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

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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.

1.3 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.

[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.

1.8 *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 µmol m^{-2} s^{-1}. (This is equivalent to µEinstein 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:

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

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*—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 µmol 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).

[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