerosol through the HEPA filter. The aerosol was generated by aspirating oleic acid into a Laskin Nozzle operating at 25 psig. An ATI 722 Particulate Detection Apparatus (Air Techniques Incorporated, Baltimore, Maryland) was used for aerosol detection. This is a linear readout photometer using a graduated 0–100 percent measurement scale.

For the test, the generator was placed at the rear of the cabinet workspace so that aerosol would be introduced upstream of the HEPA filter. The aerosol concentration was then measured using the detector probe. After adjusting the instrument to read 100 percent for the upstream measurement, a downstream measurement was obtained by moving the detector probe across the exhaust port. Several "spike" concentrations of 100 percent were detected. This finding suggested that the exhaust port released small amounts of unfiltered air into the testing room.

A report of cabinet performance tests conducted immediately prior to the elevated titer determination was reviewed. The tests conducted included a HEPA filter supply air test and an inward air flow velocity test. Results of the HEPA filter test indicated no leaks, and the average inward air flow velocity test was found to be 104 feet per minute. The report's concluding remarks stated that the cabinet offered no personal protection and should not be used for work with biohazardous materials. The basis for these conclusions could not be determined.

No test data regarding downflow air velocity or cabinet leak tightness could be found. Thus, it was unclear whether operational compatibility between downflow air velocity and inflow air velocity existed in a manner that would protect the user. Nor was it clear whether the cabinet was sufficiently leak tight so as to prevent contaminated air under positive pressure from escaping from the cabinet. No explicit statement concerning certification was made in the report prepared by the organization performing the tests.

During an interview with the microbiologist, it was learned that the individual had, on several occasions, performed sample manipulation procedures outside of the cabinet on a laboratory workbench. Exposure to the bacterium could have occurred during these activities. However, based on the individual's description of the procedures performed

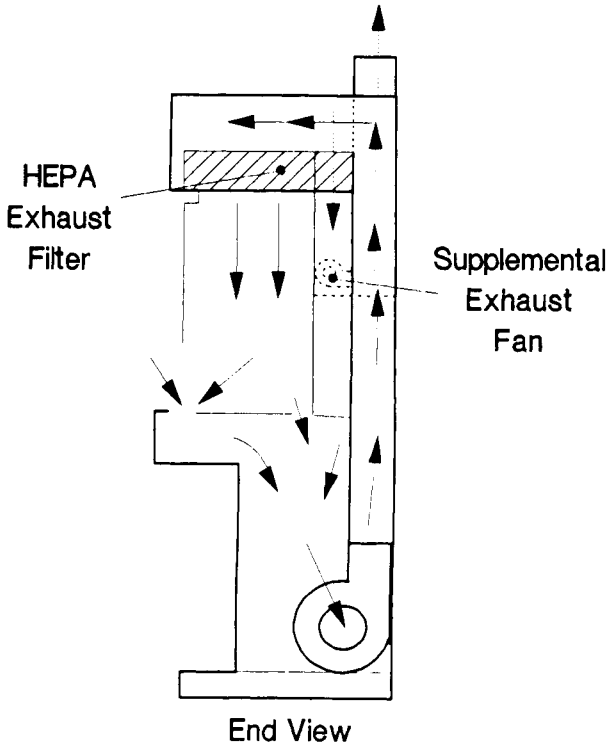

FIGURE 2. Class I biological safety cabinet.

and the manner in which they were performed, the investigators concluded that exposure during these events was possible, but not likely.

No documents were found near the physical location of the cabinet regarding intended uses or limitations, regarding past maintenance, or describing proper use of the cabinet. In addition, no label was affixed to the cabinet that described current operating effectiveness.

After consideration of all information collected, it was concluded that there were two possible exposure mechanisms. One mechanism may have involved the release of aerosolized bacteria to the workroom via the exhaust port due to inadequate filtration of cabinet air. The other mechanism may have involved the release of aerosolized bacteria through defects in the cabinet structure.

From an industrial hygiene viewpoint, it was concluded that the cabinet in question was not an appropriate design for manipulation of this bacterium. Also, the containment effectiveness of the cabinet at the time surrounding the uptake was questionable. While some data regarding cabinet performance was available, no explicit statement of operating effectiveness was documented. In addition, several important tests of BSC proper functioning (downflow air velocity and cabinet leak tightness) were apparently not conducted. There was a lack of operating information available to the microbiologist regarding cabinet use and limitations. In addition, the laboratory practices of the microbiologist increased the risk of uptake of the bacterium. Finally, no recordkeeping system for retaining maintenance and purchase records was found.

Because of this investigation, significant issues regarding use of BSCs were reviewed. Four issues of particular significance to industrial hygiene managers or investigators concern the selection, certification, user training, and recordkeeping for BSCs.

Cabinet Selection

Categories of BSCs

Three basic categories have been established for BSCs: Class I, Class II, and Class III. For Class II, there are two types: Type A and Type B. For each class, the type of protection (personal protection, product protection, or both) and the degree of protection can vary.

In the Class I cabinet (Figure 2), room air flows through a fixed front opening to prevent microbial aerosols released within the cabinet from escaping into the room. Air from this cabinet is exhausted to a dedicated facility exhaust system. This cabinet is suitable for work with low and moderate risk biological agents.[2] However, no product protection is provided by this class of cabinet. Cross contamination may result from contaminated air flowing over the cabinet work area.[2,3] A HEPA filter may be used to remove particulates before cabinet air is exhausted to the dedicated facility system.[3]

The Class II cabinet was developed to protect the investigator as well as the experiment and the environment. In terms of containment ability, the Class II design contains two features: 1) separate HEPA filters for filtering air supplied to the cabinet work area as well as air exhausted to the outside of the cabinet and 2) vertical laminar flow air movement in the cabinet workspace and inflow air movement through the front opening optimally matched to provide personal, product, and environmental protection.[2]

The Type A, Class II cabinet (Figure 3) allows for approximately 70 percent recirculation of HEPA-filtered air back to the cabinet workspace and 30 percent exhaust of HEPA-filtered air back into the laboratory atmosphere.[3] Type A cabinets are suitable for work with low to moderate risk biological agents in the absence of volatile toxic chemicals and volatile radionuclides. The Type B, Class II cabi-

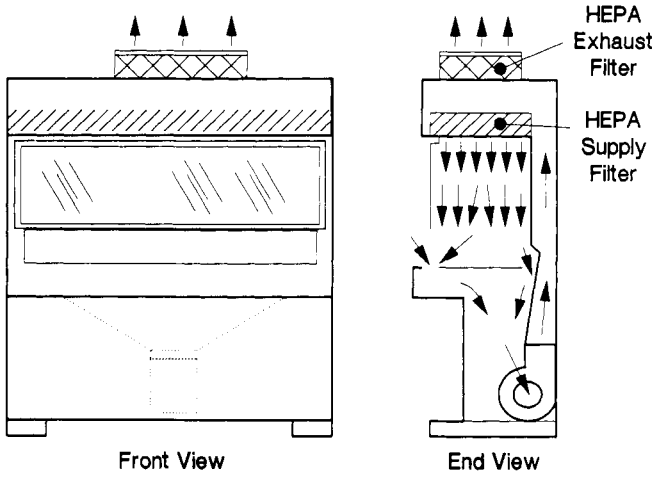

FIGURE 3. Class II, Type A, biological safety cabinet.

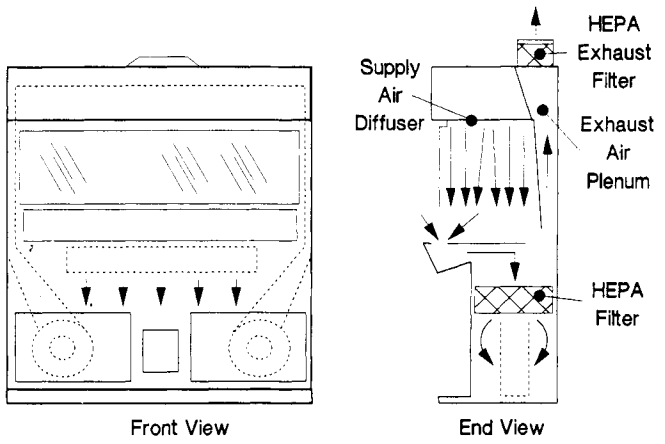

FIGURE 4. Class II, Type B, biological safety cabinet.

net (Figure 4) allows for separate exhaust (to the building's dedicated system) of up to 100 percent of HEPA-filtered air.[2] Therefore, these cabinets are suitable for work with a wider range of substances.[3] To reflect the degree of exhaust to an external system, the Type B designation is subdivided into B1–B3 by the National Sanitation Foundation (NSF).

The Class III cabinet (Figure 5) provides a totally enclosed, gastight, negative pressure, containment environment. Operations are conducted through attached arm-length rubber gloves. Room air intake and enclosure air exhaust use separate HEPA filtering systems.[2,4]

Biosafety Levels

The Biosafety Level designation reflects the degree of potential hazard to personnel and the environment. It is assigned after determination of the agent to be handled and the manipulation techniques that will be used. Four Biosafety Levels have been established by the Centers for Disease Control (CDC) and the National Institutes of Health (NIH) for classification of infectious agents.[5] For each Biosafety Level, appropriate containment equipment (biological safety cabinet) to be used in connection with agent handling is specified.

Biosafety Level 1 agents present a low biological risk and have minimal potential health effects in humans, animals, and plants. Special containment, e.g., a BSC, is generally not required for manipulation of this class of agent. Examples include *Subtilis* and *Naegleria Gruberi.*

Biosafety Level 2 agents present a moderate risk and require more containment because of known pathogenicity or synergism with other materials. Hepatitis B virus and *Salmonella* are examples of Level 2 agents, which usually require the use of a BSC.

Biosafety Level 3 agents present potentially serious lethal consequences. Manipulation of these agents is always carried out within a BSC, and access to the laboratory containing these agents is extremely limited. *Mycobacterium tuberculosis* is an example of such an agent.

Biosafety Level 4 agents present a high risk of life-threatening disease. All work is done within a Class III BSC. In addition, the laboratory facility is separated from other buildings with laboratory access strictly controlled. Lassa fever virus is an example of a Level 4 agent.[5]

Table I provides a brief summary of BSC applications. The reader should note that the terms "low," "moderate," and "high" risk biological agents employ the Biosafety Levels discussed previously. For further information on Biosafety Levels, the reader is encouraged to obtain a copy of the CDC publication *Biosafety in Microbiological and Biomedical Laboratories.* For further information on cabinet selection, the reader is encouraged to obtain a copy of the NIH publication *Laboratory Safety Monograph, A Supplement to the National Institutes of Health Guidelines for Recombinant DNA Research.*

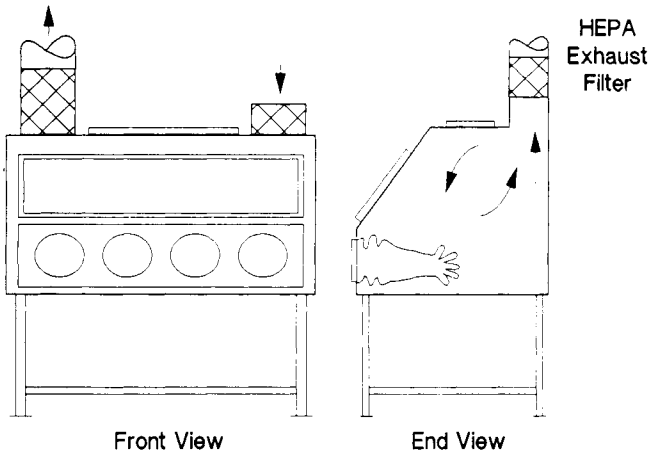

FIGURE 5. Class III biological safety cabinet.

Certification

The term certification has been applied to BSCs that meet specified design, construction, and operating performance requirements. There is no formal certification for design, construction, or performance requirements for Class I or Class III cabinetry. However, the Laboratory Safety Monograph describes recommended design and performance criteria for these two classes.[4]

For Class II cabinetry, NSF Standard 49 outlines design, construction, and performance criteria.[2] A user of a Class II BSC can consider the cabinet as being certified if it meets the requirements set out in this standard. This means that the cabinet must meet design and construction as well as certain performance criteria requirements established by the standard.

When purchase of a BSC occurs, the manufacturer should stipulate whether the cabinet meets the appropriate criteria (NSF Standard 49 for Class II cabinetry; performance criteria recommended by NIH for Class I and III). In their instruction manual accompanying the BSC, the manufacturer should state that the cabinet was tested at the factory prior to shipment. A document indicating what tests were performed should accompany the BSC. Satisfactory completion of the factory-performed tests forms the basis for

TABLE I. Classification of Biological Safety Cabinets[2,3]

Cabinet Category	Type of Protection	Circulation System	Recommended Application
Class I	Protects employee only	Exhaust 100%	Low risk biological agents where no product protection is required.
Class II, Type A	Protects employee and product	Exhaust 30% Recirculate 70%	Moderate risk agents in absence of volatile toxic chemicals and volatile radionuclides.
Class II, Type B	Protects employee and product	Exhaust up to 100% Requires external exhaust fan	Moderate risk agents or agents treated with minute quantities of toxic chemicals or trace amounts of radionuclides.
Class III	Protects employee	Custom design	High risk agents.

the initial certification (Class II cabinets) or declaration that NIH recommended criteria have been satisfied (Class I and Class III cabinets). Once the cabinet is installed on the user's premises, it becomes the responsibility of the user to maintain the BSC in conformance with the applicable performance criteria as detailed by NSF and NIH.

The cabinet should be recertified on site after installation and at scheduled intervals thereafter. For their BSCs, the CDC requires certification at the time of installation and annually thereafter. Certification is also required if the cabinet is relocated.[6] The NIH requires certification upon installation and annually thereafter. Certification is also required when HEPA filters are changed or if the cabinet is relocated.[7]

For Class I and Class III cabinetry, performance parameters have been recommended by the NIH. These parameters relate to face velocity and exhaust filter efficiency for the Class I cabinet, and negative pressure, leak tightness, and exhaust filter efficiency for Class III cabinets.[4]

For Class II cabinetry, the NIH recommends that four performance tests be performed to ascertain the operating performance. These tests are the Plenum Leak Test, the Inflow Velocity Test, the Downflow Velocity Test, and the Filter Leak Test. These tests determine the ability of these cabinets to provide both product and personnel protection.[7] For Class II BSCs, NSF Standard 49 provides details concerning 13 performance tests.

For Class II cabinetry, the NSF maintains an updated list of Class II vertical laminar flow BSCs that meet the design, construction, and performance criteria as detailed in NSF 49.[8] This document should aid the user in the selection of an appropriate Class II BSC.

Currently, there is no comprehensive list of organizations that provide certification and/or testing services for BSCs. The manufacturer of the BSC may, upon request, provide a list of organizations who can provide certification and/or testing-related services for their cabinets. The user has the option of performing the tests, but this would require the purchase of the appropriate instrumentation. For the Class II certification tests recommended by the NIH, the necessary equipment for certification includes a halogen leak detector (for the Plenum Leak Test), a thermoanemometer (for the Velocity Tests), and DOP generator and particle photometer (for the Filter Leak Test).[7]

User Information and Training

Users of BSCs must be adequately instructed in the proper use of the BSC. In particular, users should be apprised of those actions that may result in the escape of aerosols from biohazard cabinetry (primarily Class II but also Class I) and the potential exposure to the user. These high risk actions include: repeated insertion and withdrawal of workers' arms into the work chamber, opening and closing the laboratory or isolation cubicle doors, improper placement or operation of materials or equipment inside the work chamber, and briskly walking past the cabinet while in use.[4,6,9] For additional information on user training, the reader should contact the manufacturer of the BSC of interest.

As a hazard alert, cabinets should contain on the front of their surfaces explicit statements of certification (or lack thereof), limitations regarding cabinet usage, and the next date for recertification. A list of agents authorized for use within the cabinet should also be kept and prominently posted on the cabinet structure.

Formal initial and periodic training related to BSCs should be considered for all workers using these cabinets. All users of cabinets should receive basic training with subsequent periodic refresher courses regarding overall cabinet usage, biological agent manipulation techniques, proper interpretation of cabinet limitation statements (and any subsequent revisions), and certification statements. Periodic review by laboratory management to ascertain adherence to proper BSC usage, laboratory techniques, and training effectiveness should also be performed.

Recordkeeping

Records regarding cabinet purchase, intended use, agents authorized for manipulation within the cabinet, training documentation, and certification/operational status should

be readily accessible to users and to laboratory management.

Conclusions and Recommendations

This investigation uncovered the practice of manipulation of an infectious agent in a BSC that appeared to be a predecessor of the currently designated Class II BSC. This Class II-like cabinet exhausted filtered air into the testing room atmosphere. However, the filter test data indicated that small amounts of unfiltered air may have been intermittently released to the room atmosphere. Exhaust of this air may have led to the uptake of *L. Pneumophila.* Uptake could also have occurred as a result of leakage of contaminated air through defects in the cabinet structure. The information gathered suggested that the user may not have been aware of the limitations associated with cabinet usage.

The industrial hygiene manager or investigator must be aware that the type of BSC for a given application depends upon the specific agent being manipulated and the degree of protection required. Once the choice of BSC is made and the cabinet installed at the worksite, it must be maintained and tested at scheduled intervals. Personnel must be educated in the proper uses and limitations of the cabinet. Training should be updated at scheduled intervals. Documentation regarding BSC purchase, testing, and user training should be maintained and readily accessible.

Acknowledgments

The authors wish to thank Dr. Paul A. Baron and Dr. William E. Halperin, NIOSH, for their advice and consultation during this investigation.

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Received 11/22/89; review decision 2/1/90; revision 6/15/90; accepted 6/29/90