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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:

Title	Section
Referenced Documents	2
Terminology	3
Summary of Guide	4
Significance and Use	5
Safety Precautions	6
Lamps	7
Artificial Lighting	7.1
Light Sources	7.2
Construction of Artificial Light Sources that Mimic Sunlight	8
Sunlight	8.2
Visible Light	8.2
Visible Light Plus UV-B Radiation	8.3
Simulated Solar Radiation	8.4
Transmission Properties of Lamp Coverings and Laboratory Vessels	9
Lamp Coverings	9.2
Laboratory Vessels	9.3
Measurement of Light	10
Light Components	10.1
Measurement of Light Quantity	10.2
Spectroradiometry	10.3
Biologically Effective Radiation	11
Considerations for Designing Light Sources for Environmental Testing	12

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.