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Measuring Effective Vitamin D_3-Producing Ultraviolet B Radiation Using Solartech's Solarmeter® 6.4 Handheld, UVB Radiometer

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Abstract

Several types of UVB-emitting lamps were tested for their ability to generate vitamin D_3 and related isomers from precursor 7- dehydrocholesterol or provitamin D_3 in an in-vitro ampoule system. Lamp output was also measured using a spectroradiometer and two types of broadband UVB meters. UVB recorded in spectroradiograms was partitioned in several different ways, for example, as sub-bands. The various measures of UVB, including UVB meter readings, were regressed against the ampoule measurements of percent photoproduct to determine which could best explain D_3 synthesis. It was found that UVB irradiances between 280 and 304 nm, D_3 Yield Index, and Solarmeter® 6.4 readings each explained greater than 95% of ampoule D_3 synthesis. Equations are presented that allow conversion of Solarmeter® 6.4 units (IU/min) to D_3-irradiance units ($\mu W/cm^2$) or D_3 Yield Index values. Unlike other broadband UVB meters, readings by the Solarmeter® 6.4 of a variety of lamp types are directly comparable for D_3-synthesizing ability regardless of differences in spectral output among lamps.

Introduction

The recognition that ultraviolet radiation (UV) is of importance to reptiles (Laszlo, 1969) was followed by studies to determine the wavelengths involved and the quantity required. It became evident that the most important component is ultraviolet B (UVB) (280–315 nm) because of its role in vitamin D_3 (D3) synthesis and indirectly in calcium/phosphorus metabolism. UVB drives the conversion of proD3 (7-dehydrocholesterol = DHC) to preD3, which is then thermally isomerized to D3 (Chen, 1999; Holick, 2004). In the 1970s, some cases of nutritional metabolic bone disease (nutritional secondary hyperparathyroidism) in captive reptiles were recognized as resulting from an insufficiency of D3 caused by inadequate levels of UVB radiation (Frye, 1981), a condition that remains of concern to this day (Mader, 2006).

Knowing the quantity of UVB, often expressed as irradiance ($\mu W/cm^2$), emitted by natural light and various lamps is essential for the evaluation of their D3-synthesizing potential. Spectroradiometers that record irradiances at one-nm intervals across the UV and visible bands are available. Lindgren (2004) used such a spectroradiometer to measure the output from a variety of lamps used in herpetoculture; this included an analysis of UVB and the calculation of a D_3 Yield Index that was meant to accurately reflect the true D3-synthesizing potential of a lamp. Unfortunately, these meters are relatively costly and not always convenient to work with. Handheld broadband UVB radiometers manufactured by several companies are available; they are less costly and easier to use. However, it has been reported that several of these meters may give a different irradiance reading from the same light source (Gehrmann et al., 2004a, b). Part of this discrepancy is attributable to wavelengths at the red and near-infrared end of the spectrum erroneously processed as UVB readout. Thus, a meter might indicate the presence of UVB from a source known to emit none. Being made aware of this, Solartech, Inc. (Harrison Township, Michigan) created their Solarmeter® 6.2 UVB meter to reject out-of-bandwidth response, hence eliminating this unwanted input.

Wavelengths within the UVB band are not equally efficacious in producing preD3 from DHC. This is reflected in the action spectrum, a graph which relates ability to produce D3 to specific wavelengths (MacLaughlin et al., 1982). This action spectrum has now been re-evaluated and published (with full data) as the definitive pre-vitamin D_3 action spectrum (CIE, 2006). The maximum conversion occurs at about 298 nm, with wavelengths on either side of 298 nm becoming progressively less efficient in driving the conversion. About 60% is produced between 290 and 300 nm. Broadband UVB meters characteristically measure UVB wavelengths outside the effective D3-synthesizing band, making it difficult to relate the meter reading to the actual D3-synthesizing potential. Solartech, Inc. designed a Solarmeter® 6.4 that was essentially responsive only to wavelengths within the D3 action spectrum and furthermore weighted the input to reflect the efficiency for producing preD3 from DHC (Solartech, Inc., 2005). The readout was designed to reflect the D3 production rate (in IU D3/min) for human type 2 skin. How this reading is related to D3 synthesis in various reptiles and other species remains largely unknown at this time.

In the 1980s, Michael Holick's lab at Boston University Medical School developed a technique for measuring the D3-synthesizing ability of a UVB source by measuring the production of D3 and related photoproducts from DHC contained in UVB-permeable glass ampoules. This procedure represents a direct way of measuring D3-synthesizing potential that can be related to irradiance readings from meters and used to judge the validity of their output. Two such studies have been published (Gehrmann et al., 2004a, b). The use of ampoules also allows for validation of the analysis of UVB, including the D_3 Yield Index conducted by Lindgren (2004).

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Table 1. Various lamps used in current study. These lamps were acquired in 2005 and most are quite different in spectral output from lamps sold with the same brand names in 2008.

Lamp	Manufacturer	Type	Power (W)	Distance (m)
Zoologist Mega-Ray	Mac Industries Inc. (Reptile UV)	Narrow flood	100	1.22
Mega-Ray	Mac Industries Inc. (Reptile UV)	Narrow flood	100	0.30
PowerSun UV 160 W	Zoo Med Laboratories, Inc.	Spot	160	0.30
PowerSun UV 100 W	Zoo Med Laboratories, Inc.	Spot	100	0.30
Reptisun 10.0 UVB	Zoo Med Laboratories, Inc.	1219 mm (48") tube	40	0.30
Reptisun 10.0 UVB Desert	Zoo Med Laboratories, Inc.	Compact fluorescent	26	0.30
Reptisun 5.0 UVB Tropical	Zoo Med Laboratories, Inc.	Compact fluorescent	26	0.30
UVB Mystic Compact	Big Apple Herpetological (made in China)	Compact fluorescent	18	0.30

The purpose of this study is to evaluate the relationship of the D_3 Yield Index and irradiances within the UVB band for several lamps, as measured by a spectroradiometer, to the production of D3 and related photoproducts as measured in ampoules. Furthermore, we compare these results to outputs from the Solarmeter® 6.2 and Solarmeter® 6.4 meters.

Materials and Methods

Eight different UVB-emitting lamps were obtained from either Zoo Med Laboratories Inc. (San Luis Obispo, California) or Mac Industries Inc. (Cedar Point, North Carolina) (Table 1). All lamps were pre-conditioned by burning them for 100 hours prior to testing. For stability, each lamp was pre-heated for 30 minutes before actual measurements to allow it to reach its nominal working temperature. Spectral measurements were made by Suomen Aurinkosimulaattori Oy/Solar Simulator Finland Ltd. (Raisio, Finland) using IL700A Research Radiometer (International Light Inc., Newburyport, Massachusetts). All measurements were taken in free field, at a distance of 30 centimeters from the surface of the lamp. Fluorescent tubes were measured at their center point, perpendicular to the longitudinal axis of the lamp. Bulbs were measured from the direction of base longitudinal axis at a distance of 30 centimeters from the face of the lamp, except for the Zoologist Mega-Ray, whose recommended minimum distance is 122 cm; this recommendation was followed. Compact fluorescents were measured at 90-degree angle from their central axis.

The lamps used in this study were selected for their variety of output and structure as required in this study for the validation of consistent response of the Solarmeter® 6.4 in predicting ampoule response. Most of the lamps marketed at this time (2008) using the brand names in Table 1 are quite different with respect to distribution and output of UV from those characterized here. A website that offers information on a wide variety of lamps is http://www.uvguide.co.uk/index.htm.

The numerical analysis of spectral data is identical to Lindgren (2004). The UVB range was divided into two sub-bands, UVB-1 (280–304 nm) and UVB-2 (305–319 nm) to facilitate separate analysis of the bandwidth range where the D3 photosynthesis mainly takes place.

The D_3 Yield Index was obtained by first calculating the biologically effective UV irradiance (UVBE) of a source with the following equation:

$$\mathrm{UVBE} = \sum_{\lambda=252}^{313} S(\lambda)A(\lambda)\Delta\lambda$$

where:

$S(\lambda)$ = measured irradiance at wavelength λ ($\mu W/cm^2$)
$A(\lambda)$ = coefficient factor for wavelength λ, derived from action spectrum of DHC to PreD**3** photosynthesis from MacLaughlin et al. (1982)
$\Delta\lambda$ = wavelength stepping, here 1 nm.

The UVBE was converted to the final index value by a suitable proportionality constant. As in Lindgren (2004), a constant was selected which would give the reference sun (in Finland) a value of 1000. If sufficient solar data become available, a more universal reference may be specified in future work.

The ampoules were exposed for 120 minutes. The Solarmeter® 6.2 and 6.4 measurements were taken simultaneously in the same configuration. After exposure, three replicates per ampoule were analyzed using a Waters 501 HPLC pump and a 490E multiwave detector set to read at 260 nm and controlled by a Millennium 2010 Chromatography Manager program (Waters Chromatography Division, Milford, Massachusetts). The mobile phase was 8% ethyl acetate in hexane and the column was Econosphere silica, 5 µm, 250 × 4.6 mm (Alltech Associates, Inc. Deerfield, Illinois). The flow rate was 1.8 ml/min. Ampoule contents were analyzed for substrate (DHC), photoproducts (preD3 and lumisterol), and D3 concentrations. The percent of photoproducts and D3 synthesized were calculated (see Gehrmann et al., 2004b, for more details).

Results

Table 2 shows the results of the spectrophotometric analysis, ampoule production of D3 and related photoproducts, and Solarmeter® readings for the eight lamps used in this study.

The greater the effective UVB irradiance, the greater will be the amount of DHC substrate converted and the greater will be the *total* amount of photoproducts produced in ampoules

Table 2. UVB and vitamin D_3-synthesizing characteristics of various lamps used in this study.

Lamp	UVB (280–319 nm)	UVB-1 (280–304 nm)	UVB-2 (305–319 nm)	D_3 Yield Index	Total (% Product)	Prod. Rate (% / sec)	Model 6.2 ($\mu W/cm^2$)	Model 6.4 (IU/min)
Zoologist Mega-Ray	73.0	11.7	62.0	2168	28.54	46.68×10^{-6}	100	52
Mega-Ray	180.0	30.2	150.0	5657	53.98	107.81×10^{-6}	202	106
PowerSun UV 160 W	29.0	2.1	27.0	471	8.56	12.42×10^{-6}	52	17
PowerSun UV 100 W	20.0	2.8	17.0	581	11.10	16.34×10^{-6}	32	16
Reptisun 10.0 UVB	24.0	1.4	23.0	416	3.16	4.46×10^{-6}	37	9
Reptisun 10.0 UVB Desert	9.2	1.6	7.5	346	4.18	5.92×10^{-6}	11	6
Reptisun 5.0 UVB Tropical	3.4	0.6	2.8	132	0.97	1.35×10^{-6}	3	2
UVB Mystic Compact	31.0	11.7	19.0	2260	39.03	68.72×10^{-6}	51	50

during a given exposure time (see Table 2). However, the amount of photoproducts formed in ampoules exposed to lamps with higher effective irradiances will be *proportionately* less than the amount formed in ampoules exposed to lower irradiances because the rate of photoproduct formation declines as the DHC substrate concentration decreases. In order to compensate for this curvilinearity, we calculated a proportional rate that allows for a less biased comparison among lamps. We used the following equation:

$$s(t) = s(0) \times e^{-rt}$$

where:

r = the proportional rate of transformation of substrate to D3 and other photoproducts;
t = time in seconds;
s(t) = % of DHC substrate remaining after t seconds.

Solving the equation above for r, substituting 100% for s(0), and evaluating at t = 7200 sec (= 120 min) gives:

$$r = \{\ln(100) - \ln(s(7200))\} / 7200$$

See Table 2 for the calculated value of the production rate r for each of the lamps.

The calculated proportional rates in the ampoules for each of the lamps serves as the dependent variable in the regression equation calculated for each of the independent measures of UVB-D3 synthesizing ability, including the meter outputs, shown in Table 2. The coefficient of determination (R^2) associated with each regression indicates the extent to which the ampoule values are explained by the various independent UVB values. The R^2 values, multiplied by 100 to yield percent, are shown in Figure 1. It is evident that the Solarmeter® 6.4 (ST6.4), D_3 Yield Index (D3 YI), and UVB-1 each account for greater than 95% of the variation. In contrast, Solarmeter® 6.2 (ST6.2), total UVB, and UVB-2 are 80% or lower.

The output for the Solarmeter® 6.4 is in IU/min but for some purposes irradiance units ($\mu W/cm^2$) or D_3 Yield Index units might be more convenient. Accordingly, UVB-1 and D_3 Yield Index values were regressed on Solarmeter® 6.4 values, all values from Table 2, and the R^2 values and best-fit equations were determined. The R^2 values for both UVB-1 (Figure 2) and D_3 Yield Index (Figure 3) are both equal to 0.997. The prediction equation for each showing the predicted value of UVB-1 and D_3 Yield Index for various values measured by the Solarmeter® 6.4 is shown on the appropriate figure and in the conclusions.

Discussion

The quantity of UVB-synthesized D3 photoproducts in ampoules is directly related to the totality of effective wave-

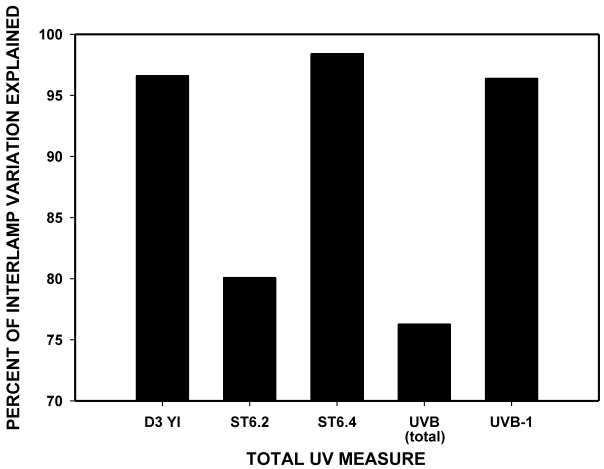

Figure 1. The extent to which variation in D3 product synthesis among ampoules exposed to different lamp irradiances is explained by various measures of UVB. UVB-2, which is not shown, is 26 %.

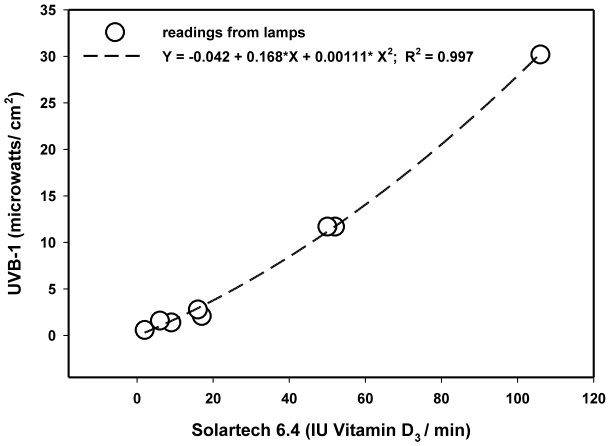

Figure 2. The quantitative relationship between the readings from a Solartech 6.4 meter and UVB irradiance contained within the 280-304 nm band. The equation and the associated R^2 value are shown at the top of the graph.

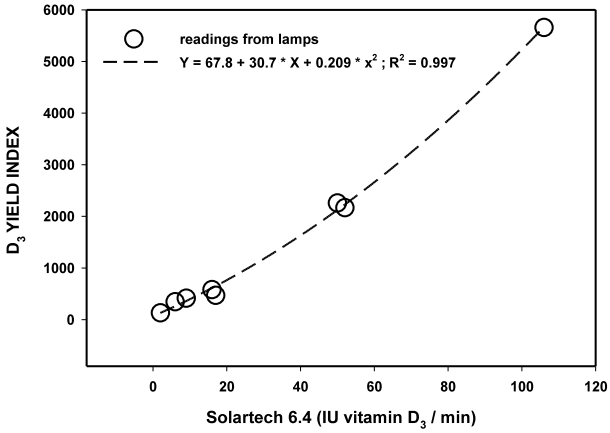

Figure 3. The quantitative relationship between readings of the Solartech 6.4 meter and the D_3 Yield Index. The equation and associated R^2 value are shown at the top of the graph.

lengths and their relative efficacy in producing preD3 from DHC. This relationship is embodied in the action spectrum, which shows that the most effective wavelengths lie between 280 and 305 nm. The observation that UVB-1, D_3 Yield Index, and Solarmeter® 6.4 readings all account for more than 95% of the variation in ampoule D3 photoproducts is expected because the UVB-1 sub-band lies entirely within the most effective D3 synthesizing band, and the D_3 Yield Index and Solarmeter® 6.4 readings are actually referenced to the action spectrum for production of pre-vitamin D3 from DHC.

The lamps in this study represent a variety of fluorescent and self-ballasted mercury vapor arc lamps. The ability of UVB-1 irradiance, D_3 Yield Index, and Solarmeter® 6.4 meters to predict ampoule D3 synthesis is expected to hold for virtually any lamp in these categories. The extent to which they will predict ampoule D3 synthesis by UVB from natural light remains to be determined but it is expected to be comparable to that of lamp-based sources of UVB.

Table 3. Relationship between the Solartech 6.4 readout (IU/min) and the UV Index (UVI) for the lamps used in this study.

Lamp	Model 6.4 (IU/min)	UV Index (6.4 reading divided by 7.14)
Zoologist Mega-Ray	52	7.3
Mega-Ray	106	14.8
PowerSun UV 160 W	17	2.4
PowerSun UV 100 W	16	2.2
Reptisun 10.0 UVB	9	1.3
Reptisun 10.0 UVB Desert	6	0.8
Reptisun 5.0 UVB Tropical	2	0.3
UVB Mystic Compact	50	7.0

The Solarmeter® 6.4 is an inexpensive broadband UVB meter that adequately describes the quantity of D3-synthesizing UVB. However, the output from the meter is referenced to the rate of D3 synthesis (IU/min) by type 2 human skin. The extent to which these units may be applied to reptile skin is unknown but their use may be confusing since most studies involving reptiles have described the amount of UVB as irradiance ($\mu W/cm^2$). For some purposes, it may be desirable to use the Solarmeter® 6.4 but convert the units to "D3 irradiance" using the equation presented herein (see conclusion 4 below).

Users of Solarmeter® 6.4 should note that the Excel-application (calculator) provided with the instrument will give slightly different results when the IU/min readout is converted to effective UVB. The values obtained by the equation given in Figure 2 are consistently lower than those obtained with the calculator. This is explained by the fact that while the calculator is based on human type 2 skin in specific circumstances, the data presented in this paper is based on the *in vitro* results of ampoule D3 synthesis.

The ampoule D3 production rates given in Table 2 can be used as a guide to estimate the rate of D3 photosynthesis in actual skin. The ampoules are a good approximation of photosynthesis taking place in skin, but the formation of the actual vitamin D3 is a multi-stage process. Its speed is largely temperature-dependent and there are significant differences in speed of the entire process in various species of animals. For example, in comparison to *in vitro* results, the speed of thermal isomerization of preD3 to D3 can be more than 10 times faster in actual skin samples of humans, frogs (*Rana temporaria*) and iguanas (*Iguana iguana*) (Holick et al., 1995). The rates have also been shown to differ among species of lizards of the gen-

era *Anolis*, *Sceloporus* and *Hemidactylus* (Ferguson et al., 2005).

Solartech, Inc. has designed a meter (Solarmeter® 6.5) to measure the Ultraviolet Index (UVI) directly (www.solarmeter.com/model65.html). It is essentially a Solarmeter® 6.4 with the IU/min dimensions internally divided by 7.14 to produce a readout in UVI units (see Table 3 for values associated with the lamps used in this study). The UVI is a universally recognized measurement and is appropriate for describing the UVB environment globally. The World Health Organization booklet www.who.int/uv/publications/en/GlobalUVI.pdf. offers information about the UVI and lists links to sites that cover specific geographic areas. For example, annual time series of UVI values from natural light for selected cities in the USA can be found at www.cpc.ncep.noaa.gov/products/stratosphere/uv_index/uv_annual.shtml. These values can be used as a guide to determine the maximum allowable UV irradiation. However, it is important to consider the reptile's natural habitat and activity patterns when evaluating readings taken in vivaria illuminated with lamps. The meteorological readings are always taken unobstructed and out in the open, but very few reptile species spend any length of time in exposed areas under full sunlight.

Conclusions

1. Both the unweighted UVB irradiance between 280 and 304 nm and the D_3 Yield Index calculated from spectroradiograms explain greater than 95% of the variation in D3 synthesis in ampoules.

2. The broadband UVB Solarmeter® 6.4 explains greater than 95% of the variation in D3 synthesis in ampoules.

3. A major advantage of the Solarmeter® 6.4 is that readings from a wide variety of UVB sources may be compared directly for D3-synthesizing potential without compensation for differences in spectral output among lamps.

4. The readout from the Solarmeter® 6.4 in IU/min can be converted to D3 irradiance in $\mu W/cm^2$ by use of the equation:

$$D3\ Irrad = 0.00111 \times (IU/min)^2 + 0.168 \times IU/min - 0.042$$

5. The readout from the Solarmeter® 6.4 in IU/min can be converted to D3 Yield Index by use of the equation:

$$D_3\ Yield\ Index = 0.209 \times (IU/min)^2 + 30.7 \times IU/min + 67.8$$

6. The readout from the Solarmeter® 6.4 as IU/min can be converted to the UV Index by dividing IU/min by 7.14. This value of UVI will be the same as the UVI output from the Solarmeter® 6.5, which can therefore be used in place of the Solarmeter® 6.4.

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An Unusual Microhabitat for an American Toad (*Anaxyrus americanus*)

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North American toads (genus *Anaxyrus*) do not typically climb trees. However, a recent report described a southern toad (*Anaxyrus* [as *Bufo*] *terrestris*) in a tree cavity approximately 1.6 m above the ground in a situation where it was likely that it had climbed the vertical trunk (Kornilev, 2007). This reminded me of a similar case involving an American toad (*A. americanus*).

I recorded the following observation at the Nelson-Trevino Bottoms of the Chippewa River, Buffalo County, Wisconsin (T23N,R14W,S27) (Cochran, 2001). On 23 August 1997, I discovered an adult American toad sitting partially embedded in a slight depression of rotted wood on top of a vertical tree stump approximately 25 cm in diameter and 1 m above the floodplain forest floor. One possibility, however unlikely, is that the toad climbed to this position. An alternative explanation is that it reached the top of the stump by swimming during the spring high water period, but it is not clear why the toad would have remained there during the subsequent months. Heavy shading by the forest canopy may have kept temperature and moisture within acceptable limits.

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