Photobiology 102: UV Sources and Dosimetry - the Proper Use and Measurement of "Photons as a Reagent"

Francis P. Gasparro and Douglas B. Brown

Photobiology Laboratory, Department of Dermatology & Cutaneous Biology, Thomas Jefferson University, Philadelphia, Pennsylvania, U.S.A.

The goal of these comments is 3-fold: (i) to prevent the propagation of serious experimental errors in photobiology; (ii) to improve the quality and relevance of photobiology experiments; and (iii) to save authors, reviewers and editors time. Recently several manuscripts have been received in which the title describes the use of ultraviolet radiation (UVR) to induce some effect. It is not uncommon for the description of the UVR to be limited to one or two sentences. Often the sources are described all together incorrectly. This is inexcusable for any submission, and it is an especially egregious flaw in a manuscript in which UVR is the principal reagent! The most common examples involve studies in which "sunlamps" are employed. These lamps are often described as a source of UVB. Some authors give a peak wavelength, e.g., 300 or 313 nm or some variation of these. Some provide the percent of UVB and UVA (almost always omitting any mention of UVC). This is at a minimum inaccurate and probably just plain wrong. The most common sunlamps are broad spectrum sources that emit radiation starting in the UVC near 270 nm and ending in the UVA near 360 nm (see **Fig 1**). The percentages of wavelengths in the UVA, UVB, and UVC regions are 43%, 54%, and 3%, respectively (Brown *et al*, submitted).

The optimal source for skin photobiology experiments is the sun; however, for a host of self-evident reasons, it is not considered for laboratory studies and hence the need for artificial sources like fluorescent sunlamps (FS lamps). These so-called "sunlamps" provide a poor representation of the solar spectrum for several reasons. Foremost among these is that a small, but significant, portion of its spectral output contains shorter wavelength UV photons, in particular UVC photons and also some very short UVB photons, that never reach the surface of the Earth. A perusal of the photobiology literature would inform authors of the need to filter these wavelengths (Learn *et al*, 1993). Kodacel (Eastman Kodak, Rochester, NY) filtering effectively eliminates all of the 3% UVC from the FS lamp output (see **Fig 1**). As is also shown in **Fig 1**, the remaining spectrum is over-represented by UVB when compared with sunlight as it contains 43.3% UVB photons and 56.7% UVA compared with 4.1% and 95.9% for sunlight. Learn *et al* (1995) demonstrated that the use of unfiltered sunlamps may inadvertently result in the monitoring of a UVC effect that can then be mistaken for a UVB effect. For example, the human UVC- and UVB-induced erythemal effects seem to be due to different physiologic responses. Learn *et al* (1995) noted the biologic effectiveness spectrum of sunlamps can be calculated by taking the product of the output in a given region and the action spectrum for a specific event caused by those photons. **Table I** summarizes their findings, showing the effects of the relatively small amounts of UVC in these sunlamps. In another example, Roberts *et al* (1996) showed that relatively small amounts of nonsolar UVR photons (i.e., UVC and/or shorter UVB wavelengths, 290–295 nm, usually not found in terrestrial sunlight) contribute to the induction of immune suppression. Specifically, 36.5% and 3.5% of the total immunosuppressive UV energy from sunlamps and Kodacel-filtered sunlamps (KFS), respectively, fall below 295 nm. They also showed that immune suppressive effects occured at much lower doses using unfiltered sunlamps as the UVR source. Finally, the overrepresentation of the UVB wavelengths means that potential additive, synergistic or antagonistic effects seen with sunlight may be altered with sunlamps even if Kodacel filtering is employed.

Thus, while carefully performed in all other respects, the disregard for rigor in photobiology may mean that some experiments, in the end, contribute little to our understanding of photobiology events in human skin caused by solar exposure. In a worst case scenario, such results could be erroneous. Different sources, the lack of proper filtering, different or inappropriate meters, and a disregard for precision and accuracy could also make comparisons of data from different laboratories difficult if not impossible.

Therefore, it is essential that a set of photobiology standards be established so that we are all talking about the same thing when we say UVB or sunlight in a manuscript. First it must be appreciated that there is no routinely available substitute for the complete UV portion of sunlight. The inadequacy of sunlamps was described above. Even solar simulators fall short, although by a much

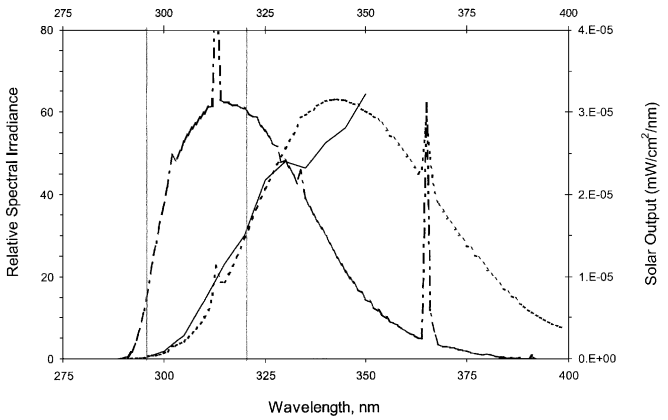

Figure 1. Spectral irradiance of UVR sources discussed. The spectral irradiances for Kodacel-filtered sunlamps (broken line) and UVA-340 lamps (dotted line) are compared with a typical solar irradiance (solid line, right axis). The vertical lines at 290 and 320 mark the UVC/UVB and UVB/UVA boundaries, respectively.

Manuscript received January 10, 2000; accepted for publication January 10, 2000.

Reprint requests to: Dr. Francis P. Gasparro, Photobiology Laboratory, Thomas Jefferson University, Room 428 (BLSB), 233 South 10th Street, Philadelphia, PA 19107. Email: francis.gasparro@mail.tju.edu

Abbreviations: FS lamp, fluorescent sunlamp; KFS, Kodacel-filtered sunlamp.

Table I. Effects of UVC portion of sunlamp spectrum

Observed effect	UVC contribution	Reference
Murine ear edema	10.4%	Learn *et al*, 1993
Human erythema	11.1%	Learn *et al*, 1993
	16.7%	Learn *et al*, 1993
Immune suppression	36.3%	Roberts *et al*, 1996

Table II. ''Metered dose'' to produce 750 CPD per mb

Source	''Metered dose'' for 750 CPD per mb	Slope CPD per mb per mJ per cm^2
FS	18	41
KFS	58	13
UVA-340	302	2.5

narrower margin than sunlamps; however, they are not routinely available because of their cost. There is one relatively inexpensive fluorescent lamp that comes close. In a recent paper (Beasley *et al*, 1996), UVA-340 lamps (Q-Panel, Cleveland, Ohio) were shown to provide output quite comparable with the short wavelength end of the solar spectrum (see **Fig 1**). With only slight differences from the sun in the short wavelength region of the spectrum, these lamps provide a reasonable and relatively inexpensive source of solar UVB photons (despite being called UVA-340 lamps). Clearly the latter are superior to FS lamps for photobiology experiments. Recently we demonstrated different effects in cells exposed to UVR from these three sources (FS, KFS, and UVA-340) (Brown *et al* submitted). The difference in end points could be attributed to different levels of DNA photoproduct formation even though all of the sources were measured with the same UVB probe. The extent of cyclobutane pyrimidine dimer (CPD) formation is a true measure of the dose of delivered UVR. **Table II** shows that dose required from each lamp to induce 750 CPD per megabase (mb).

This raises the issue of the kind of radiometer employed to determine the amount of incident energy delivered to the cells or specimens. It is essential that a high quality research device with calibration traceable to the National Bureau of Standards (or equivalent) be employed. While it is tempting to save precious research funds, the devices in most laboratory supply catalogs are toys compared with the research devices available from manufacturers such as International Light (Newburyport, MA), Optronics (Orlando, FL), or Solar Light (Philadelphia, PA). These manufacturers provide radiometric devices with detectors designed specifically for different kinds of UVR sources. In addition they provide calibration services that are necessary because of drift that occurs in all of these instruments with the passing of time.

There are numerous examples of these deficiencies and omissions in recent publications. Three will be summarized here. Pavey *et al* (1999) described the induction of the expression of p16CDKN2A in human skin after exposure to UVR. As they note in their introduction, CDNK2A encoding the inhibitor protein p16 has been identified as a tumor suppressor gene in which mutations have been reported in the germline of members of familial melanoma kindreds and to a lesser degree in sporadic melanomas. This recent study using human foreskins expanded on previously published work in cultured cells; however, this important step to a more realistic tissue to mimic real human skin exposure has been negated by an improper selection of the UVR source for the treatment of the skin specimens. The FS-20 UVB lamp they described, although known as a sunlamp, is a poor representation of the solar spectrum for several reasons. The first was described above – the lamp is a source of nonsolar shorter wavelength photons. Second, although many assume that UVB exposure and melanoma are linked in a causal fashion, there is little experiential data to support this assumption (Atillasoy *et al*, 1998). In fact, in appears that longer UVA wavelengths (beyond those also contained in ''sunlamps'') may have an important role in melanomagenesis (Setlow, 1996; Moan *et al*, 1999). Third, in their description of UV-induced damage the authors completely overlook the potential significance of UVA-induced oxidative damage to DNA (Kvam and Tyrrell, 1999). Thus, while carefully performed, these experiments have little potential to contribute to our understanding of photobiology events in human skin exposed to sunlight.

In another example, Kuhn *et al* (1999) described the activation of insulin-like growth factor in keratinocytes exposed to ''UVB radiation''. Unfiltered sunlamps were employed (DF Spandau, personal communication) and no radiometric device of any kind was described. It was claimed that growth factor deprivation lowered the threshold of the UVB dose required to induce apoptosis. Furthermore, in these studies it appears that the keratinocytes were irradiated in media. Since media contains a variety of UVR absorbing compounds, it is difficult to attribute the effects to that of ''UVB'' alone on the cells.

Shahmolky *et al* (1999) used UVR from unfiltered FS lamps to characterize the expression of mRNA encoding the ribosomal subunit L13A in rat keratinocytes. There are several problems with this report. First, in the title the authors claim the effects are due to UVB, yet the unfiltered source they employed also emitted UVC and UVA radiation. This oversight is compounded by the authors' awareness of this issue as indicated by their discussion in the opening paragraph of the Introduction. Second, their radiometry is incorrect. In a recent study we compared the ability of three UVR sources (see above) to induce the formation of CPD. It is important to note that the number of CPD formed is the real measure of delivered UVR. **Table II** shows the dose required to induce an equivalent number of CPD (750 per mb). All of the bulbs were used in the same apparatus operated under identical conditions and measured with the same UVB probe. Clearly these cannot all be ''correct'' doses. UVR probes are designed with a predetermined spectral response curve. If the source output matches the spectral response curve then accurate dosimetry can be performed; however, if there is a mismatch, the dosimetry will be inaccurate. The output of unfiltered FS lamps contains wavelengths which a SEE-240 probe is not designed to detect. It is likely that the effects described by Shahmolky *et al* as occuring at a dose of 9 mJ per cm^2 really correspond to a much higher effective dose (~2–5 times higher). Third, the experiments were performed at a single UVR dose with no rationale given for its selection. Thus, while the report appears to describe a quite novel finding, it is difficult to appreciate its application to human skin photobiology.

Fortunately there have not been similar problems in recent JID articles; however, as mentioned in the opening paragraph, this is not because of what is being submitted. These errors are caught during the review and editorial processes.[1] Yet it would be preferable if the issue of photobiology techniques (radiation and metering) were not in need of scrutiny and vigilance, hence the *raison d'etre* for this piece (see Box for suggestions for authors).

Other errors in photobiology occur frequently. Sometimes it is claimed that a UVB dose employed is equivalent to some multiple of a minimal erythemal dose (MED). For example, in the report by Pavey *et al* (1999), 875 J per m^2 was described as being equivalent to one MED, yet in fact this dose for the most common type of Caucasian skin is almost three MED (Baron *et al*, 1999). Furthermore, although the radiometer was not described it is unlikely to be able to respond to the nonsolar photons contained in their unfiltered source. Hence their meter reading is not an accurate measure of the ''reagent photons'' added in their system. The authors correctly note the limited penetrative ability of UVC in the interpretation of their results; however, they curiously state that this does not explain why responses observed with UVC are not detected with irradiation using the longer wavelength UVB. The

[1]Since July 1999, the photobiology section editor has sought this kind of supplemental information from authors before recruiting reviewers for the manuscript.

"SUGGESTIONS" FOR POTENTIAL JID SUBMISSIONS

1 At a minimum, in the *Materials and Methods*, the authors should describe the source employed and the manufacturer. The authors should explicitly state the spectral output of their source. Appropriate filtration should also be described.

2 Authors should not rely on manufacturer's data sheets for source characteristics other than as a rough guideline. There can be variations from lot to lot. Therefore for the highest integrity, spectroradiometry should be employed to determine the spectral output of the lamps used to perform the experiments.

3 Similarly for source dosimetry, the lamps need a brief warm-up period (15 min) prior to obtaining an intensity reading through any filter or lid that might be covering the specimen or cells. The plastic used to make tissue culture dishes will strongly attenuate the UVB region of the spectrum and may also have an effect in the UVA range.

4 While spectroradiometric measurements need only be performed at relatively infrequent intervals (new lamps or after 6 months of use), radiometric measurements need to be performed for each experiment. Variations in line voltage from day to day will cause changes in the energy output of the lamps.

5 Lastly, authors should be aware of the need to perform all irradiation of cells in a colorless, transparent medium (e.g., PBS or a Hanks buffer) devoid of organic compounds that could act as photosensitizers.

answer quite simply is that the photochemistry is different at the different wavelengths. Not only does the yield of photoproduct vary with wavelength (Tommasi *et al*, 1997), but the local DNA sequences at which they form may also vary (Denissenko *et al*, 1997; Drouin and Therrien, 1997). While their explanation of different effects in different cells types may be part of the answer it is very likely only a small part.

Ultimately it must be kept in mind that the action spectrum for any measured effect is not expected to be a flat line as a function of wavelength. To apply *in vitro* studies with an artificial source to effects that occur when human cells are exposed to sunlight, it is essential that sources and meters reflect this real world situation. Furthermore, while a given study may examine one particular effect, it must be appreciated that UVR may affect other related phenomenon (perhaps with a different action spectrum). In summary, while exposing cells or skin to UVR may be easily achieved, it must be done using parameters that are relevant to human photobiology. No one would add another reagent to skin cells and consider the implications for human biochemistry if human skin were never exposed to that agent. The same must be true for UVR exposure.

The assistance of Adam E Peritz, PhD with filter transmission measurements and spectroradiometry is gratefully acknowledged. The authors also gratefully acknowledge: Lou D'Aloisio, Marvin Arocena, and Donald Forbes from Primedica-Argus for providing the FS-40 lamps and Kodacel and allowing access to their facilities for measuring lamp emission spectra; Patrick Brennan from Q-Panel for providing a set of UVA-340 lamps and the data shown in ***Fig 1****; Robert Angelo from International Light and Robert Sayre from Rapid Precision Testing (Memphis, TN) for solar spectral data shown in* ***Fig 1****; David L. Mitchell and Stephanie Chiarello from MD Anderson (Smithville, TX) for the CPD analyzes. FG was supported in part by a grant from Therakos, Inc.*

REFERENCES

Atillasoy ES, Seykora JT, Soballe PW, *et al*: UVB induces atypical melanocytic lesions and melanoma in human skin. *Am J Pathol* 152:1179–1186, 1998

Baron ED, Stern RS, Taylor CR: Correlating skin type and minimum erythemal dose. *Arch Dermatol* 135:1278–1279, 1999

Beasley DG, Beard J, Stanfield JW, Roberts LK: Evaluation of an economical sunlamp that emits a near solar UV power spectrum for conducting photoimmunological and sunscreen immune protection studies. *Photochem Photobiol* 64:303–309, 1996

Denissenko MK, Chen JX, Tang M-S, Pfeifer GP: Cytosine methylation determines hot spots of DNA damage in the human p53 gene. *Proc Natl Acad Sci USA* 94: 3893–3898, 1997

Kuhn C, Hurwitz SA, Kumar MG, Cotton J, Spandau DF: Activation of the insulin-like growth factor-1 receptor promotes the survival of human keratinocytes following ultraviolet B irradiation. *Int J Cancer* 80:431–438, 1999

Kvam E, Tyrrell RM: The role of melanin in the induction of oxidative DNA base damage by ultraviolet A irradiation of DNA or melanoma cells. *J Invest Dermatol* 113:209–213, 1999

Learn DB, Beard J, Moloney SJ: The ultraviolet C energy emitted from FS lamps contributes significantly to the induction of human erythema and murine ear edema. *Photodermatol Photoimmunol Photomed* 9:147–153, 1993

Learn DB, Beasley DG, Giddens LD, Beard J, Stanfield JW, Roberts LK: Minimum doses of ultraviolet radiation required to induce murine skin edema and immunosuppression are different and depend on the ultraviolet emission spectrum of the source. *Photochem Photobiol* 62:1066–1075, 1995

Moan J, Dahlback A, Setlow RB: Epidemiological support for an hypothesis for melanoma induction indicating a role for UVA radiation. *Photochem Photobiol* 70:243–247, 1999

Pavey S, Conroy S, Russell T, Gabrielli B: Ultraviolet radiation induces p16CDKN2A expression in human skin. *Cancer Res* 59:4185–4189, 1999

Roberts LK, Beasley DG, Learn DB, Giddens LD, Beard J, Stanfield JW: Ultraviolet spectral energy differences affect the ability of sunscreen lotions to prevent ultraviolet-radiation-induced immunosuppression. *Photochem Photobiol* 63:874–884, 1996

Setlow RB: Relevance of in vivo models in melanoma skin cancer. *Photochem Photobiol* 63:410–412, 1996

Shahmolky N, Lefebvre DL, Poon R, Bai Y, Sharma M, Rosen CF: UVB and γ-radiation induce the expression of mRNAs encoding the ribosomal subunit L13A in rat keratinocytes. *Photochem Photobiol* 70:341–347, 1999

Tommasi S, Denissenko MF, Pfeifer GP: Sunlight induces pyrimidine dimers preferentially at 5-methylcytosine bases. *Cancer Res* 57:4727–4730, 1997

Note added in Proof This is not the first time such questions have been raised in the Journal. In a special supplement in 1959 this issue was addressed at a time when the concern revolved round delineating physiologic effects in skin (*J Invest Dermatol* 32s:132–391, 1959). One contributor wrote "there is an urgent need for a generator which will emit a constant light comparable to the wavelengths emitted by the sun's rays at noon, on a clear day in June, in the mid-west" (Kesten, pp. 165–166). Another wrote "Of the available emitters, the fluorescent 'sun' lamps seem to have come closest to the short radiation from the sun, are inexpensive, easy to operate, can be calibrated exactly and show little change of spectrum or intensity with time. Wider use and study of the biologic applications of this type of uV emitter is seriously recommended" (Urbach, pp. 167–169). A concern then was delivering a sufficient amount of energy to discern a perceptible physiologic effect in skin. Today with the tools of molecular biology, this is no longer the substantive issue it was then. Now the goal of photobiology studies in the skin should be to elucidate key molecular events which occur when skin (or skin cells) are exposed to physiologically and biologically relevant artificial ultraviolet radiation.

While to some it may seem overly ambitious to expect today's investigators to be aware of a debate from more than two decades ago, it is important to remember the age-old axiom – those who do not study history are doomed to repeat it! In 1978, Claud Rupert succinctly addressed the issue of this editorial in a commentary (Rupert CS: The biologic effectiveness of ultraviolet light. *Natl Cancer Inst Monogr* 50:85–89, 1978) "The effect of a polychromatic illumination depends on its wavelength distribution, weighted by the effectiveness of each wavelength (the action spectrum) under the conditions employed." Unfortunately these words did not have the desired impact at that time.