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Standard Guide for Use of Lighting in Laboratory Testing¹

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1. Scope

1.1 The use of artificial lighting is often required to study the responses of living organisms to contaminants in a controlled manner. Even if the test organism does not require light, the investigator will generally need light to manipulate the samples, and the test might be conducted under the ambient light of the laboratory. One will need to consider not only whether the particular test organism requires light for growth, but also whether the environmental compartment relevant to the test is exposed to light and, if so, what the attributes of light are in that compartment. The light could affect growth of the organism or toxicity of a contaminant, or both. For instance, it has been shown that the toxicity of some organic pollutants is enhanced dramatically by the ultraviolet (UV) radiation present in sunlight (1, 2).² Furthermore, the level of ambient lighting in the laboratory (which might affect the test) is not standardized, nor is it comparable to natural environments. It is thus important to consider lighting in all forms of environmental testing. When light is used in the test, one should determine whether the spectral distribution of the radiation source mimics sunlight adequately to be considered environmentally relevant. Also, the container or vessel for the experiment must be transparent, at the point of light entry, to all of the spectral regions in the light source needed for the test.

1.2 It is possible to simulate sunlight with respect to the visible:UV ratio with relatively inexpensive equipment. This guide contains information on the types of artificial light sources that are commonly used in the laboratory, compositions of light sources that mimic the biologically relevant spectral range of sunlight, quantification of irradiance levels of the light sources, determination of spectral outputs of the light sources, transmittance properties of materials used for laboratory containers, calculation of biologically effective radiation, and considerations that should go into designing a relevant light source for a given test.

¹ This guide is under the jurisdiction of ASTM Committee E50 on Environmental Assessment, Risk Management and Corrective Action and is the direct responsibility of Subcommittee E50.47 on Biological Effects and Environmental Fate.

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² The boldface numbers in parentheses refer to the list of references at the end of this guide.

1.3 Special needs or circumstances will dictate how a given light source is constructed. This is based on the requirements of the test and the environmental compartment to which it is targeted. Using appropriate conditions is most important for any experiment, and it is desirable to standardize these conditions among laboratories. In extreme cases, tests using unusual lighting conditions might render a data set incomparable to other tests.

1.4 The lighting conditions described herein are applicable to tests with most organisms and using most chemicals. With appropriate modifications, these light sources can be used under most laboratory conditions with many types of laboratory vessels.

1.5 The attributes of the light source used in a given study should list the types of lamps used, any screening materials, the light level as an energy fluence rate (in W m^{-2}) or photon fluence rate (in $\mu\text{mol m}^{-2} \text{s}^{-1}$), and the transmission properties of the vessels used to hold the test organism(s). If it is relevant to the outcome of a test, the spectral quality of the light source should be measured with a spectroradiometer and the emission spectrum provided graphically for reference.

1.6 The sections of this guide are arranged as follows:

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1.7 The values stated in SI units are to be regarded as the standard. The values given in parentheses are for information only.

1.8 This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use. Specific precautionary statements are given in Section 6.

1.9 This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.

2. Referenced Documents

2.1 ASTM Standards:³

E943 Terminology Relating to Biological Effects and Environmental Fate

E1218 Guide for Conducting Static Toxicity Tests with Microalgae

E1415 Guide for Conducting Static Toxicity Tests With *Lemna gibba* G3 (Withdrawn 2021)⁴

E1598 Practice for Conducting Early Seedling Growth Tests (Withdrawn 2003)⁴

IEEE/ASTM SI 10 Standard for Use of the International System of Units (SI): The Modern Metric System

3. Terminology

3.1 *Definitions*—The words “must,” “should,” “may,” “can,” and “might” have very specific meanings in this guide. “Must” is used to express an absolute requirement, that is, to state that the conditions ought to be designed to satisfy appropriate lighting, unless the purpose of a test requires a different design. “Must” is only used in connection with factors that directly relate to the acceptability of specific conditions. “Should” is used to state that a specified condition is recommended and ought to be met if possible. Although violation of one “should” is rarely a serious matter, violation of several will often render the results of a test questionable. Terms such as “is desirable,” “is often desirable,” and “might be desirable” are used in connection with less important factors. “May” is used to mean is (are) allowed to, “can” is used to mean is (are) able to, and “might” is used to mean could possibly. Thus the classic distinction between may and can is preserved, and might is never used as a synonym for either “may” or “can.”

3.2 *Descriptions of Terms Specific to This Standard* (see also Terminology **E943**):

3.2.1 *fluence*, *n*—amount of light per unit area, expressed as energy (J m^{-2}) or photons (mol m^{-2}). This is sometimes equated to light dose.

3.2.2 *fluence rate*, *n*—flow rate of light, flux of light, or the amount of light per unit area per unit time. It is sometimes

referred to as light intensity, although this is not a desirable term because intensity refers to the amount of radiation in a unit angle. The energy fluence rate (also irradiance, energy flow rate, or power) is usually given in units of $\text{J m}^{-2} \text{s}^{-1}$ or W m^{-2} ($1 \text{ J s}^{-1} = 1 \text{ W}$). The photon fluence rate (flow rate on a quantum basis) is usually given in the unit $\mu\text{mol m}^{-2} \text{s}^{-1}$. (This is equivalent to $\mu\text{Einstein m}^{-2} \text{s}^{-1}$. An Einstein is Avogadro’s number (a mole) of photons and was used for quantum measurements but is no longer an SI — supported unit (see **IEEE/ASTM SI 10**).) The conversion between energy fluence rate and photon fluence rate is as follows:

$$\mu\text{mol m}^{-2} \text{s}^{-1} = \text{W m}^{-2} \times \lambda(\text{nm}) \times 8.36 \times 10^{-3} \quad (1)$$

3.2.2.1 *Discussion*—This illustrates an inherent problem of converting between light units: the energy is wavelength (λ) dependent, so conversion between energy and quantum units requires knowledge of the spectral distribution of the light source (see **10.2.4** for conversion guidelines).

3.2.3 *fluorescence*, *n*—emission of light by an excited atom or molecule.

3.2.4 *foot-candle*, *n*—lumen per ft^2 (see **3.2.8**).

3.2.5 *frequency* (ν), *n*—description of radiation as the number of wave peaks passing a point in space per unit time. Units are normally cycles s^{-1} or Hz.

3.2.6 *IR*, *n*—infrared radiation (wavelength range, 760 nm to 2000 nm).

3.2.7 *irradiance*, *n*—quantity of radiant energy received by a unit area per unit time. This is the same as the energy fluence rate.

3.2.8 *lumen*, *n*—light emitted by a point source of 1 cd. It is a unit of luminosity or brightness used in photography and stage lighting and is irradiance based on sensitivity of the human eye (maximum sensitivity at 550 nm). It has the same dimensions as watts because it is equivalent to irradiance by definition. However, the lumen as a measurement is wavelength dependent (1 lm at λ 560 nm is 1.5 mW, and 1 lm at λ 430 nm is 127 mW) (see **10.2.3**), so extreme care should be used with this unit. If possible, light levels based on lumens should be converted to an appropriate light unit for environmental studies (for example, W m^{-2} or $\mu\text{mol m}^{-2} \text{s}^{-1}$) (see **10.2.4** for conversion guidelines).

3.2.9 *lux*, *n*—lumen per m^2 (see **3.2.8**).

3.2.10 *photon*, *n*—one quanta (or single indivisible packet) of light or radiant energy. A mole of photons (an Einstein) equals Avogadro’s number (6.022×10^{23}). The energy of a photon is related to its frequency or wavelength and is given by $E = h\nu = hc/\lambda$, where h = planks constant ($6.6 \times 10^{-34} \text{ J s}$), c = speed of light ($3 \times 10^8 \text{ m s}^{-1}$), ν = frequency, and λ = wavelength (if c is used in m s^{-1} , then λ must also be in m).

3.2.11 *spectral distribution*, *n*—a description of a light source as the quantity of light at each wavelength. An energy spectral distribution is the energy of a light source given as a function of wavelength. A photon spectral distribution is the number of photons in a light source as a function of wavelength.

3.2.12 *UV-A*, *n*—ultraviolet A radiation (wavelength range, 320 nm to 400 nm).

³ For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard’s Document Summary page on the ASTM website.

⁴ The last approved version of this historical standard is referenced on www.astm.org.

3.2.13 *UV-B*—ultraviolet B radiation (wavelength range, 290 nm to 320 nm).

3.2.14 *UV-C, n*—ultraviolet C radiation (wavelength range, 200 to 290 nm).

3.2.15 *visible light, n*—the spectral region visible to humans (wavelength range, 400 nm to 700 nm). This is the photosynthetically active region of the spectrum as well.

3.2.16 *wavelength (λ), n*—the description of radiation (or radiant energy) as the distance between two consecutive peaks in an electromagnetic wave. Units are normally in nm. The energy of a photon is inversely proportional to its wavelength. Also, frequency \times wavelength = speed of light.

4. Summary of Guide

4.1 This guide provides information on several types of laboratory light sources and the need for standardized lighting. The varieties of commercially available light sources and the spectral quality of their outputs are presented first. The ways in which different lamps can be assembled to mimic sunlight are then summarized. There is a discussion of the methods for measuring the amounts and spectral quality of light, and the need for accurate standardized methods. Finally, a discussion on biologically effective radiation is included.

5. Significance and Use

5.1 The information in this guide is designed to allow investigators conducting research or tests of environmental relevance to select appropriate light sources.

5.2 Investigators will be able to make reasonable selections of light sources based on cost, the requirements of the test organisms, and the properties of the test chemicals.

5.3 These methods have major significance for the comparison of results between laboratories. Investigators at different sites will be able to select similar light sources. This will provide standardization of a factor that can have major impact on the effects of hazardous chemicals.

6. Safety Precautions

6.1 Many materials can affect humans adversely if precautions are inadequate. Therefore, eye and skin contact with radiation (especially UV) from all light sources should be minimized by such means as wearing appropriate protective eyewear, protective gloves (especially when washing equipment or putting hands in test chambers or solutions), laboratory coats, and aprons. Special precautions, such as enclosing test chambers and their light sources, and ventilating the area surrounding the chambers, should be taken when conducting tests. Care should be taken when using light measuring equipment to prevent the accidental breakage of light sources into test vessels to ensure organism health. Information on toxicity to humans (3-5), recommended handling procedures (6-8), and chemical and physical properties of the test material and light source should be studied before a test has begun. Special procedures might be necessary with UV light sources, radio-labeled test materials, and materials that are, or are suspected of being, carcinogenic (9-11).

6.2 *Ozone*—Many UV light sources (those emitting UV-C) produce ozone. For instance, xenon (Xe) arc lamps produce significant amounts of ozone. Adequate ventilation should be provided to remove the ozone.

6.3 *Ultraviolet Radiation*—Any light source producing UV-B or UV-C is harmful to eyes and skin. In particular, contact with eyes is to be avoided, even for very short periods of time. Eyes can be shielded with appropriate eyewear (safety glasses or goggles that absorb UV radiation) available from most scientific supply companies. The spectral quality of the eyewear should be checked periodically with a UV/vis spectrophotometer. Transmission should be less than 0.1 % for all wavelengths below 330 nm. Contact with skin is also to be prevented. In general, all light sources that generate UV-B will generate some UV-C as well.

6.4 *Heat*—Many light sources, especially short-arc lamps, create a high fluence rate of IR radiation. Skin, clothing, and other materials exposed to high levels of IR radiation are subject to severe burns or may ignite.

6.5 **Warning**—Mercury has been designated by EPA and many state agencies as a hazardous material that can cause central nervous system, kidney and liver damage. Mercury, or its vapor, may be hazardous to health and corrosive to materials. Caution should be taken when handling mercury and mercury containing products. See the applicable product Material Safety Data Sheet (MSDS) for details and EPA's website – <http://www.epa.gov/mercury/faq.htm> - for additional information. Users should be aware that selling mercury and/or mercury containing products into your state may be prohibited by state law.

7. Lamps

7.1 *Artificial Lighting*—The development of artificial lighting stems from two needs: (1) the requirement for inexpensive commercial and public lighting and (2) specialized lighting for research and technology (see Table 1 for a listing of some of the light sources available). There are essentially two ways that light can be generated for toxicity testing: (1) electric discharge lamps, those that are based on photon emission from an electronically excited gas (for example, fluorescent and short-arc lamps); and (2) thermal lamps, those that are based on photon emission from a heated filament (for example, incandescent lamps) (12, 13). Laser sources are not practical for most toxicology studies and are not discussed in this guide.

7.2 *Light Sources:*

7.2.1 *LED Lamps*—Light-emitting diode (LED) lamps are composed of diodes made of semi-conductive materials, such as silicon or selenium, that contain two electrodes (an anode and cathode) that produce light upon introduction of an electric current. Heat produced by LED lamps is absorbed into a heat sink, which prevents performance issues and also any heat issues for users. LED lamps are more efficient than incandescent and fluorescent lamps, and they are also directional, meaning they emit light in a specific direction in comparison to incandescent and fluorescent lamps which produce light and heat in all directions. They do not typically 'burn out' or fail like incandescent or fluorescent lamps. Instead, 'lumen depreciation' occurs, and the lifetime of an LED light is considered

TABLE 1 Light Sources

Lamp	Spectral Regions	Fluence Rate ^A	Approximate Cost ^B		Manufacturers ^C
			Lamp	Fixture ^D	
Fluorescent	visible	20–400	5–20	10–30	E,F,G
	UV-A	1–50	10–40	10–30	H,I
	UV-B	1–50	10–40	10–30	H,I,J
	UV-C	1–50	10–40	10–30	H
	visible + UV-A	20–400	20–50	10–30	K
Short-arc					
Hg	UV-B, UV-A, visible	500–2000	150–1000	2000–6000	L,M,N
Xe	UV-B, UV-A, visible	500–2000	150–1000	2000–6000	L,M,N,O
Metal halide	UV-A, visible	300–1000	100	1000	K
Sodium vapor	visible	300–1000	100	1000	K
Microwave	UV-B, UV-A, visible	500–2000	2000	10000	P,Q
Incandescent	visible	100–1000	5–100	10–1000	E,F,G
LED	UV-C, UV-B, UV-A, visible		4-10	10-30	E,F,G

^A In $\mu\text{mol m}^{-2} \text{s}^{-1}$.
^B In U.S. dollars (in 1994).
^C These are representative manufacturers but are by no means the only manufacturers. This listing should in no way be considered an endorsement.
^D Power supply and lamp holder.
^E General Electric; this company markets through local electrical suppliers.
^F Philips; this company markets through local electrical suppliers.
^G Westinghouse; this company markets through local electrical suppliers.
^H Southern New England Ultraviolet Co., Brantford, CT, 203-483-5810.
^I Local theatrical lighting suppliers.
^J National Biological Corp., Twinsberg, OH, 216-425-3535.
^K Dura-Test Corp., Fairfield, NJ, 800-289-3876.
^L Oriol Corp., Stratford, CT, 203-377-8282.
^M Ealing Electro-Optics Inc., South Natick, MA, 617-651-8100.
^N Photon Technology Inc., South Brunswick, NJ, 908-329-0910.
^O Heraeus DSET Laboratories, Inc, Phoenix, AZ, 602-465-7356.
^P Fusion Lighting, Rockville, MD, 301-251-0300.
^Q Hutchins International Ltd, Mississauga, Ont., 416-823-8557.

complete when the brightness of the LED lamp depreciates by 30 % or more. Common LED colors are red, green, and blue, with white light produced using a combination of different colored LED lights and/or a phosphor material that converts the light color to white. The LEDs generate narrow-banded, spatially uniform light at five wavelengths (275 nm, 309 nm, 348 nm, 369 nm, and 406 nm), with irradiances that are stable and easily adjusted to desired levels (Fig. 1 and Fig. 2). Initial investment in LED can be more expensive than other lamps, but the cost savings over time and longevity ultimately make them a less expensive option. LEDs also have many advantages over incandescent light sources, including lower power consumption, longer lifetime, improved physical robustness, smaller size, and faster switching. They also have a wide range of controllable colour temperatures, wider operating temperature, no low temperature startup problems, and a low maintenance cost. LEDs do not contain mercury and have a low health impact as a result of low ultraviolet radiation (UV). LEDs can very easily be dimmed either by pulse-width modulation or lowering the forward current. This pulse-width modulation is why LED lights, particularly headlights on cars, when viewed on camera or by some people, seem to flash or flicker. This is a type of stroboscopic effect. (14, 15).

7.2.2 Fluorescent Lamps—Fluorescent lamps are based on excitation of low-pressure Hg gas by an electric current. When the Hg atoms relax back to ground state, they emit photons at

254 nm (that is, in the UV-C). The 254 nm photons are absorbed by a phosphor coating on the inside of the tube, and the phosphor emits (fluoresces) at longer wavelengths (280 nm to 750 nm). The spectral output of the lamp (Figs. 3 and 4) will thus depend on the composition of the phosphor coating. The most common phosphors are halophosphates, for instance, barium titanium phosphate, manganese-activated magnesium gallate, and calcium halophosphate, which emit mostly in the visible region of the spectrum. Many different types of fluorescent lamps are commercially available (Table 1). The major benefits of fluorescent lamps are the availability of inexpensive fixtures and bulbs, low heat (IR) output, long life, and stable spectral quality. However, the irradiance levels of fluorescent lamps are relatively low; it is difficult to build a fluorescent lighting system with more than approximately $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ (only approximately 20 % of full sunlight). Most fluorescent light sales will be banned by 2020 as per the United Nations Environment Program Minamata Convention (16).

7.2.2.1 Visible Light Fluorescent Lamps—The most common is the cool-white fluorescent type, with a blue light (450-nm) to red light (600-nm) ratio of 1 to 2 on a photon basis (Fig. 3). Two other common types of fluorescent lamps are warm-white, with a higher relative level of red light, and daylight, with a higher relative level of blue light (Fig. 3). Also, lamps with more balanced spectral distributions in the visible region are available (Table 1 and Fig. 3).

7.2.2.2 Ultraviolet Fluorescent Lamps—UV fluorescent lamps have phosphors that emit at approximately 300 nm (tanning lamp) and 350 nm (black light) (Fig. 4). Low-pressure Hg lamps without a phosphor are also available (germicidal lamps). They emit a sharp line at 254 nm and are used in laminar flow hoods and clean rooms to sterilize surfaces prior to use. All of these UV fluorescent lamps are quite common and are available from numerous manufacturers (Table 1).

7.2.3 Metal Vapor Arc Lamps—The basis of short-arc lamps is similar to fluorescent lamps, except that a phosphor is not required. The gas in the lamp is excited by a high electric potential. The gas then completes a direct current (dc) circuit between a cathode and an anode by forming an arc. As the gaseous atoms in the arc relax to ground state, they emit radiation. The two most common gases used are mercury (Hg) and Xenon (Xe). These lamps have very high outputs, with photon fluence rates in the visible spectral region exceeding $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$. However, the lamps, lamp holders, and stable high-voltage dc power supplies are generally expensive.

7.2.3.1 Mercury Short Arc Lamps—High-pressure Hg-arc lamps have five major emission bands: at 365 nm, 405 nm, 436 nm, 546 nm, and 578 nm (Fig. 5). In addition, they have a lower-level emission that is a continuum from 280 nm to 800 nm. These lamps have very high outputs in the five main emission bands, without a great deal of IR. They can thus be very useful for specific applications in which high fluence rates are required.

7.2.3.2 Xenon Short Arc Lamp—Xenon-arc lamps emit with a great deal more lines than an Hg lamp. As such, this source provides a continuum of radiation from approximately 260 nm (UV-C) to 1100 nm (IR) (Fig. 5). In fact, Xe-arc lamps have a very close spectral match to sunlight. This, combined with a

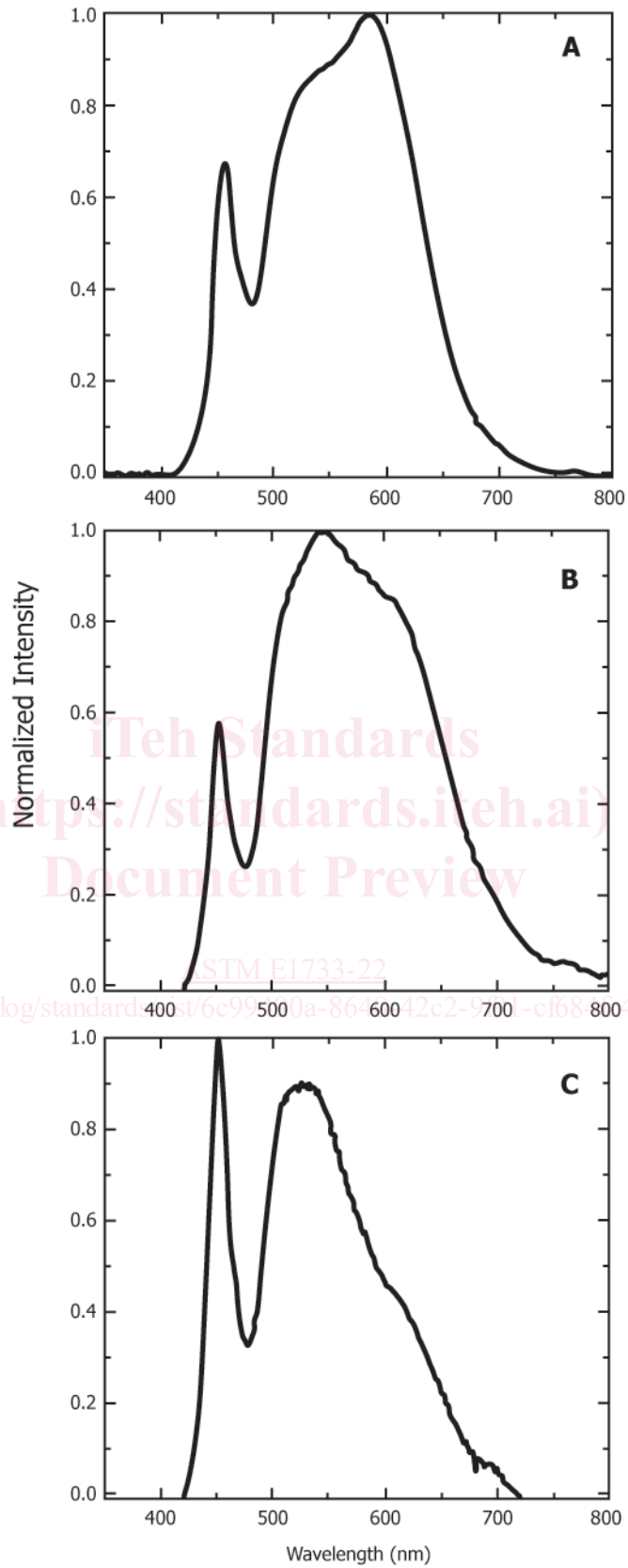


FIG. 1 Spectral Outputs of LED Lamps: (A) Warm White; (B) Bright White; and (C) Daylight

Light emitting diode-1

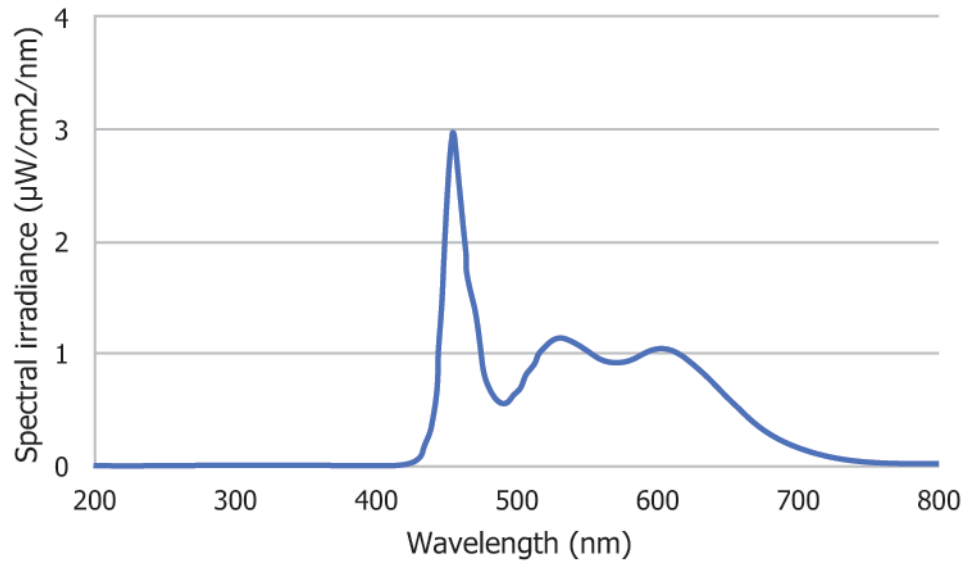


FIG. 2 Spectral Output of LED lamp

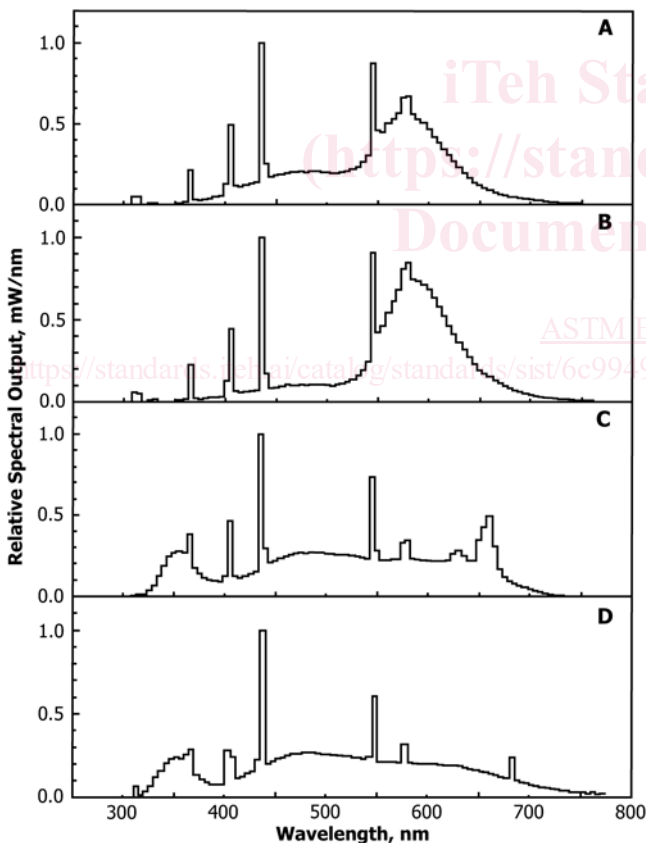


FIG. 3 Spectral Output of Visible Light Fluorescent Bulbs: (A) Cool White; (B) Warm White; (C) Day Light; and (D) Color Classer

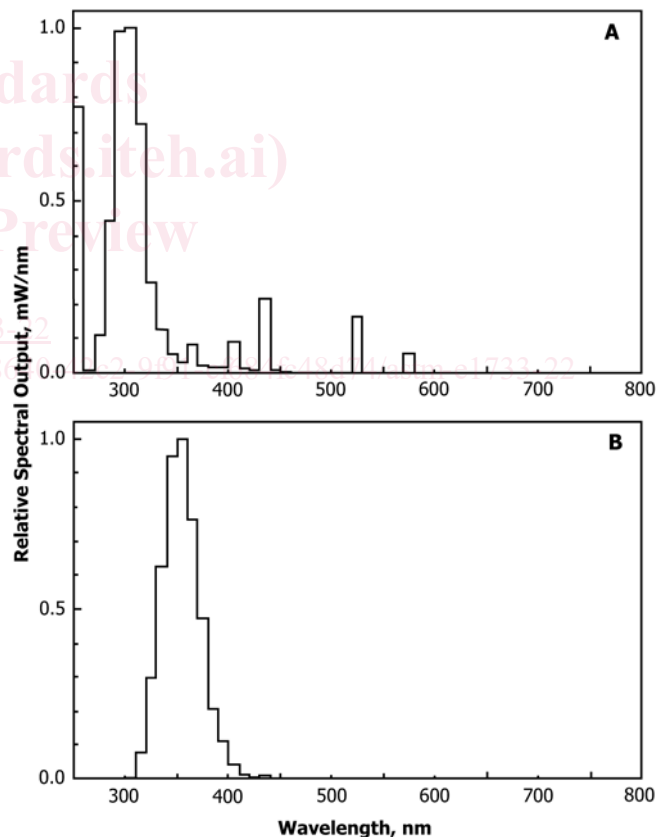


FIG. 4 Spectral Output of UV Fluorescent Lamps: (A) UV-B Lamp; and (B) UV-A Lamp

very high output and an ability to light relatively large areas, makes this source highly attractive for environmental studies. However, the amount of IR in the source can be problematic. Well-cooled chambers and appropriate IR filters might be thus required.

7.2.3.3 Metal Halide Lamps—These are Hg arc lamps doped with the halide salt of another metal. Iodine is the most common halide, and common metals are sodium, scandium, dysprosium, and thallium. The presence of the mixed metal vapors greatly increases the number of spectral emission lines

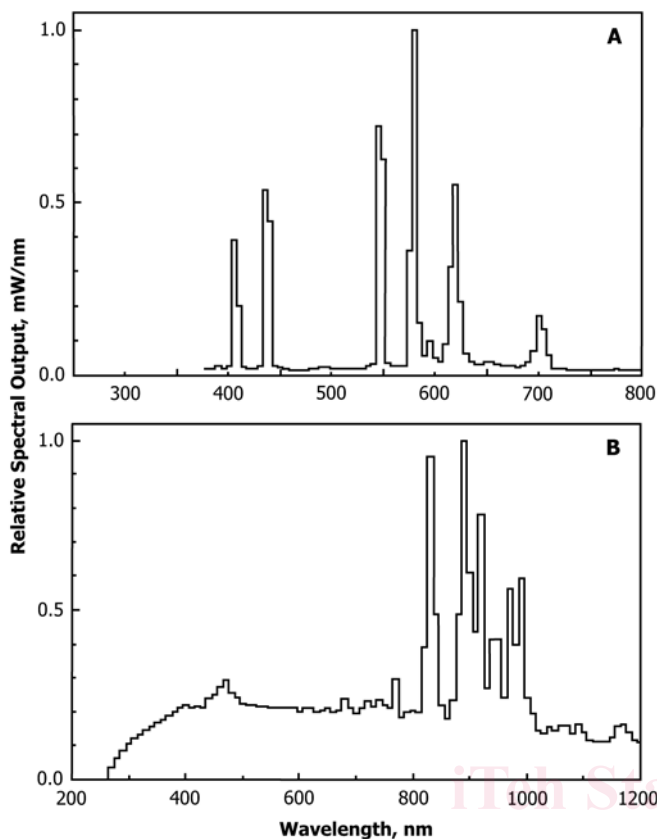


FIG. 5 Spectral Outputs of Short-Arc Lamps: (A) Hg Arc Lamp; and (B) Xe Arc Lamp

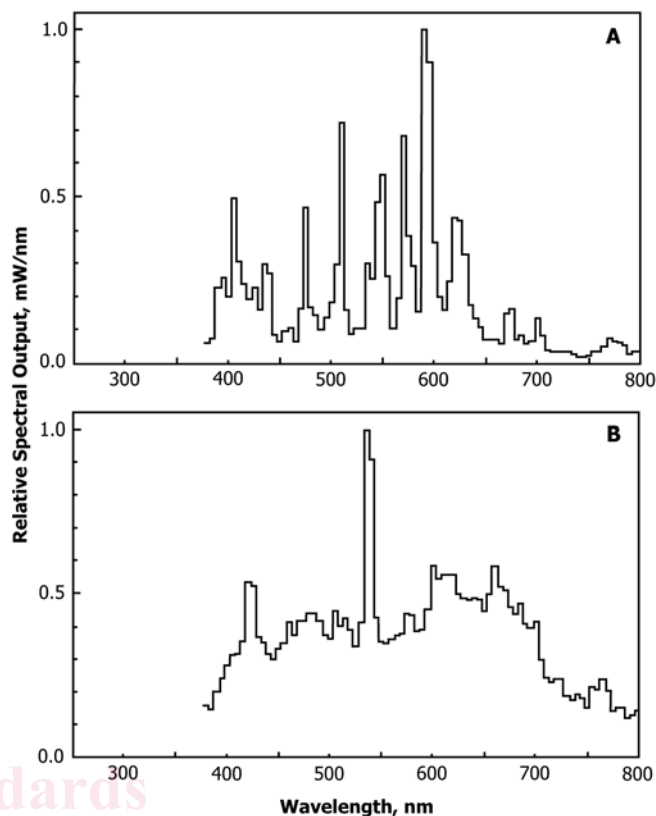


FIG. 6 Spectral Outputs of Metal Halide Lamps: (A) Sodium-Scandium Lamp; and (B) Dysprosium-Thallium Lamp

relative to Hg alone (Fig. 6). These lamps have high outputs and very good spectral distributions in the visible region, giving them an excellent “color” quality. The lamps also have relatively high outputs and low IR. They are commonly used in stadium and arena lighting for these reasons. They are a good alternative to Xe arc lamps for laboratory purposes. Also, the power supply for these lamps is an alternating current (ac) ballast, which is much less expensive than the dc power supplies required for Hg and Xe short arc lamps.

7.2.4 Sodium Vapor Lamps—Low-pressure sodium (Na) vapor lamps emit a sharp band at 589 nm (orange light) (Fig. 6). High-pressure Na vapor lamps also emit around at 589 nm, but with a much wider emission band (approximately 100 nm) (Fig. 7). Although these sources have limitations due to their monochromatic nature, they are relatively inexpensive, they can reach high fluence rates, and the light quality is near the peak wavelength for human vision. They are thus an excellent light source for street lighting. Although not necessarily the best source for biological testing, especially when plant growth is involved, they are nonetheless used to achieve high fluence rates without heat problems. This is because orange light can be used reasonably efficiently for photosynthesis (17).

7.2.5 Microwave-Powered Light Sources—An emerging technology is the microwave-powered lamp, which work by microwave excitation of an elemental sulphur powder inside a small spherical bulb. The microwave-excited sulphur emits visible photons. These lamps have very high fluence rates in the visible spectral region from 400 nm to 700 nm, and they

mimic sunlight accurately in this spectral region (Fig. 8). They are also a focusable point source. Therefore, they are an excellent choice for many applications, especially plant growth. Microwave lamps coincidentally have little IR, preventing most heat creation problems associated with high irradiance lighting. Also, they have little UV, and the addition of these wavelengths to a test is thus at the choice of the investigator. The bulb life for these lamps is very long, approximately 10 000 h. Bulbs containing powders of different composition that emit in the UV-B or UV-A have also been developed. The only disadvantage at present with microwave lamps is cost, due partly to the expense of new technology; however, development is under way to bring down the cost.

7.2.6 Incandescent Lamps—These lamps contain a solid body (filament) that is heated by an electric current. The heated filament emits in a continuum with a spectral quality described by the temperature of the filament. The higher the temperature of the filament, the shorter the wavelengths that are emitted by the lamp (Fig. 9). The most common filament is tungsten; this metal is strong, and has a high melting temperature and low vapor pressure at high temperature. This gives the filament a long life. To minimize evaporation of the metal, the bulb is generally filled with inert and stable gases (such as 90 % argon, 10 % nitrogen). A small amount of a halogen gas (at approximately 1 %) is often used as well (thus the name tungsten-halogen lamps); this causes evaporated tungsten to redeposit on the filament, further increasing the life of the lamp (up to 2000 h). The lamps have excellent light quality in the visible region