Standard Guide for
Use of Lighting in Laboratory Testing[1]

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.

1.3 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 μmol m^{-2} 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:

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

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

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 and health practices and determine the applicability of regulatory limitations prior to use.* Specific precautionary statements are given in Section 6.

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

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*—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*—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 $J\ m^{-2}\ s^{-1}$ or $W\ m^{-2}$ (1 J s^{-1} = 1 W). The photon fluence rate (flow rate on a quantum basis) is usually given in the unit $\mu mol\ m^{-2}\ s^{-1}$. (This is equivalent to $\mu Einstein\ m^{-2}\ s^{-1}$. 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:

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

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*—emission of light by an excited atom or molecule.

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

3.2.5 *frequency, (ν)*—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*—infrared radiation (wavelength range, 760 nm to 2000 nm).

3.2.7 *irradiance*—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*—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 $\mu mol\ m^{-2}\ s^{-1}$) (see 10.2.4 for conversion guidelines).

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

3.2.10 *photon*—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 (6.6×10^{-34} J s), c = speed of light (3×10^8 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*—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*—ultraviolet A radiation (wavelength range, 320 to 400 nm).

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

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

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[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.

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

3.2.16 *wavelength (λ)*—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 × 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 eyeware, 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. 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 eyeware (safety glasses or goggles that absorb UV radiation) available from most scientific supply companies. The spectral quality of the eyeware 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 *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 to 750 nm). The spectral output of the lamp (Figs. 1 and 2) 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 µmol m^{-2} s^{-1} (only approximately 20 % of full sunlight).

7.2.1.1 *Visible Light Fluorescent Lamps*—The most common is the cool-white fluorescent type, with a blue light

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

[A] In µmol m^{-2} 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] Oriel 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.
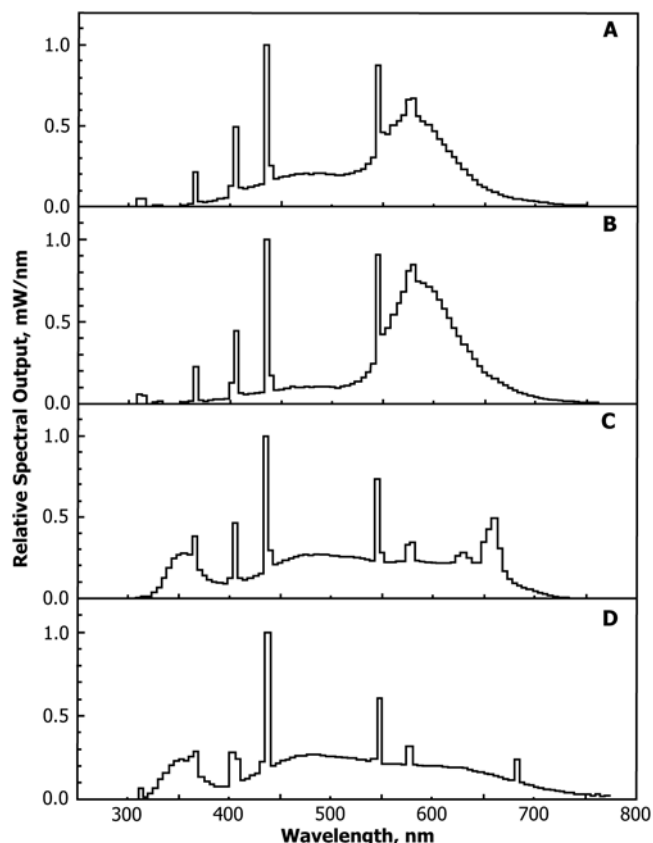[Q] Hutchins International Ltd, Mississauga, Ont., 416-823-8557.

FIG. 1 Spectral Output of Visible Light Fluorescent Bulbs: (A) Cool White; (B) Warm White; (C) Day Light; and (D) Color Classer

(450-nm) to red light (600-nm) ratio of 1 to 2 on a photon basis (Fig. 1). 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. 1). Also, lamps with more balanced spectral distributions in the visible region are available (Table 1 and Fig. 1).

7.2.1.2 *Ultraviolet Fluorescent Lamps*—UV fluorescent lamps have phosphors that emit at approximately 300 nm (tanning lamp) and 350 nm (black light) (Fig. 2). 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).

7.2.2 *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 µmol m^{-2} s^{-1}. However, the lamps, lamp holders, and stable high-voltage dc power supplies are generally expensive.

7.2.2.1 *Mercury Short Arc Lamps*—High-pressure Hg-arc lamps have five major emission bands: at 365, 405, 436, 546, and 578 nm (Fig. 3). In addition, they have a lower-level

emission that is a continuum from 280 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.

7.2.2.2 *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. 3). In fact, Xe-arc lamps have a very close spectral match to sunlight. This, combined with a 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.

7.2.2.3 *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 relative to Hg alone (Fig. 4). 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)
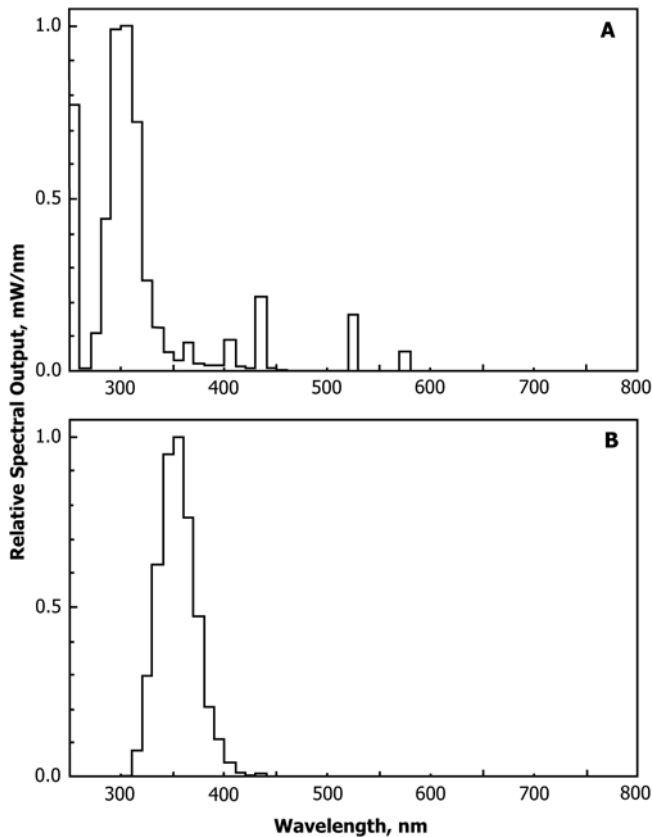
4

FIG. 2 Spectral Output of UV Fluorescent Lamps: (A) UV-B Lamp; and (B) UV-A Lamp
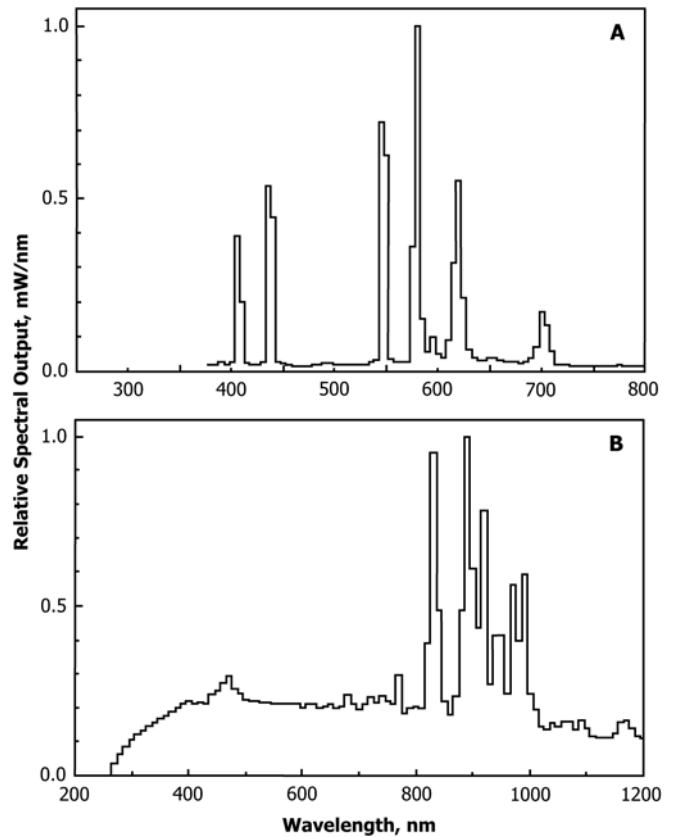

FIG. 3 Spectral Outputs of Short-Arc Lamps: (A) Hg Arc Lamp; and (B) Xe Arc Lamp

ballast, which is much less expensive than the dc power supplies required for Hg and Xe short arc lamps.

7.2.3 *Sodium Vapor Lamps*—Low-pressure sodium (Na) vapor lamps emit a sharp band at 589 nm (orange light) (Fig. 4). High-pressure Na vapor lamps also emit around at 589 nm, but with a much wider emission band (approximately 100 nm) (Fig. 5). 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 (14).

7.2.4 *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 to 700 nm, and they mimic sunlight accurately in this spectral region (Fig. 6). 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.

7.2.5 *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. 7). 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 of the spectrum and have high outputs, but they also emit a great deal of IR, which can create a heat problem.

8. Construction of Artificial Light Sources that Mimic Sunlight

8.1 *Sunlight*—Radiation from the sun with wavelengths greater than 290 nm can reach the surface of the earth (15).
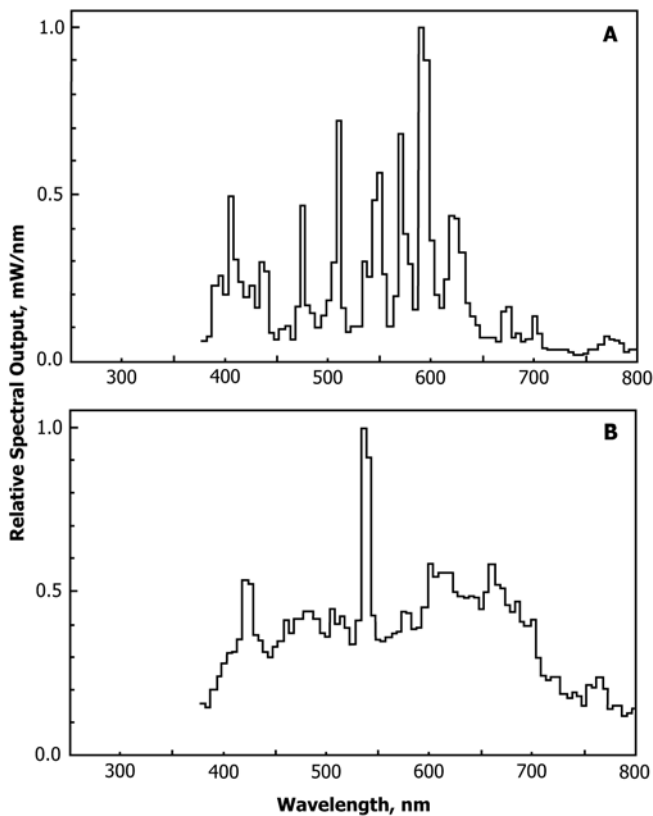
FIG. 4 Spectral Outputs of Metal Halide Lamps: (A) Sodium-
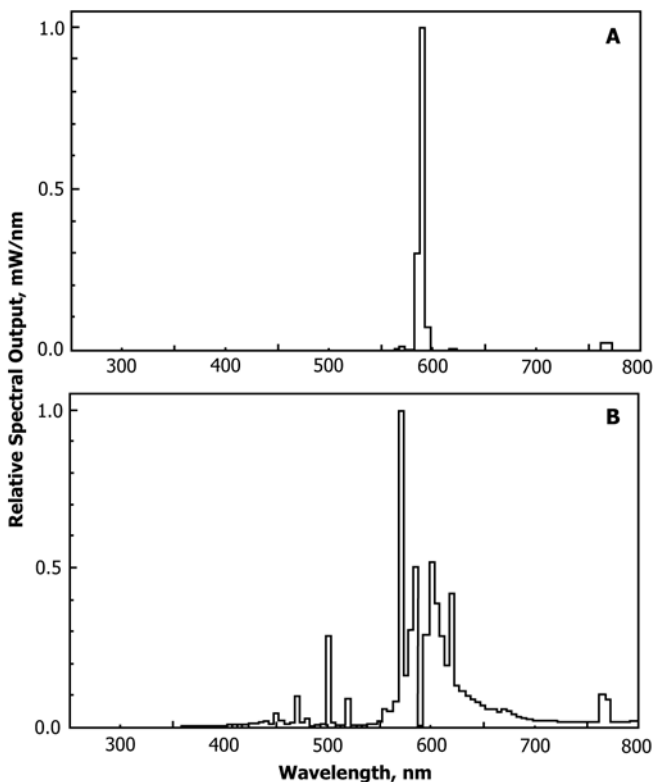Scandium Lamp; and (B) Dysprosium-Thallium Lamp

FIG. 5 Spectral Outputs of Na Vapor Lamps: (A) Low-Pressure
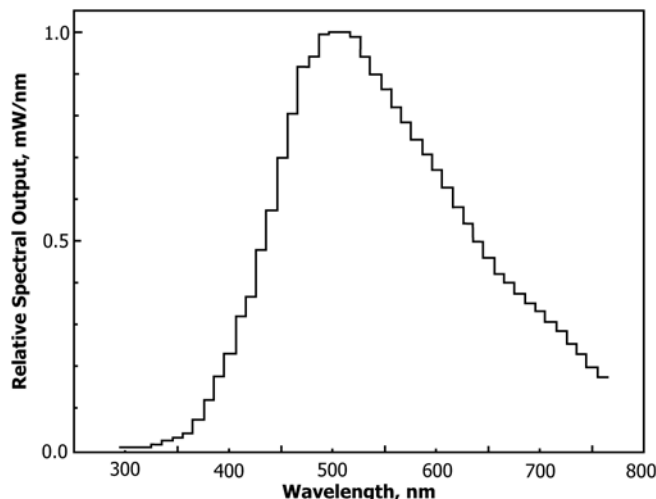Na Vapor Lamp; and (B) High-Pressure Na Vapor Lamp

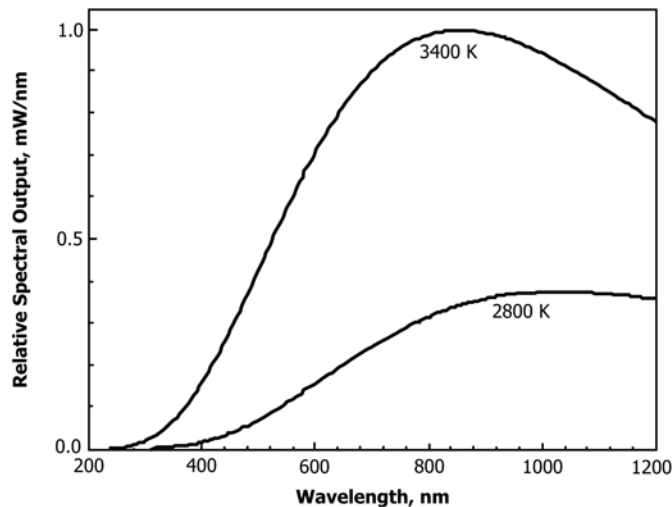FIG. 6 Spectral Output of a Microwave Lamp

FIG. 7 Spectral Outputs of Incandescent Lamps

Radiation below 290 nm is absorbed by the various gases in the atmosphere and is not of environmental concern. At the surface of the earth, the molar ratio of visible:UV-A:UV-B is approximately 100:10:1; however, the content of UV-B is highly variable. For example, on a clear day in late summer, the UV-B:visible ratio at latitudes corresponding to southern Canada and the northern United States is approximately 0.5 % of visible on a photon basis (Fig. 8(A)), while the UV-B level is much higher closer to the equator or at higher elevations; as high as 1.5 % of visible (16, 17). Also, the amount of UV-B is increasing due to depletion of the stratospheric ozone layer (18). The amount of UV-B varies with time of day, peaking at solar noon, and the fraction of UV-B in solar radiation changes with season, exhibiting maximal levels around the summer solstice and minima around the winter solstice (16-19). One should take these factors into consideration when designing a laboratory light source that will mimic sunlight.

8.2 *Visible Light*—Any of the light sources described above can be used if only visible light is required for a test. The best choice is fluorescent lighting if low fluence rates are required.
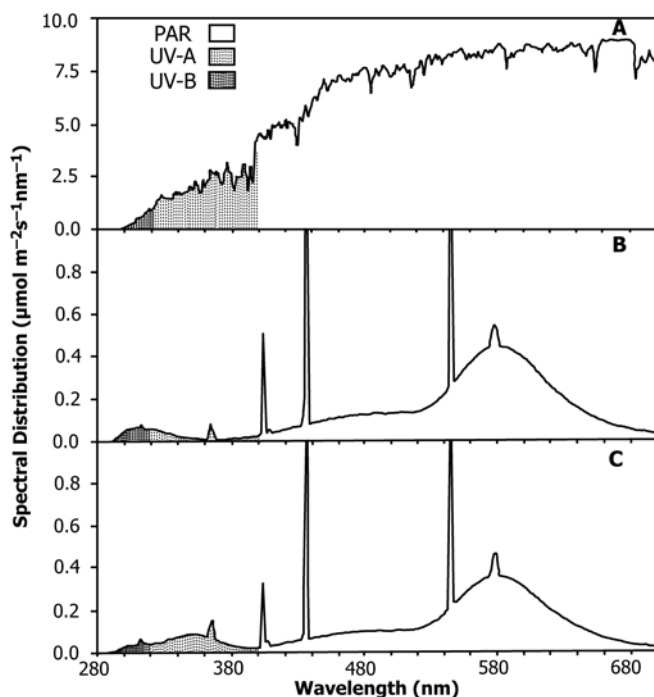
FIG. 8 Spectral Distribution of Sunlight; a Visible Light Plus UV-B Source and a SSR Source: Panel A, Sunlight Measured on Cloudless Day on Lake Erie 23 Miles North of Cleveland, OH (12:21 p.m., 13 July 1994); Panel B, Emission Spectrum of a Visible Plus UV-B Source Filtered Through Cellulose Diacetate; and Panel C, SSR Source Filtered Through Polystyrene

The investigator will have to balance the pros and cons of other types of lamps for higher irradiance lighting. For example, sodium vapor lamps could be used if the full visible spectrum is not required. For the entire visible region, incandescent lamps can be used to supplement fluorescent lamps as long as the refrigeration or cooling system has enough capacity to handle the excess heat. A microwave lamp would be ideal if the budgetary resources are available.

8.3 *Visible Light Plus UV-B Radiation*—A light source with UV-B at approximately 1 % of visible light on a photon basis can be built inexpensively (Fig. 8(B)). This visible plus UV-B source contains cool-white fluorescent lamps (visible light) and a UV-B fluorescent lamp [20]. The radiation from the UV-B lamp is filtered through cellulose diacetate (0.08 mm) to remove extraneous UV-C (<290 nm) [21]; the Hg gas in fluorescent tubes emits at 254 nm, and this UV-C radiation is not quantitatively removed by the glass and phosphor in UV-B lamps. UV-C is much more damaging to biological molecules than UV-B and must be quantitatively removed unless the investigator is interested specifically in the effects of UV-C. The UV-B lamp also can be screened with cheese cloth to achieve the desired visible:UV-B fluence ratio. To mimic loss of the ozone layer, the UV-B level can be raised by removing successive layers of cheese cloth from the UV-B lamp or adding extra UV-B lamps. It has been found that many plants (for example, *B. napus* (canola), rye, soybean, and *L. gibba* (a duckweed)) can be grown under a visible/UV-B source similar to that described above [1, 20, 22-25]. However, this type of lamp arrangement will have relatively low fluence rates (<400

$\mu mol \ m^{-2} \ s^{-1}$ of visible light). Some plants (for example, peas) do not grow well in the presence of UV-B if the visible light level is low [26]. Therefore, a preliminary assessment of satisfactory growth of the test organism under a given visible/ UV-B light source should be performed.

8.4 *Simulated Solar Radiation*—The visible plus UV-B light source described in 8.3 provides only UV-B and visible light. In many cases, it is desirable to have UV-A present as well to better replicate the solar spectrum. For instance, it is likely that the level of blue light and UV-A relative to UV-B is important, as these spectral regions activate deoxyribonucleic acid (DNA) repair via photolyase [27, 28]. Also, the UV-A/blue light level is important in plants for synthesis of protective pigments like carotenoids. Thus, full spectrum artificial lighting, even at relatively low fluence rates, might compensate for the need for high fluence rate visible light, as mentioned in 8.3. The visible:UV-A:UV-B ratio should be approximately 100:10:1 in a simulated solar radiation (SSR) source [15, 19]. Unlike UV-B, the level of UV-A is relatively constant in the environment, not varying greatly with latitude, altitude, or season. Also, UV-A will not increase as the ozone layer is depleted. In general, the IR can be left out of the light source since it does not activate many biological processes and it creates excess heat that is problematic to remove from environmental growth chambers, exposure chambers, and incubators.

8.4.1 *Simulated Solar Radiation with Fluorescent Lamps*—One can construct a light source that mimics sunlight with respect to the relative amounts of visible and UV (a visible:UV-A:UV-B ratio of 100:10:1; Fig. 8(C)) using fluorescent lamps [1]. One such SSR source contains two cool-white fluorescent lamps, one 350-nm fluorescent lamp, and one 300-nm fluorescent lamp. The 300-nm lamp is filtered through cheese cloth to bring the UV-B level down to 1 % of visible. The light is also filtered through cellulose acetate or polystyrene to remove all of the incident UV-C (200 to 290 nm). While the spectral output shown in Fig. 8(C) does not replicate sunlight precisely, the visible:UV-A:UV-B ratio corresponds approximately to that of terrestrial sunlight in the 290 to 700-nm wavelength range from mid-spring to mid-fall in temperate latitudes corresponding to southern Canada and the northern United States [15, 19]. *B. napus* (canola), *Spirodela oligorrhiza* (a duckweed), and *Lemna gibba* (a duckweed) have been found to grow well under this source, exhibiting no overt signs of UV-B stress. The UV-B content of the source can be raised to simulate ozone depletion by removing successive layers of the cheese cloth.

8.4.2 *Simulated Solar Radiation with Fluorescent and Incandescent Lamps*—One can build a light source, as in 8.3, but obtain a better spectral balance in the blue and red regions by adding incandescent lamps. This can also increase the total fluence rate of the source. The other adjustment that should be made is to raise the UV levels, so that the UV-B and UV-A will still be at 1 % and 10 % of visible, respectively. One might also need to control the heat reaching the sample with refrigeration systems or IR filters.

8.4.3 *Simulated Solar Radiation with a Xenon Arc Lamp*—A Xe arc lamp can be used alone to mimic solar radiation. One

simply uses a cut-off filter (such as Schott WG300; >90 % transmittance above 310 nm, 50 % transmittance at 300 nm, and no transmittance below 290 nm) to remove all radiation below a given wavelength. It is also important to use an IR filter (such as a water filter or IR reflector) to remove heat. This source provides high fluence rates over relatively large areas (~200 cm in diameter).

9. Transmission Properties of Lamp Coverings and Laboratory Vessels

9.1 Various clear media can be used to cover lamps to alter their spectral qualities. There are also many types of clear laboratory containers. One can thus remove specific spectral regions or generate light from a single spectral region. One caution that should be taken with coverings and laboratory vessels is that transmittance properties vary with thickness according to Beer's Law. Therefore, if a 1-mm thickness of a given material has 50 % transmission at a certain wavelength, then a 2-mm thickness of the material will transmit only 25 % at that wavelength.

9.2 *Lamp Coverings:*

9.2.1 *Removal of Ultraviolet*—UV radiation can be removed with a variety of plastics or window glass. Cellulose acetate film and polystyrene have cutoffs at 290 nm, therefore absorbing all of the UV-C. To prevent damage to the test organism, the UV-C usually needs to be quantitatively removed from UV emitting lamps. Polyester based clear plastic and most types of window glass have cutoffs between 330 and 380 nm and therefore can be used to remove UV-B and UV-C from a light source. Plastic filters will degrade over time in UV, and they should therefore be checked periodically with a spectrophotometer to be certain that they have maintained satisfactory spectral quality (usually less than 10 % change in any spectral region) **(21)**.

9.2.2 *Isolation of Spectral Regions*—Except for specialized tests, monochromatic or partially monochromatic light is not necessary for environmental work. Broad band regions of the visible spectrum can be isolated with colored theatrical grade celluloids (spectral band widths approximately 50 to 100 nm). If narrower bandwidths are needed, interference filters can be used (5 to 20-nm bandwidths); however, interference filters will greatly limit the total fluence reaching the test organism. For more information on isolating specific spectral regions, see Refs **(12, 13)**.

9.3 *Laboratory Vessels:*

9.3.1 *Borosilicate Glass*—Borosilicate glass is the most common form of glassware. It is used to hold organisms during toxicity tests. In particular, it is used for algae and *L. gibba* growth because it is transparent to the visible light needed for photosynthesis. It is appropriate for many needs at thicknesses found in common flasks and petri plates (wavelength cutoff at 275 nm and 50 % transmission at 295 nm). It therefore can be used for any test in which UV-B is required. Of course, if wavelengths around 295 nm are needed, the investigator needs to be sure that the amount of incident 295-nm radiation is high enough to account for absorbance by the borosilicate glass. Also, very thick glass and low-grade borosilicate glass will

absorb UV-B. The transmittance properties of the material to be used should therefore be checked with a spectrophotometer.

9.3.2 *Polystyrene*—Polystyrene is the plastic generally used in petri dishes, culture bottles, and multi-well dishes. It has very good transmission properties (common thicknesses have a cutoff at 288 nm and 50 % transmission at 300 nm). It is therefore useful with almost any environmentally relevant light source. It also offers the advantage of absorbing all of the incident UV-C. The only precaution that needs to be taken is that all light measurements should be made with polystyrene over the light sensor to account for any radiation absorbed by the plastic because this varies with the thickness of the plastic. Also, UV-C and UV-B degrade the plastic after long exposures (approximately two weeks), so the plastic should be checked periodically during testing and it should be discarded after each test is complete.

9.3.3 *Acrylic*—Acrylic is used for many applications in environmental testing. It is especially useful for building larger vessels for applications such as microcosm containment. Its absorbance cutoff is around 385 nm, but this of course varies with the thickness of the plastic. For instance, 1-cm thick acrylic has 50 % transmittance at 386 nm and 10 % transmittance at 379 nm. Therefore, if UV is needed in the test, the top of the container needs to be made with a different material, such as cellulose acetate, which transmits UV.

9.3.4 *Other Materials*—Other types of containers can be used, but they must be transparent to the spectral regions important to the test. The transmission properties of the material can be checked reliably with a spectrophotometer. The spectral quality of the light source can then be adjusted to compensate for absorbance by the vessel.

10. Measurement of Light

10.1 *Light Components*—There are two components to measuring light: quantity and quality **(12, 13)**. Both are important aspects of a light source. When light is a concern, the quantity of light must be measured each time a laboratory test is performed. It is analogous to checking the pH of a solution. Since the spectral distribution of a lamp is often available from the manufacturer, the spectral quality does not require routine measurement. However, if different types of lamps are combined into a single light source, the spectral output of the assembled radiation source should be measured with a spectroradiometer (see 10.3).

10.2 *Measurement of Light Quantity*—Light quantity can be measured in three ways: radiometric, quantum, and photometric methods **(12)**. In essence, each method uses the same type of instrumentation, a light-sensitive detector (thermopile or photodiode) that converts an absorbed photon into a voltage or a current and an amplifier to detect the voltage or current change (for other light measurement techniques see Refs **(12)** and **(13)**). The amount of radiant energy should be reported as a fluence rate, although total fluence is appropriate under certain circumstances.

10.2.1 *Radiometric Methods*—This is a measure of light quantity in units of energy (joules) or power (watts). Therefore, a fluence rate by this method will be $J\ m^{-2}\ s^{-1}$ or $W\ m^{-2}$. The instrument used is called a radiometer. The detector in a

radiometer is a thermopile, which converts radiant energy to heat and in turn generates an electromotive force. This results in a voltage change that is proportional to the amount of energy absorbed. The voltage change is converted by an amplifier to a calibrated output in J m^{-2} s^{-1} or W m^{-2}. One can purchase radiometers with filters over the sensor that allow only certain spectral regions to pass. For instance, radiometers with filters that transmit only visible light are used by plant physiologists because this is the photosynthetically active region (PAR) of the spectrum; thus the name PAR meters. Also, one can buy radiometers that have filter bundles that are specific for UV-A or UV-B. These are used by meteorologists to make daily UV-B readings that are now commonplace in weather reports. One can also buy very accurate and sensitive radiometers that detect over a very broad range (for example, from 200 to 2000 nm) and calibrate the radiometer for its sensitivity at each wavelength. The calibration is crucial because the sensitivity of all detectors is wavelength dependent. With appropriate filters, the irradiance at a chosen wavelength can then be made.

10.2.2 *Quantum Methods*—This is a measure of light as the number of photons present. Therefore, the result will be a photon fluence rate with units generally given as µmol m^{-2} s^{-1}. (Note that this is equivalent to µEinstein m^{-2} s^{-1}; see 3.2.2.) The instrument used is called a quantum sensor. The sensor is a light-sensitive diode (or photovoltaic cell), which converts absorbed photons to an electric current that is proportional to the number of photons absorbed. The amplifier then converts this information to a reading in µmol m^{-2} s^{-1}. However, the sensitivity of the sensor is wavelength dependent, so a filter bundle is placed above the diode to quantum correct the reading. The most common type of quantum sensor is balanced for visible light and, like the PAR meter, is commonly used by plant physiologists to obtain an accurate reading of photosynthetically active radiation. Specialized quantum sensors are also available (for example, for UV-B and UV-A). Again, care should be used to ensure that the meter is appropriate and accurate for the spectral region of interest.

10.2.3 *Photometric Methods*—This is another measure of the amount of light energy present. It therefore has similarities to radiometry. However, it is a method of measuring light that is specific for human vision and is used mostly by photographers and lighting engineers (**12, 13**). The basic unit is the lumen (which is the amount of light emitted by a point source of 1 cd). The amount of light is usually given as lux (lm m^{-2}) or foot-candles (lm ft^{-2}). The thermopile is protected by a filter bundle that limits the region of the spectrum to an approximate gaussian distribution of light centered at 550 nm, with limits of detection at 400 and 700 nm (Fig. 9). These instruments therefore detect only visible light, but they are highly wavelength dependent even within that spectral region. Photometry is quite good for photography because that industry has standardized on measuring light in this way. However, this method is generally not appropriate for measuring lighting used in toxicity testing. If this is the only option that an investigator has for measuring light, the data should be converted to an appropriate radiometric or quantum unit (see 10.2.4). In addition, many photometric light meters are of low grade since they are designed for amateur photographers. Great
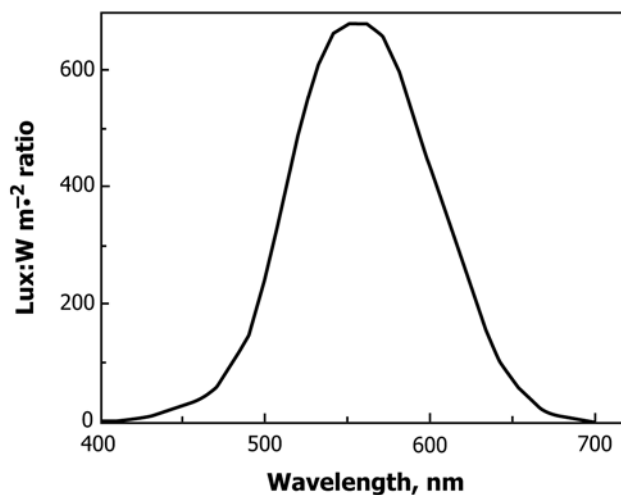
Note 1—To convert lx to W m^{-2} at a given wavelength, divide lx by the number on the y axis at the wavelength of interest; that is, if a light source had its primary output as red light centered at 600 nm, lx is converted to W m^{-2} by dividing by 420.

FIG. 9 Ratio of lx to W m^{-2} as a Function of Wavelength

care should thus be used when the data are collected by a photometric instrument.

10.2.4 *Conversion of Light Measurement Units*—The amount of light energy present is dependent on the spectral distribution of the photons that make up that light source. This is because the energy of a photon is inversely proportional to its wavelength. Therefore, to convert between units of light quantity, one must know something about the spectral output of the light source (see 10.3). However, many of the light sources used in an environmental toxicology study will be in the visible region, and it is often generated with fluorescent lighting. In these instances, the spectral output of the light source will usually be centered at approximately 550 nm (see Fig. 1), and this wavelength can be used as the "average wavelength" for conversions of the total fluence rate of a light source (Table 2). Some of the conversion factors given in Table 2 assume an average wavelength of 550 nm. If a large amount of the radiation is from spectral regions other than the visible and the spectral output of the source is not centered at 550 nm, the spectral distribution is required for reliable conversions. In particular, to convert lux to radiometric or quantum units, care should be used because lux as defined varies greatly with

TABLE 2 Conversion Factors for Light Measurements

Convert	to	Multiply by[A]
µmol m^{-2} s^{-1}	W m^{-2}	119.6/(λ nm)
W m^{-2}	µmol m^{-2} s^{-1}	8.36 × 10^{-3} × (λ nm)
lx (at 550 nm)	W m^{-2}	1.47 × 10^{-3}
lx (at 550 nm)	µmol m^{-2} s^{-1}	6.91 × 10^{-3}
fc	lx	10.75

[A] For conversion between µmol m^{-2} s^{-1} and W m^{-2}, any wavelength can be used. Since many common laboratory light sources for visible light (for example, cool-white fluorescent lamps) have an average wavelength of 550 nm, this wavelength can be used for many conversions. For conversions from lx to µmol m^{-2} s^{-1} and W m^{-2}, an average wavelength of 550 nm is assumed. However, if the light source has a spectral quality that is not centered at 550 nm, the assumed wavelength for conversion of lx to another unit will have to be altered. See Fig. 9 for the wavelength dependence for conversion of lx to W m^{-2}.

wavelength relative to W or µmol of photons. The wavelength dependence for the ratio of lx to W m^{-1} is provided in Fig. 9. Also, it should be clear from Fig. 9 that lux cannot be used as a measurement for UV radiation. Note that if SSR is used, only approximately 10 % of the radiation comes from the UV, and therefore conversions for total radiation present in the source can still be based on an average wavelength of 550 nm, and the initial measurement can be made in lux as long as the visible:UV fluence rate ratio is known.

10.3 *Spectroradiometry*—A spectroradiometer is used to measure the spectral distribution of light. This expands greatly on broad band irradiance measurements by determining the fluence rate at each wavelength. The data are plotted as W m^{-2} nm^{-1} or µmol m^{-2} s^{-1} nm^{-1}. In fact, the data shown in Figs. 1-7 are spectroradiometric measurements of various light sources. The need for such instrumentation in plant biology and environmental photobiology is immense since light is often a primary "reagent." Some spectroradiometers are very accurate, using a double monochrometer design to achieve highly monochromatic light (<1 nm optical resolution without stray light, and spurious spectral lines or higher order wavelengths (for example, integral multiples of λ)). Also, the detector is very sensitive over a broad waveband. These instruments are quite expensive (>$50 000), and their accuracy is beyond the needs of most users. In the case of less expensive instruments ($15 000 to $25 000), they are nonetheless accurate enough for most needs. They often have detectors with a limited wavelength range (for example, only 300 to 850 nm) and have only one monochrometer. This will be adequate for some applications. Another approach is a diode array detector-based spectroradiometer (available for $25 000). An efficient holographic grating (spectrometer) spreads the light as a function of wavelength along a 512- or 1024-diode array (1-nm resolution from 250 to 1100 nm). With a diode array, the whole spectrum is thus collected simultaneously. Modern holographic gratings generally have minimal interference from higher order wavelengths. These instruments are thus also sufficiently accurate for most needs.

11. Biologically Effective Radiation

11.1 *Description of Biologically Effective Radiation*—The biologically effective dose often needs to be considered when one is assessing the fluence rate in a light source (29-31). This is a weighting of the total irradiance applied to an organism to account for the biologically active content for a particular response. It is thus a weighting of a spectrum based on the wavelengths that are absorbed and the efficiency of each wavelength at initiating a specific biological response. For instance, if a response is caused exclusively by blue light (400 to 500 nm), it makes no difference how much red light (600 to 700 nm) is present in the light source. Biologically effective radiation is calculated as the cross-sectional overlap between an action spectrum for a biological response and the spectral output of the light source. That is, this manipulation of spectral data indicates the amount of the total incident radiation in the light source that can actually be used to promote a specific response. It is commonly used for UV-B effects because UV-B is highly damaging and the amount of damage increases

dramatically as the wavelength decreases. Typical action spectra used for biologically effective UV-B are the general plant damage spectrum (29), inhibition of photosynthesis spectra (32, 33), in vitro and in vivo DNA damage spectra (34, 31), and the erythema spectrum (35). Other in vivo plant action spectra that can be used include flavonoid synthesis (36, 37), degradation of the D1 photosystem II reaction center protein (14), and curvature of seedlings (38). For instance, if biologically effective radiation for plant acclimation to UV-B is needed, flavonoid synthesis can be used since it is associated with protection of plants from UV-B. If biologically effective radiation for plant damage is required, the inhibition of photosynthesis action spectrum might be used. If biologically effective radiation for photosynthetic activity is needed, an action spectrum for photosynthesis may be used (14).

11.2 *Determination of Biologically Effective Radiation*—To determine the content of biologically effective UV radiation, an action spectrum is normalized to unity at a given wavelength (for instance, 300 nm). The spectral output of the light source is multiplied by the normalized action spectrum at each wavelength. This weighted spectrum is integrated to yield a biologically effective fluence rate (in J m^{-2} s^{-1} or µmol m^{-2} s^{-1}). These data are sometimes converted to a daily dose.

12. Considerations for Designing Light Sources for Environmental Testing

12.1 *Plants and Phytoplankton*—Naturally, visible light is required for photosynthesis. However, in general, one should also try to mimic the spectral quality of natural sunlight in the environmental compartment relevant to the test. Thus, if one is conducting a test on plants or algae (see Guides E1218 and E1415, and Practice E1598), the light source should really simulate sunlight. Not only should the growth of the plants under spectrally accurate lighting be considered, but many contaminants are photoactive as well. Furthermore, many plants require UV-A for optimal growth.

12.2 *Terrestrial Animals*—SSR will be relevant to terrestrial animals (wildlife and domesticated animals) that live above the surface of the ground. In general, one should attempt to mimic the spectral quality of sunlight, especially if there is a toxicant under study that is potentially photoactive. The wavelengths absorbed by the chemical should be in the light source in that case.

12.3 *Aquatic Organisms*—Transmission of light through the water column should be considered for aquatic toxicology. The amount of UV-B relative to visible light drops as the depth increases (15). Therefore, an SSR spectrum will be a function of distance from the surface. Also, the light quality reaching the test organism is highly dependent on water quality. Thus, the spectrum of light relevant to a test will be highly variable. An absorbance spectrum of a relevant water sample should be used, if possible, as a guide to design a light source.

12.4 *Sediment Testing*—Sediment testing only needs a light component for sediments from shallow clear waters. In this case, the relevant spectrum would be based on the spectral quality of sunlight that reaches the sediment in a natural setting. In general, however, the same rules apply as for aquatic

testing. Also, one should consider whether particulate matter from the sediment is exchanging into the water column and whether the particles will be exposed to significant amounts of sunlight. If this is the case, any contaminants adsorbed onto the sediment could be modified photochemically. Such photomodification reactions can have a profound effect on toxicity (1).

13. Keywords

13.1 action spectrum; artificial lighting; simulated solar radiation; spectral distribution; sunlight; ultraviolet radiation; visible light

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