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Research Techniques Made Simple

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Research techniques made simple: Experimental UVR exposure

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Abstract

Ultraviolet radiation (UVR) exposure is a widely applied technique in clinical and pre-clinical studies. Such experimental conditions provide crucial information on the biological responses of skin and cell models, which may then be extrapolated and interpreted, for example in the context of equivalent daylight exposures. It is therefore important to fully understand the characteristics of UVR and the principles behind correct and appropriate UVR exposure in experimental settings. In this *Research Techniques Made Simple* article, we discuss the relevant background information and the best practices for accurate, transparent and reproducible experimentation and reporting of UVR exposure.

Summary points

o What information the assay or technique provides:
These measurement techniques provide a comprehensive characterisation of UVR exposure in experimental and clinical settings. The information gives clarity, robustness, and encourages reproducibility of the exposures carried out.

o Limitations of the assay or technique:
The quality of measurements and information provided depends on the equipment available to the user. Simple measurements are inexpensive and still very useful; however, a full characterisation of light sources may require more specialist tools. If these tools are unavailable it is advised to contact other centres with photobiology clinical and/or laboratory expertise, who may be able to assist and therefore produce a more complete understanding of the relevant UVR exposure.

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Introduction

The effects of ultraviolet (UV) radiation (UVR) are diverse and far-reaching. Controlled UVR exposure is commonly required in both pre-clinical and clinical research settings in order to address questions about its biological effects on skin or surrogate skin models. Experimental studies of both natural daylight and artificial UVR exposure have underpinned many of the landmark discoveries in dermatological research and our understanding of physiological and pathological processes. For example, pre-clinical and clinical models of UVR exposure have profoundly& Jaenicke increased our understanding of the wavelength dependency of photocarcinogenesis, the characteristics of UVR-induced immunosuppression and the characteristics of UVR-induced erythema. These experimental models of UVR exposure have also advanced our understanding of drug-induced photosensitivity and of photoprotective agents, and have informed our development of photodermatological practices, both photodiagnostic and phototherapeutic. For example, the pioneering work of Parrish & Jaenicke (Parrish and Jaenicke 1981) informed our understanding of the action spectrum for clearance of psoriasis and in turn the development of narrowband UVB phototherapy, facilitating effective clearance of psoriasis, whilst minimising erythemal risk.

All too often contrasting and conflicting data appear in the literature, which may arise due to a lack of understanding of the characteristics of UVR and the principles of its interactions with *in vivo* or *in vitro* skin or equivalent models. Photophysics underpins all aspects of UVR exposure and there are several ways of quantifying this radiation. It is critical that these are defined and understood (Table 1). Adhering to the following *photophysics core principles* will help ensure that the UVR exposure is understood and correctly undertaken.

Photophysics

Core principle 1: Safety

It is key that risk assessment is performed prior to working with UVR and that control measures are used to prevent exceeding exposure limit values. Exposure limits for UVR are published by ICNIRP (ICNIRP 2004), and each country should have incorporated these into their own legislation. In the hierarchy of control measures, engineering controls are the most effective protection measure (e.g. UVR enclosed within a box) followed by administrative controls (e.g. staff training and standard operating procedures (SOPs)). Personal protective equipment (PPE) such as UV protective glasses are the least effective and, while their use may be required, should not be relied upon as the only risk control.

Core principle 2: UV Spectrum

UVR (100-400 nm) can be subdivided: UVA (315-400 nm), UVB (280-315 nm) and UVC (100-280 nm), with UVA further subdivided into UVA1 (340-400 nm) and UVA2 (315-340 nm). It is worth noting that these ranges are commonly used but can vary depending on convention. UVC wavelengths are absorbed by the atmosphere and are therefore not relevant when considering solar exposures at terrestrial level, however there is a growing

interest in using artificial UVC sources (~222 nm) to disinfect surfaces, including human skin. UV sources are often referred to by their respective wavebands, e.g. 'UVA lamp'. While this naming convention may be convenient, it is not descriptive enough and does not include enough detailed information to fully understand the characteristics of any experimental irradiation procedure performed with that source.

The emission spectrum of a light source is essential, providing critical information on wavelength-dependent biological mechanisms and enabling action spectra to be defined (Liebel et al. 2012). In many instances, the dose is the only quantity reported in studies, however without the prerequisite information on UV spectrum and irradiance, the dose can be misleading.

Example

A publication may report that a UVA lamp with a dose of 100 J m^{-2} caused erythema in the skin of 10 subjects and may then make a broad statement that UVA radiation causes erythema. However, without detail on the spectrum of the lamp, it is unclear which wavelengths of radiation caused the erythema. Take, for example, the UVA fluorescent lamp spectrum in Figure 1. If the spectrum is combined with the CIE erythema action spectrum (Figure 2) it is clear that the most effective wavelengths at causing erythema in this lamp are the UVB wavelengths that are emitted, some of which are not even visible in the original non-logarithmic plotted spectral irradiance.

The principles described by this example apply not only to erythema but any other biological effect. Very low UVR emissions at a given wavelength may be the cause of the biological effect if they are more effective than higher UVR emissions at other wavelengths.

Core principle 3: Calibration

Key to the accurate measurement of light is use of a calibrated detector (Moseley et al. 2015), traceable to National Standards (e.g. National Physical Laboratory, NPL). If using a broadband light detector to measure irradiance it is crucial that this is done with knowledge of the output spectrum of the light source and is calibrated to each light source measured. Although more costly, using a calibration laboratory that is ISO 17025 accredited for optical calibration will provide confidence that the calibration is appropriate for the task. Always describe the process you are undertaking to the calibration laboratory and ask if they can provide an appropriate calibration.

Example of ideal reporting of UVR exposure

"The skin was exposed to *100 J* m^{-2} of ultraviolet radiation (*280-400 nm*) from a *fluorescent lamp (PL-L 36W/09/4P, Philips Lighting, Amsterdam, Netherlands)*. A stable irradiance of *2 W* m^{-2} was measured with a *calibrated radiometer (ILT2400 meter, SEL033 detector, UVA filter, Teflon diffuser, calibrated 05th March 2020)*. Exposure time was *50 seconds* and the *spectral irradiance* of the lamp is shown in Figure 1."

Core principle 4: Uniformity of Illumination

It is important to measure the uniformity of UVR distribution within the irradiation area (Moseley et al. 2015). Different regions within an irradiation field, e.g. a multi-well plate, may be subject to a higher or lower irradiance than others, resulting in different doses being delivered to different sites within the field. Uniformity may be measured at the irradiation field by measuring irradiance in multiple locations across the irradiation field (Figure 3). For example, the centre of the plate and at the corners. Uniformity may then be characterised by observing the mean, median, standard deviation and coefficient of variation (CoV), which is the standard deviation expressed as a percentage of the mean. The CoV should be kept as low as possible, and the difference between any two measurement values should not be more than 10%.

It can be difficult to improve the uniformity of light irradiation. Changing the distance between light source and irradiation field will typically change the uniformity but will also change the irradiance. Caution should be exercised in both maintaining an appropriate irradiance for the study and a realistic irradiation time.

Core principle 5: UVR source stability

The output of a UVR source may not be stable and will typically decrease during its lifetime. It is important to characterise the source output stability prior to use. This can be done by performing measurements at set time periods (e.g. every 30 seconds) for the anticipated maximum exposure time of the experiment plus a “warm-up” period. It is common practice for a “warm-up” period to be defined as the length of time required for the lamp emission to become relatively stable for the exposure time of the experiment (e.g. less than a 5% change in measured irradiance). Once stable, a single irradiance measurement can be multiplied by the exposure time to determine the radiant exposure. With unstable lamps it can also be possible to leave a detector in-situ and measure the cumulative exposure. Such a process should be undertaken with caution, ensuring the detector does not influence, and is representative of, the UVR incident on the experimental setup.

Pre-clinical testing

When investigating the effects of UVR in cell culture models *in vitro*, endpoints that can be considered as indicators of the biological effects of UVR include the induction of cell cycle arrest or apoptosis and quantification of DNA damage. UV-induced DNA damage can be assessed by measuring DNA “comets” or quantifying cyclobutane pyrimidine dimers (CPD) and 6-4 photoproduct formation. These photoproducts can be detected using a variety of techniques including molecular, chromatographic and mass spectrometry strategies (Figueroa-González and Pérez-Plasencia 2017). Detection and quantification of these indicators of DNA damage can also be undertaken in animal models and in human skin after UV-irradiation.

If investigating the effects of drug and light interactions *in vitro,* regulatory requirements state that any drug with a molar absorption coefficient (MEC) >1000 L mol^{-1} cm^{-1} at any wavelength between 290-700 nm must undergo photosafety evaluation (2015), with the

first step being the *in vitro* 3T3 Neutral Red Uptake Phototoxicity Test (1981). The initial assessment of phototoxic potential can also include photoreactivity assessments measuring the formation of type I (superoxide) and type II (singlet oxygen) reactive oxygen species (ROS) following irradiation with UV-visible radiation (Onoue et al. 2008; 2015). Whichever light source is used, it is important to measure both singlet oxygen and superoxide species to avoid the production of false negatives (Ceridono et al. 2012), particularly as negative pre-clinical results indicate a low probability of phototoxicity and can support a decision not to undertake further photosafety evaluation in humans.

In vitro UV irradiation in cell models or skin equivalents, whether involving a drug or other intervention, follows the same broad principles outlined above alongside appropriate controls. This may include at least four study arms with each subject to the same variations in drug concentration and radiant exposure:

1. unirradiated cells with no drug/intervention applied
2. irradiated cells with no drug/intervention applied
3. unirradiated cells with drug/intervention applied
4. irradiated cells with drug/intervention applied

Pre-clinical studies in cell models may be difficult to interpret in terms of extrapolation to the human setting and thus it may be necessary to undertake UV irradiation studies in animal models. The same principles remain as to fully understanding the rationale behind choice of irradiation source and the characteristics of UV exposure.

Clinical phototesting

Many clinical research studies require phototesting. There may be very different research questions posed, such as in drug phototoxicity studies, photoprotection studies, studies to understand biological effects of UV and wavelength-dependency. Once the study objectives are outlined, the key aspects of clinical phototesting are to decide on which UV wavelengths should be used: should it be a narrowband or broadband source, is it single exposures or iterative testing, which dose ranges and irradiances to use, are normal range values available?

The endpoint in clinical phototesting is often a measurement of the minimal erythema dose (MED). We define this as the minimum dose of light that produces just perceptible reddening of the skin at 24h post-irradiation (Ouinn et al. 1994). MED gives a measure of the threshold erythemal sensitivity of skin after exposure to any given UVR light source and will be influenced by the emission spectrum, irradiance and incremental dose-series used during the MED assessment. The MED can be used as a quantitative measurement of the UVR dose required to cause erythema that is just perceptible to the naked eye and in clinical practice this is routinely used in many phototherapy units as a baseline measurement in each patient as an indicator of sensitivity to the phototherapeutic light source, in order to ensure that the dose regimen used is individualised and safe. The MED is also used to identify abnormal photosensitivity and to characterise the action spectrum for photosensitivity. Experimentally, the MED allows objective measurement of individual threshold erythemal sensitivity, thus enabling study conditions to be investigated, such as

the effects of a photosensitising drug, a photoprotective agent, of changing the characteristics of the light source or of investigating parameters such as the influence of body site for example.

Reflectance devices may also be employed to obtain quantitative data, defining the erythema index (EI), in turn enabling objective measurements of both threshold erythemal responses, based on MEDs, but also the slope of dose-response curves and degree of induced erythema at doses above threshold. It should be noted however that there are variations concerning visual or quantitative measurements of erythema, arising primarily from assessor training, ambient lighting conditions and variability between measurement devices. Additionally, due to light absorption by melanin, reflectance devices are generally unreliable when used on darker skin phototypes or when there is significant UV-induced pigmentation. Typically, iterative provocation testing is performed at a larger test site than that used to obtain an MED, which is again important to document.

Examples of phototesting options available for clinical studies

Narrowband phototesting: This is only available through specialist photodiagnostic centres. Usually an irradiation monochromator with broadband xenon arc lamp is utilised. Light from this source is filtered by a diffraction grating monochromator, allowing specific wavebands from the spectral range of the xenon arc lamp to be selected and used to irradiate the skin (Moseley and Ferguson 2010). Thus, the term monochromator is misleading as whilst the filtered emission spectrum is relatively narrow, it is not in fact single wavelength emission (typically full-width half maximum of 5 – 30 nm). These specific wavebands may be used to determine wavelength-specific MEDs. It is important to have a normal population reference range when measuring MEDs across the UV spectrum, as it will allow deviations from the normal range of MEDs to be detected, although this normal range must be relevant to the population under study. Reporting must also include spectral emission and irradiances.

Solar simulator: Despite the terminology, these light sources are often not a mimic of the solar spectrum (Figure 4). There is no agreed standard of what constitutes a solar simulator for clinical phototesting, so it is important to understand the spectral output of the source being used and reporting using our Core Principles, particularly if extrapolating findings to equivalent daylight exposures. The daylight spectrum is broadband and variable, so care should always be taken in equating the results from solar simulator exposures to hypothetical daylight exposures. These devices may be used either to define MEDs or as a provocation source. Unfiltered solar simulators may miss UVA-weighted phenomena, such as drug phototoxicity, and therefore care should be taken when interpreting wavelength-dependent mechanisms (Moseley and Ferguson 2010). Filters may be used to ‘remove’ certain wavebands. Similarly, metal halide lamps are often used in combination with filters to achieve the desired spectral output. However, care should be taken – for example, erythema may be induced at ~10000x lower dose with UVB than with long wavelength UVA. Even residual exposure at certain wavelengths through use of inefficient filters may confound results and create false positives (Figures 1 and 2).

Often erythemally-weighted exposures from solar simulators are equated to a duration of exposure from daylight at solar noon in mid-June, in terms of duration. In making such a comparison however, the reference daylight spectrum used should be provided, and understood in the context of the results presented.

The skin's response to UV-irradiation varies depending on body site and skin phototype and is also influenced by chronic sun exposure, with an MED on a sun-exposed site typically being higher than that on a non-exposed area. For example, an MED on the lower leg is on average four times higher than that on the trunk in lighter skin phototypes (Olson et al. 2016). Provocation should ideally be carried out on consistent body sites (e.g. back) and on clear skin, to allow for interpatient comparison. Alternatively, it is often most appropriately performed on a body site that may be of particular interest, e.g. where rash is induced with natural exposure. This information should always be included in reporting, and caution should be exercised if comparing results to a normal range which may have been derived using a different irradiation source, body site and/or patient population.

Conclusions

The effects of UVR on clinical and pre-clinical models are of fundamental importance. However, these underlying mechanisms may only be accurately interpreted through the practice of robust and reproducible UVR exposure. Increasingly, researchers are interested in the effects of visible and near infrared radiation, and all of the principles outlined above also apply when carrying out such exposures. Care should always be taken to ensure that irradiances and doses are biologically relevant, and that appropriate experimental controls and safety precautions are observed.

Reporting UV exposure is important in all preclinical and clinical experimental settings and in a transparent manner, which allows the reader sufficient information not only to understand fully the UV exposure carried out and its effects and to appropriately interpret the data, but also to then be able to reproduce the exposures under the same experimental conditions.

Conflict of interest statement

P. O'Mahoney has no conflicts of interest to declare.
E. Eadie, V. McGuire, R.S. Dawe and S.H. Ibbotson provide unpaid consultancy to Spectratox, a not-for-profit company that offers commercial clinical and *in-vitro* phototoxicity testing as well as UV meter calibration. R.S. Dawe and S.H. Ibbotson are also unpaid directors of Spectratox.

CRediT Statement

P. O'Mahoney: Writing – original draft, Writing – review & editing, Visualisation,
V. McGuire: Writing – review & editing
R. Dawe: Writing – review & editing

E. Eadie: Writing – review & editing, Visualisation
S. Ibbotson: Writing – review & editing, Conceptualisation

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Multiple choice Q&As

1. Of what benefit is a logarithmic plot of spectral irradiance?
    a. It allows for calculations of uniformity of irradiance
    b. It reveals smaller peaks in the spectrum which may be more biologically effective
    c. It allows the UV exposure to be better replicated
    d. It can be used in the safety assessment of the lamp

    Answer (b.) – The logarithmic plot makes smaller peaks more visible, which may be more effective depending on the biological mechanism to be investigated. The normal spectral irradiance plot is sufficient for: allowing replication of experiments; and for a safety assessment of the lamp. Neither plot is required for measuring uniformity of irradiance.

2. In an experiment a sample is irradiated with a UVA lamp. The irradiance of the lamp is 4 W m^{-2} and the sample is irradiated for 1 minute. What is the total radiant exposure delivered in this example?
    a. 4 J m^{-2}
    b. 240 J m^{-2}
    c. 0.25 J m^{-2}
    d. 15 J m^{-2}

    Answer (b.) – The radiant exposure delivered is equal to the irradiance (W m^{-2}) multiplied by the exposure duration (seconds).

3. Regulatory requirements state that any drug absorbing light between _________ nm must undergo photosafety evaluation
    a. 100 – 400 nm
    b. 315 – 400 nm
    c. 290 – 700 nm
    d. 290 – 315 nm

    Answer (c.) - Regulatory requirements state that any drug absorbing light between 290-700 nm must undergo photosafety evaluation. This range covers from UVB through the visible spectrum, encapsulating most of the primary wavebands of interest.

4. What is the main advantage of monochromators for phototesting?
    a. They are portable

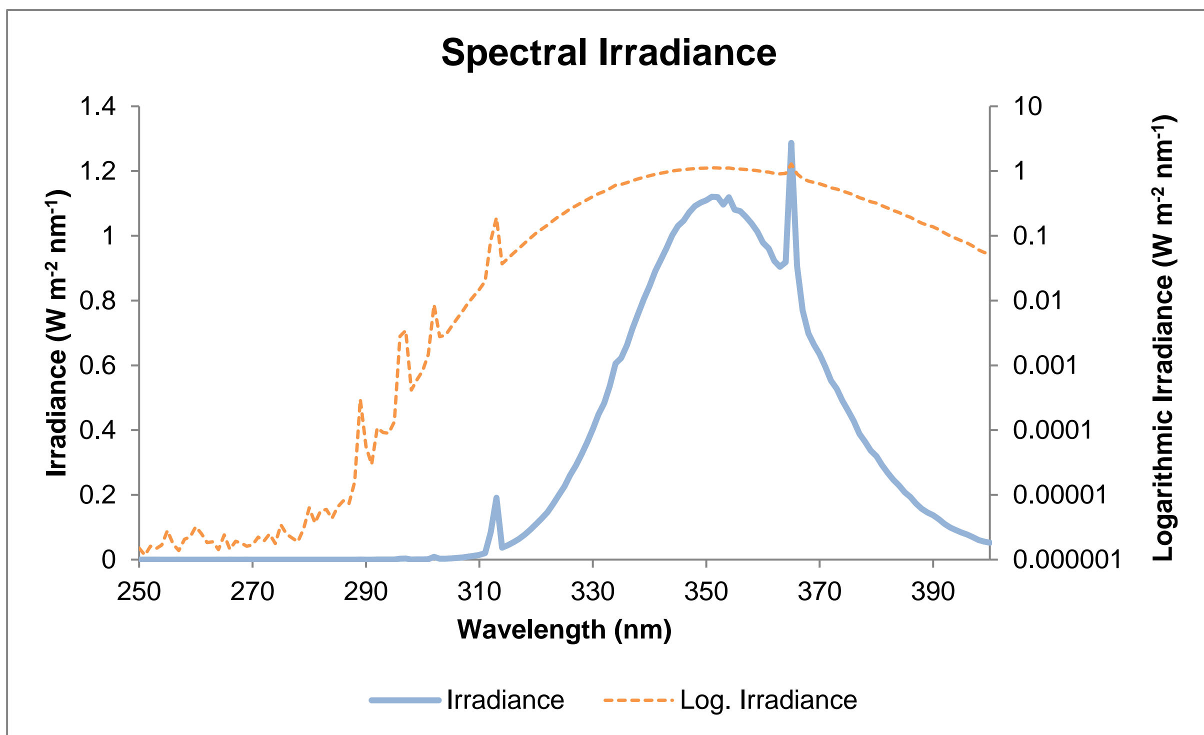
b. Any waveband from the spectral range of the xenon arc lamp may be selected for testing
c. The xenon arc lamp has a very long lifetime
d. They can irradiate multiple areas of the skin at once

Answer (b.) – Monochromators allow the user to select specific wavebands from the xenon arc lamp for testing. They tend not to be portable devices and the lamps are checked regularly and replaced when outputs decrease beyond a predetermined limit. They are typically only able to irradiate one area of skin at a time.

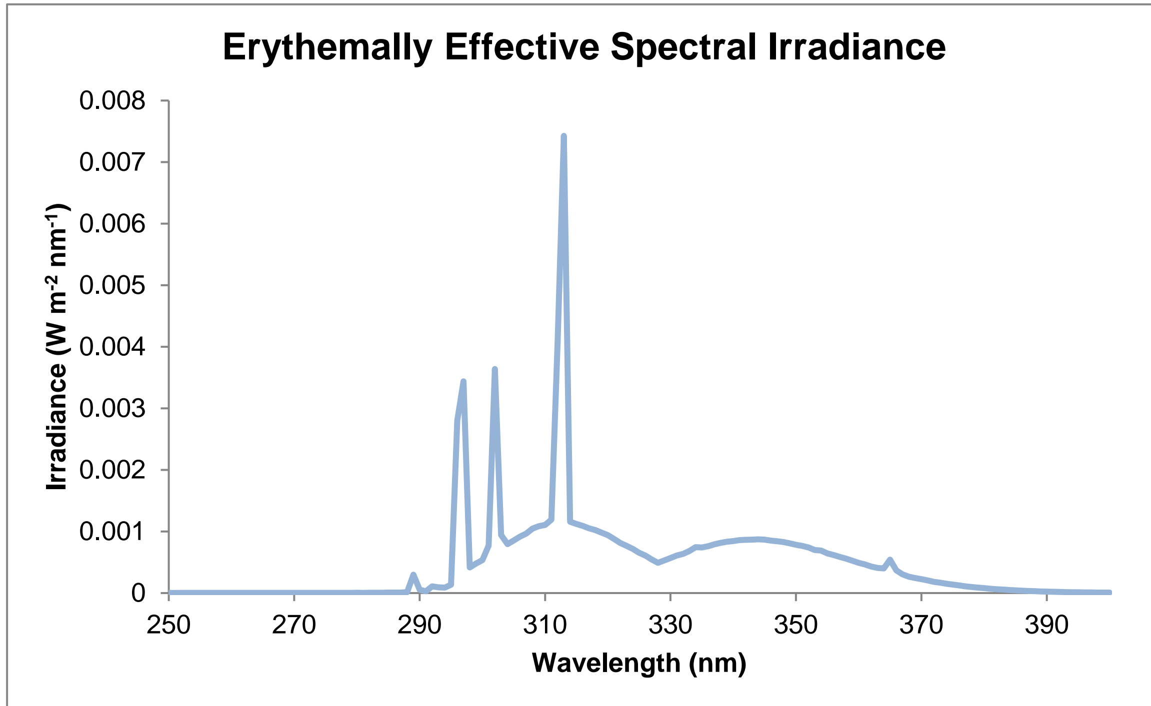
5. How is the minimal erythema dose defined?
    a. The minimum dose of UVB radiation required to cause just perceptible reddening of the skin
    b. The minimum irradiance of light required to cause just perceptible reddening of the skin
    c. The minimum dose of light required to cause just perceptible reddening of the skin
    d. The minimum dose of UVA radiation required to cause just perceptible reddening of the skin

Answer (c.) – The definition of the MED is not specific to any waveband, however an MED measured on the skin will be specific to the provocation source used. The MED is quantified as a dose of light (usually J cm^{-2}) though irradiance would typically be recorded alongside an MED.

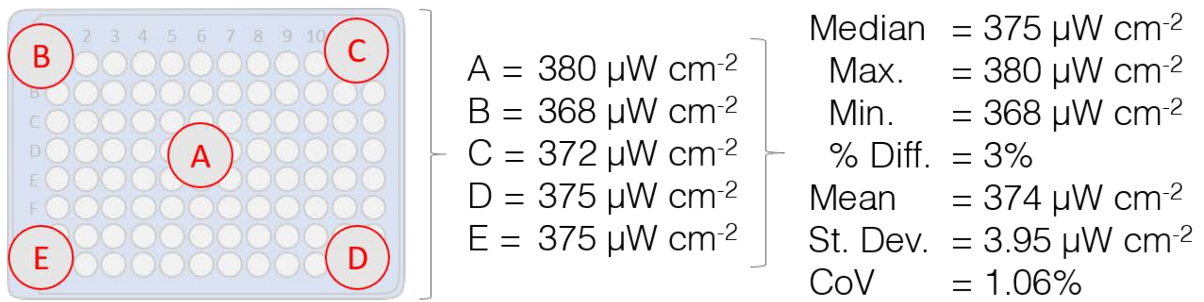
Figures, tables and captions



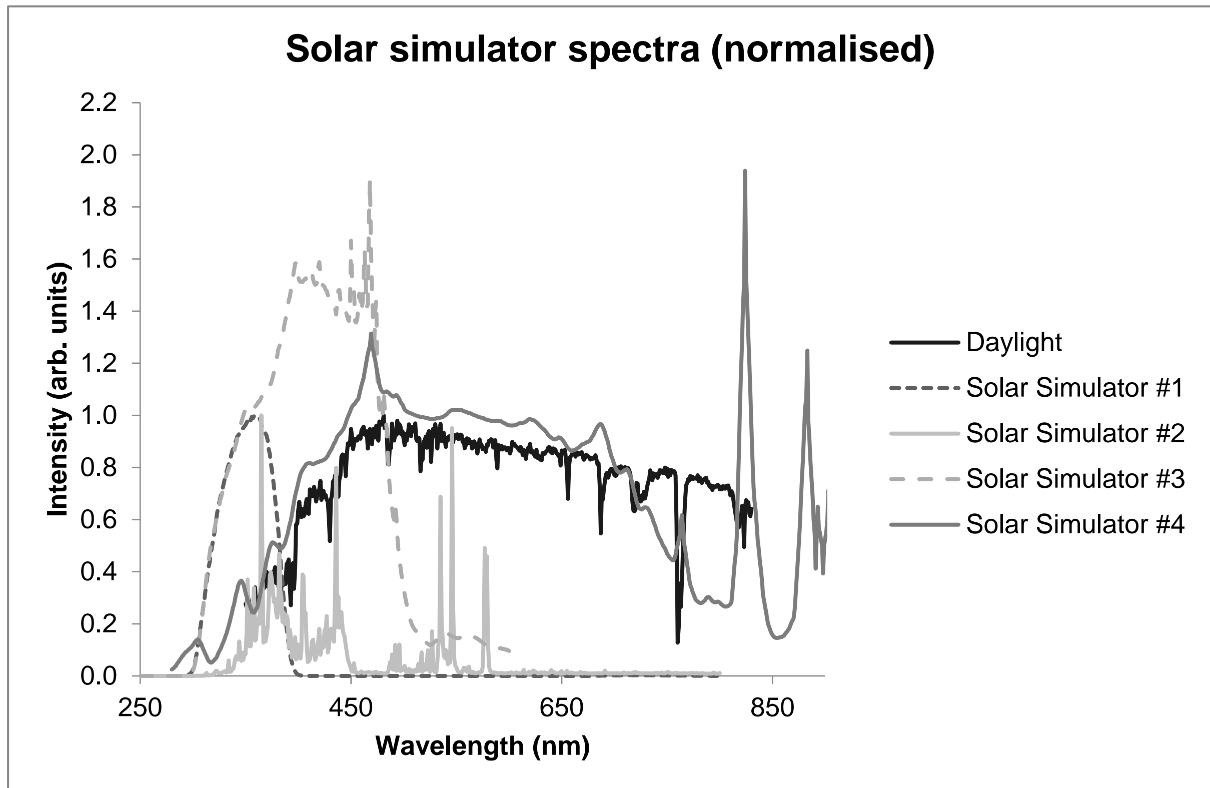
[Figure 1. Fluorescent lamp (PL-L 36W/09/4P, Philips Lighting, Amsterdam, Netherlands) spectral irradiance on non-logarithmic and logarithmic scale.]



[Figure 2 Demonstrates the importance of knowing the lamp spectrum over the full wavelength range and plotting the spectrum on a logarithmic scale. If only Figure 1 had been reported, it would have been missed that there are lower irradiance emissions in the UVB which are extremely effective at causing erythema. 298 nm radiation is 1,400 times more effective at causing erythema than 350 nm radiation.]



[Figure 3. An example of carrying out an assessment of uniformity of light distribution.]



[Figure 4. Comparison of daylight with a variety of solar simulators used for provocation testing in photobiology.]

	Description
Power (W)	In this context relates to the total optical output of the light source in all directions, measured in Watts (W). Not normally useful to report with non-coherent UVR exposures.
Irradiance (W m^{-2})	Power falling on a surface per unit area (W m^{-2}). It should always be reported alongside information on the spectral range to which it applies, i.e. Irradiance = 10 W m^{-2} (280-400 nm)
Spectral irradiance (W m^{-2} nm^{-1})	Irradiance per unit wavelength (W m^{-2} nm^{-1}) normally displayed in graphical format (Figure 1). Should ideally be displayed as both logarithmic and non-logarithmic y-axis.
Radiant Exposure (J m^{-2})	Often referred to as "dose". Radiant exposure (J m^{-2}) = Irradiance (W m^{-2}) x Exposure time (s)
Reciprocity	When the biological effects depend upon the radiant exposure only and not the way it was delivered. i.e. Same biological effect from 10 J m^{-2} delivered as 10 W m^{-2} in 1 second or 1 W m^{-2} in 10 seconds Reciprocity cannot always be assumed.
Wavelength	Refers to a specific wavelength of light per nm, e.g. 350 nm
Waveband	Defines a range of wavelengths, e.g. 315-400 nm, though sometimes written as the full-width half maximum (FWHM) about a central wavelength, e.g. 350 ± 10 nm

[Table 1. Key photophysics parameters]