Article

Applied Biosafety, 11(4) pp. 222-227 © ABSA 2006

Use of Ultraviolet Lights in Biological Safety Cabinets: A Contrarian View

Paul J. Meechan[1] and Christina Wilson[1, 2]

[1]Merck Research Laboratories, West Point, Pennsylvania and [2]Muhlenberg College, Allentown, Pennsylvania

Abstract

In the U.S., the use of ultraviolet lights (UV) has been a source of friction between the research community, who desire them in their biosafety cabinets (BSCs), and the biosafety community, who have largely been agnostic or openly hostile to their use. This paper examines some of the claims on both sides of the issue, provides data regarding the actual irradiance inside and near BSCs at a large pharmaceutical research and development site, and makes recommendations that both protects users from the adverse effects of UV as well as supports its continued use as a useful adjunct to good laboratory hygiene.

Introduction

The use of ultraviolet (UV) lights in biological safety cabinets (BSCs) has enjoyed a long history, although it would be difficult to tell from the biosafety literature. The current version of the NSF International Standard 49 dismisses the use of UV in a BSC. The current standard, as have previous versions, states that the use of UV lights in cabinets, according to Section 5.25.2, is not recommended, although a purchaser could request it. The CDC and NIH, in their joint pamphlet "Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets, 2nd Ed." have taken a similar stand. Regarding the use of UV lights in a BSC, the pamphlet states:

> "Ultraviolet (UV) lamps are not required in BSCs. If installed, UV lamps must be cleaned weekly to remove any dust and dirt that may block the germicidal effectiveness of the ultraviolet light. The lamps should be checked periodically with a meter to ensure that the appropriate intensity of UV light is being emitted. UV lamps must be turned off when the room is occupied to protect eyes and skin from UV exposure, which can burn the cornea and cause skin cancer."

ABSA has, to date, made no official pronouncement. Curiously, researchers continue to request their cabinets be outfitted with the lights and the major manufacturers offer them as an option in nearly all cabinets. This suggests a disconnect in the risk/benefit analysis made by both researchers and biosafety professionals.

To start the analysis, one needs to understand the physical properties of UV light, the effects of UV light on biological organisms, including humans, and the current limits of exposure of humans to UV light. One also needs to know the limitations of UV as a disinfectant, as well as its ability to exert its effect outside the desired area, e.g., the ability to be transmitted through the BSC glass or be reflected off the work surface and pass through the opening in the cabinet. Only after a thorough analysis of these items may one compare UV light to chemical disinfectants and determine the relative value of UV light as part of a total strategy of good laboratory hygiene.

Properties of Ultraviolet Light

Physical Properties of Ultraviolet Light

Ultraviolet light is, by definition, light with shorter wavelengths than may be observed by the human eye. Physicists and photobiologists generally break the region into three distinct subgroups: UVA, consisting of light with a wavelength between 400 and 320 nm; UVB, consisting of wavelengths between 320 and 290 nm; and UVC, with wavelengths between 290 and 200 nm, although other groups have slightly different division points (www.merckmedicus.com/pp/us/hcp/thcp_dorlands_content.jsp?pg=/ppdocs/us/common/dorlands/dorland/dmd-u-002.htm#1135839, www.gelighting.com/na/home_lighting/ask_us/pop_glossary.html#U). For disinfection purposes, the optimal wavelengths reside within the UVC range and low pressure mercury lamps provide a nearly monochromatic 254.6 nm output (for representative spectra, see www.gelighting.com/na/business_lighting/education_resources/literature_library/product_brochures/specialty/downloads/germicidal/germicidal_tech_sheets.pdf). For the remainder of this paper, the term "UV" shall represent only wavelengths within the UVC band.

UV Effects on Biological Molecules and Microorganisms

DNA appears to be the critical target for killing by UV, although the debate concerning the "lethal lesion" has existed for decades. Two major adducts are formed by UV light, both of which are bimolecular adducts of pyrimidine bases. The most common adduct is the (cis-syn) (5-6) cyclobutane pyrimidine dimer, although a "minor" adduct, known as the (6-4) or PyC dimer, may actually be the lethal lesion in vegetative cells. It has, however, been known for more than three decades that either lesion is a block to DNA and RNA polymerases that inhibits both replication on either DNA strand and transcription on the antisense strand (for a review, see Friedberg, Walker and Siede, 1995).

Ultraviolet light has been used in the research laboratory as an effective germicide and virucide. UV inactivation doses have been determined for a variety of organisms and UV is a fairly efficient disinfectant for most vegetative organisms and viruses. Even at the minimum acceptable irradiance in a biosafety cabinet of 40 $\mu W/cm^2$ (U.S. Department of Health and Human Services et al., 2000), it takes 12.5 minutes to reach 30,000 $\mu J/cm^2$ (1 W = 1 J/sec), which has been listed as germicidal for spore forming organisms by one UV manufacturer (www.uvp.com/pdf/ab-115.pdf). UV does not penetrate well. Although UV can disinfect an empty biosafety cabinet (BSC), it will only disinfect the outer surface of any material stored in a BSC.

UV Damage to Humans and Current Limits of Exposure to UV

The American Conference of Governmental Industrial Hygienists (ACGIH) has set a threshold limit value (TLV) of 6.0 mJ/cm^2, which is based on the only observed acute effect: erythema to a "fair skinned" individual (ACGIH, 2005). Damage to the eye or skin is significantly affected by the UV wavelength utilized. Penetration into the dermis does not occur until wavelengths of greater than 300 nm have been reached; for wavelengths within the UV-C band, penetration of no more than approximately 50 µm into the epidermal layer occurs (Jagger, 1985; Suess, 1982). In contrast to the data known for UV-B and UV-A, the link between erythema and the most severe long-term stochastic effect, skin cancer, has not been quantitated and there is no current link between UV and the most severe form of skin cancer, melanoma (Gilchrest, Eller, Geller and Yaar, 1999; Alam and Ratner, 2001; Rubin, Chen, and Ratner, 2005). It must be noted, however, in their 11th Report on Carcinogens the National Toxicology Program (NTP), classifies UV-C as a probable (reasonably anticipated to be) human carcinogen. It should also be noted that a TLV is defined as "...conditions under which it is believed that nearly all workers may be repeatedly exposed, day after day, over a working lifetime, without adverse health effects." suggesting that adherence to the TLV should preclude **any** adverse effects, stochastic or deterministic (ACGIH, 2005).

Although not included in any regulatory framework, in terms of human risk, the risk of keratoconjunctivitis ("corneal burn") would be considered to be a risk to a worker and his or her ability to perform their daily routine, since UV-C band wavelengths are capable of only penetrating the cornea (Jagger, 1985). Although the data are fragmentary, the threshold for keratoconjunctivitis from UV in humans, according to one web site, is approximately 70 mJ/cm^2 (www-med-physik.vu-wien.ac.at/uv/actionspectra/as_eye/eye.htm). The damage is usually noticed within six to 12 hours after exposure and recovery is essentially complete within seven days (Jagger, 1985).

It must be acknowledged that skin cancer, including the potential for melanoma, must be considered as part of the risk from exposure to UV. However, no reputable studies have been reported regarding the risk to humans from UV, as it is not currently possible to separate the effect of workplace exposure to 254 nm radiation from solar spectrum UV, which causes an estimated one million cases of skin cancer annually in the U.S. alone (CDC, 2006).

Current Objections

A series of objections have been raised to the use of UV bulbs in a BSC in a paper submitted to ABSA for consideration as an ABSA position paper (Burgener, personal communication). It has been argued that in addition to putting researchers at risk from ocular damage and cancer, which has been discussed previously in this paper, the light generates ozone that can damage materials in the cabinet, is ineffective at high humidity levels and must be cleaned on a weekly basis to prevent a drop in output. Each is briefly explored below:

The risk of ozone potentially generated from the use of UV-C bulbs has not been quantitated in a biosafety cabinet. Since the standard low-pressure mercury, quartz-enveloped bulbs emit 95% of their energy at 254 nm and less than 3% of the energy at an ozone-generating wavelength, 149 nm (www.gelighting.com/na/business_lighting/education_resources/literature_library/product_brochures/specialty/downloads/germicidal/germicidal_tech_sheets.pdf), the potential ozone hazard to materials within the cabinet is small, especially if the length of time the UV bulb is energized is minimized. Moreover, if the cabinet blower is active during UV disinfection of the work area, any potential ozone within the cabinet would be exhausted. Although the data are not shown, GE

claims that their bulbs do not generate ozone which, if correct, makes the entire discussion moot. The authors acknowledge that 254 nm radiation can directly interact with plastics and cause crazing and potential weakening, but these are direct events and can be eliminated by good biosafety cabinet practices, specifically, by minimizing the amount of material left in a cabinet.

Since the CDC/NIH measurement protocol (U.S. Department of Health and Human Services et al., 2000) allows one to measure the irradiance in the center of the cabinet at ambient relative humidity, temperature and air flow, this irradiance should be the major determinant in deciding whether the UV is capable of killing the agents introduced into the cabinet. It is recognized that bulb cleanliness and temperature affects UV bulb output, but the actual measurement in the cabinet accommodates these factors. Data that suggest killing is reduced at high relative humidity need to take into account two factors:

1. Most laboratories in this country are air conditioned and the relative humidity is unlikely to be significantly above 70% most of the year; and
2. I. L. Shechmeister, in his chapter on UV irradiation, states "There are also inconsistent results in the attempted correlation of susceptibility of airborne bacteria to UV at different relative humidities." (Shechmeister, 1991), suggesting that the data are not beyond dispute.

Therefore, humidity in most laboratories is not a significant issue and killing at high relative humidity may not actually be drastically curtailed.

Cleanliness has been raised as an issue, including the requirement in the NIH/CDC pamphlet that the bulb be cleaned weekly with ethanol to remove dust and dirt, although no citation is given demonstrating the need in a BSC (U.S. Department of Health and Human Services et al., 2000). Considering that the bulbs reside in, effectively, a Class 100 atmosphere, the source and amount of any such dust remains an open issue and one not further addressed in this paper. As a physical agent, it must be conceded that areas hidden from the light are not disinfected. Boxes of pipette tips left in a BSC will not have the tip disinfected through the case, will not have the area under them disinfected, nor will areas in shadows cast by the boxes be adequately disinfected. It must also be recognized that UV light will damage many materials which may be used within a biosafety cabinet, including many plastics and rubber-based materials, which could result in other hazards (e.g., leak in aspirator tubing or gas burner tubing). However, the NIH/CDC pamphlet on the selection and use of biosafety cabinets strongly discourages the storage of any materials within the cabinet and thus should not be a major concern in a lab which adheres to good laboratory practices.

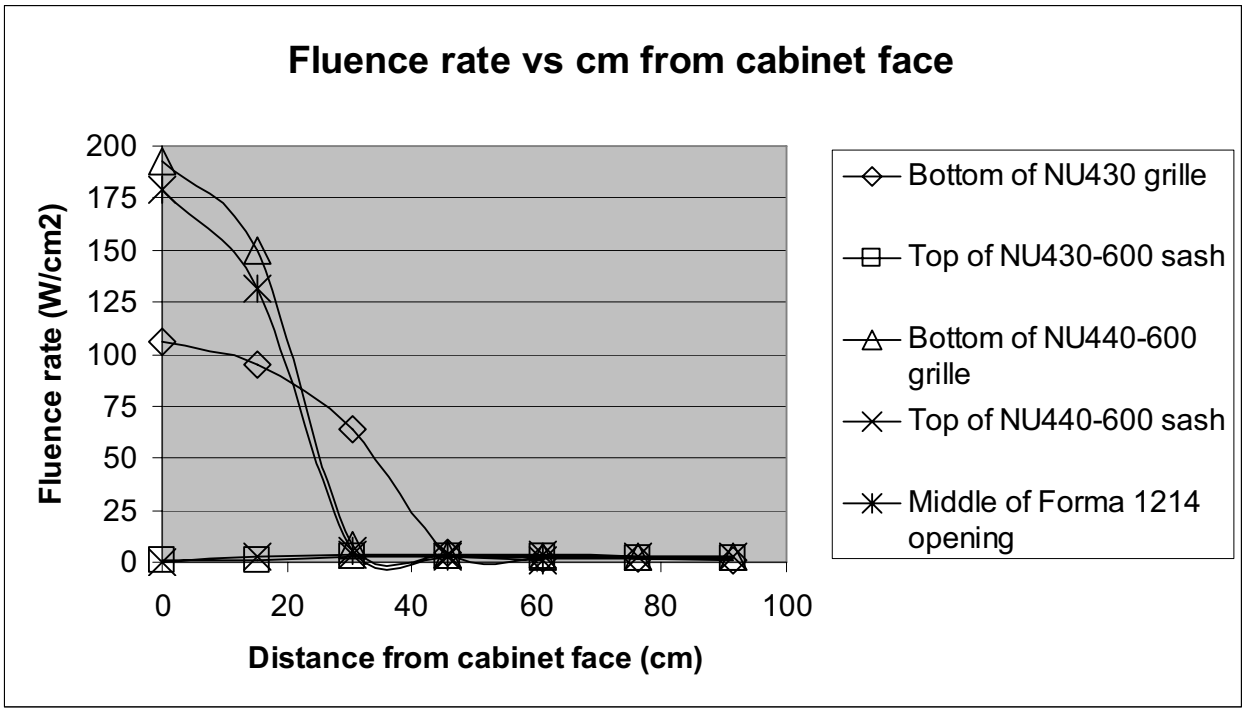
Results

Three major cabinet manufacturers were contacted and asked what percentage of the 254 nm radiation escaped through the glass. None could provide documented data. Therefore, a calibrated UV photometer (UVP UVX radiometer, with a 254 nm probe [UVX-25]) was used to determine the irradiance through the glass. After measuring 45 cabinets from three major cabinet manufacturers onsite (NuAire, Baker, and Forma), the irradiance observed through the glass was found to be 0.9 +/- 0.8 $\mu W/cm^2$ (mean +/- 2 SD), with a range of 0.2 to 1.8 $\mu W/cm^2$. With the sash closed, it would take, on average, over 6667 seconds (111 minutes) for uncovered skin in contact with the BSC glass to reach the ACGIH TLV. Even at the worst performing cabinet, it would take 3333 seconds (55.6 minutes) of direct contact to reach the TLV.

However, the open area below the sash provides no glass to attenuate the radiation. During the survey, it was observed whether the cabinet was interlocked and the irradiance at the center of the open area, in the plane of the sash, was measured for the cabinets without interlocks. Thirty-five of the 45 were not interlocked and the mean flux at the center of the open area for these cabinets was 118.2 +/- 93.8 $\mu W/cm^2$. This allowed only an average of 50 seconds before reaching the TLV. With a 8-12 inch opening and 4 to 6 foot length, the irradiance could be expected to decrease in a roughly linear fashion with increasing distance at the same height as the opening, anticipating that the open area functioned as a plane source. However, data obtained from three different cabinets did not agree with that approximation (Figure 1). Within 15 cm of the opening, a significant irradiance was obtained (>90 $\mu W/cm^2$). However, by 30 cm in two cabinets, and 45 cm in the other, the irradiance was approximately 4 $\mu W/cm^2$. At that distance, one could have bare skin exposed for 1500 seconds (25 minutes). At the top of the glass (142 and 136 cm), the maximum irradiance (3.1 and 4.1 $\mu W/cm^2$, respectively) was observed 45.7 cm (18") from the plane of the sash. This would allow a person to stand 18" from the cabinet, facing the cabinet and not reach the TLV at the eyes for at least 1460 seconds (>24 minutes). Moving in either direction lowered the irradiance. Taller individuals would receive, as expected, lower doses to the eyes.

The use of PPE also plays a role in dose reduction. The publication of the data provided below are intended to demonstrate the degree of conservatism to one of the objections, and should not be construed as furnishing an excuse for an individual to intentionally expose themselves to UV radiation. Entry into any research laboratory at our facility requires the use of safety glasses. As shown in Table 1, placing commercially-available (UVEX) poly-

Figure 1
Irradiance with open sash at varying distances from cabinet face.



carbonate safety glasses in front of the UVX-25 probe while the probe was held in the center of the open face of a BSC reduced the irradiance by 97%, from 179 to 5.6 $\mu W/cm^2$. This reduction in irradiance would allow an individual to place their face in the opening and protect their face for over 1000 seconds (17.9 minutes). Although not recommended as a UV protection device, a single thickness of either nitrile or latex gloves wrapped around the UV-25 probe and placed in the center of a BSC reduced the irradiance to background (Table 1). Use of Tyvek® arm shields or coveralls, or lab coats, disposable or reusable, did not reduce the exposures to background. Moreover, a disposable lab coat allowed a significant leakage of UV through it, allowing only a 114 second exposure to an otherwise bare arm in the center of a BSC.

Discussion

The unpublished and unsubstantiated claim by some biosafety professionals that researchers do not need and do not use UV is undercut at our site by finding that during our survey, we only found 4 of 45 biosafety cabinets not equipped with UV lights (8.8%). The vast majority of our UV-equipped cabinets were using the light to assist with disinfecting their cabinets. Users of these BSCs also used chemical disinfectants, with isopropanol being the most common disinfectant in our tissue culture areas (data not shown).

The results are similar to results obtained by Noll (Noll, 1995), although, in this study, the time needed to reach the TLV was longer. Without knowing additional experimental details used by Noll, there can be no discussion of why there was relatively good agreement regarding the time to reach the TLV (0.83 minutes in this study vs. 0.47 to 0.73 in the Noll study) at hand level, and in the "room center" (34 to 83 minutes in this study at 91 cm from the cabinet vs. 32 to 84 minutes in the Noll study), with poorer agreement at eye level (24 to 32 minutes in this study at 45.7 cm from the cabinet vs. 13 to 24 minutes at an unknown distance in the Noll study).

There are also specific advantages to the use of 254 nm radiation as an adjunct to disinfection. They include:

1. Neither major lesion generated by UV allows polymerases used in PCR to "read through" and amplify the damaged template. This makes it an ideal disinfectant to prevent cross contamination of PCR samples. Researchers performing PCR would be precluded from using biosafety cabinets to prepare samples if UV lights were banned.
2. Unlike most disinfectants, use of a physical disinfectant leaves no residue. The disinfecting action stops upon de-energizing the bulb.
3. Mutation to a fully-resistant phenotype is virtually impossible. Unlike chemical disinfectants, it is not possible to activate an efflux pump or degrade the active agent biochemically. Vegetative organisms do possess effective DNA repair processes, including photoreactivation, exci-

Table 1

Irradiance through different types of PPE.

Type of glove	**µW/cm2**
Latex	background
Nitrile (blue)	background
Nitrile (green)	background
Nitrile (teal)	background
Nitrile (purple)	background
Other PPE	
Tyvek® arm shield	background
Tyvek® coverall	1.1
Disposable lab coat	52.5
Lab coat	2
Unshielded fluence rate	282 µW/cm2
Polycarbonate safety glasses	5.6
Unshielded fluence rate	179.3 µW/cm2

sion and recombination processes, but they are the wild type organisms and the doses required to inactivate many of them have been determined experimentally (www.uvp.com/pdf/ab-115.pdf).

4. Ultraviolet light is an effective germicide and virucide for organisms directly exposed to the UV light. As stated in the Background section, the UV inactivation doses have been determined for a variety of organisms. Compared to the data used to support listing by the EPA as a virucide, UV is more efficient for most vegetative organisms and viruses. Even using the NIH/CDC criterion of the minimum acceptable irradiance in a biosafety cabinet of 40 µW/cm^2, it takes 12.5 minutes to reach the 30,000 µJ/cm^2 found to inactivate spore forming organisms. Use of a UV light in excess of an hour or overnight is massive overkill. However, use of UV to disinfect the interior surface or contents of a container is likely to be futile, as UV has little penetrating power.

We do not, however, intend to discourage the use of chemical disinfectants even though they have several limitations of their own. It is not uncommon to find:

1. The incorrect disinfectant being used, such as isopropanol against adenovirus; and
2. Inadequate disinfection time for the agent, agent load, or organic material present.

It is extremely uncommon to find a biosafety cabinet wetted for 10 minutes, as is done in the Association of Official Analytical Chemists (AOAC) disinfection tests (5). Inadequate disinfection time or failure to wipe beneath pipette boxes or other materials left in a biosafety cabinet results in the same false sense of security as relying solely on UV as a disinfecting agent.

One may choose to argue that a ban on the use UV light should be viewed as part of an "ALARA" (As Low as Reasonably Achievable) program for non-ionizing radiation. If one accepts a linear, no-threshold approach to the stochastic effects (e.g., skin cancer), then this is not a difficult decision. However, this needs to be explicitly stated as a goal and balanced against the benefits of UV and the risks involved in using flammable (ethanol or isopropanol) or oxidizing chemicals (bleach).

Rather than simply eliminate the use of ultraviolet light, a more useful approach is to recognize the benefits and risks of the radiation. Since the only significant leakage of UV from a biosafety cabinet is from the front opening, taking steps to eliminate that leakage is the key to eliminating exposure. Requesting the manufacturer to interlock the light with the sash is a simple technical fix to the problem. ABSA could also request that such an interlock be included in the revision to NSF 49. For those cabinets with fixed sashes, an opaque covering could be provided that allowed air flow while minimizing UV exposure. Some cabinetmakers do manufacture retrofit kits to interlock the sash and UV bulb. An additional precaution would be the addition of a timer to the UV light, a feature recently added by at least one of the major manufacturers to their "digital" model. This would allow adequate time for disinfection without the potential for a person in the same room to reach the TLV for 254 nm radiation under any circumstances.

Even the most ardent supporters of the use of UV must also be willing to concede that UV lights in BSCs with open sashes are potential hazards and staff members must be informed of those risks and advised not to loiter

near the cabinets. Nearly all biosafety professionals can recall episodes during which a researcher has shown ignorance or complete indifference to the immediate and long-term risk from UV exposure, or the limitations of UV radiation. Attempting to culture cells while UV lights are on, attempting to sterilize the inside of a container by irradiating the exterior of the container with UV, or leaving the UV on all night are only a few examples of abuse of UV. However, an absolute prohibition of their operation in the presence of staff, is, however, equally not supported by the data. The prohibition for the operation of UV lights in cabinets with interlocks and no open areas while staff is present can also no longer be supported by experimental data and should be eliminated. Limited use of UV in BSCs with required safety features such as interlocks and timers is a reasonable compromise.

Acknowledgements

The authors wish to thank Mr. Brian Petuch, RBP and Mr. Joseph Gyuris, RBP for their assistance and expert advice during the review of this manuscript. The expert reviewers are thanked for their constructive criticisms.

References

American Conference of Governmental Industrial Hygienists (ACGIH). (2005). TLVs and BEIs based on the documentation of the threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati: Author.

Alam, M., & Ratner, D. (2001). Cutaneous squamous-cell carcinoma. *New England Journal of Medicine, 344*(13), 975-983.

AOAC International. (1999). *Official methods of analysis* (16th ed., 5th rev.). P. Cunniff (Ed.). Gaithersburg, MD: Author.

Block, S. (1991). *Disinfection, sterilization and preservation* (4th ed.). Malvern, PA: Lea & Febiger.

Centers for Disease Control and Prevention. (2006). Skin Cancer: Preventing America's Most Common Cancer. www.cdc.gov/cancer/nscpep/about2004.htm

Friedberg, E. C., Walker, G. C., & Siede, W. (1995). *DNA repair and mutagenesis*. Washington, DC: ASM Press.

Gilchrest, B. A., Eller, M. S., Geller, A. C., & Yaar, M. (1999). The pathogenesis of melanoma induced by ultraviolet radiation. *New England Journal of Medicine, 340*(17), 1341-1348.

Jagger, J. (1985). *Solar-UV actions on living cells*. New York: Praeger Scientific.

National Toxicology Program. (2005). *UVC*. In Report on Carcinogens (11th ed.). http://ntp.niehs.nih.gov/ntp/roc/eleventh/profiles/s183uvrr.pdf

Noll, M. L. (1995). Ultraviolet radiation exposures in biomedical research laboratories. *Applied Occupational and Environmental Hygiene, 10*(12), 969-972.

NSF International. (2004). *Class II (Laminar Flow) biohazard cabinetry*. NSF49-2004a. Ann Arbor, MI: Author.

Rubin, A. I., Chen, E. H., & Ratner, D. (2005). Basal-cell carcinoma. *New England Journal of Medicine, 353*(21), 2262-2269.

Shechmeister, I. L. (1991). *Sterilization by ultraviolet irradiation*. In S. S. Block (Ed.), Disinfection, sterilization and preservation (4th ed.). Philadelphia: Lea & Febiger.

Suess, M. J. (Ed.). (1982). *Nonionizing radiation protection*. Geneva, Switzerland: World Health Organization.

U.S. Department of Health and Human Services, Centers for Disease Control and Prevention & National Institutes of Health. (2000). *Primary containment for biohazards: Selection, installation and use of biological safety cabinets* (2nd ed.). J. Y. Richmond & R. W. McKinney (Eds.). Washington, DC: U.S. Government Printing Office. Available at www.cdc.gov/od/ohs/biosfty/bsc/bsc.htm