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Biological Impact of Shorter Wavelength Ultraviolet Radiation-C†

Chikako Nishigori*[1,2], Nozomi Yamano[2], Makoto Kunisada[2], Aiko Nishiaki-Sawada[3], Hiroyuki Ohashi[3] and Tatsushi Igarashi[3]

[1]Japanese Red Cross Hyogo Blood Center, Kobe, Japan
[2]Division of Dermatology, Department of Internal Related, Graduate School of Medicine, Kobe University, Kobe, Japan
[3]Ushio Inc., Chiyoda-ku, Tokyo, Japan

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ABSTRACT

Life on earth has constantly coped with the impact of solar radiation, especially solar ultraviolet radiation (solar UV). Various biological mechanisms protect us from solar UV. New devices emitting shorter wavelengths UV-C, *i.e.* <254 nm emitted by conventional UV germicidal lamps, have emerged. These shorter wavelength UV-C emitting devices are useful for various purposes, including microorganism inactivation. However, as solar UV-C does not reach the earth surface, biological impacts of UV-C has been studied using 254 nm germicidal lamps, and those using shorter wavelength UV-C is rarely known. To balance the utility and risk of UV-C, the biological effect of these new UV-C emitting devices must be investigated. In addition, our knowledge of biological impacts of the wavelength-dependent entire UV (100–400 nm) must be enhanced. In this review, we briefly summarize the biological impacts of shorter wavelength UV-C. Mechanisms of UV-C-induced cellular damage and factors affecting the microorganism inactivation efficiency of UV-C have been discussed. In addition, we theoretically estimate the probable photocarcinogenic action spectrum of shorter wavelength UV-C. We propose that increasing the knowledge on UV-C will facilitate the adoption of shorter wavelength UV-C emitting new devices in an optimal and appropriate manner.

INTRODUCTION

Ultraviolet radiation C (UV-C) is defined as UV falling within the wavelength range of 100–280 nm. As it is absorbed by ozone layer, solar UV-C cannot reach the earth surface. Conventional germicidal lamps that primarily emit 254 nm UV are routinely utilized for surface sterilization because this wavelength is effective in killing microorganisms, however, they are not safe to human skin due to their genotoxic effects. Recently developed much shorter (<254 nm) wavelength UV-C emitting devices are less hazardous to mammalian cells, in addition to being efficient in microorganism disinfection. Recently, shorter wavelength UV-C ranging from 200 to 230 nm is sometimes called "Far UV-C". Issues concerning the use of UV-C emitting devices are becoming more relevant in the "COVID-19 Era" to medical professionals and people working in public spaces. This has led to a burst in investigations related to usefulness and safety of various shorter wavelength UV-C, especially regarding Far UV-C; they are efficient in killing various microorganisms.

Solar radiation is beneficial: it gives us warmth, brightness, activates vitamin-D3, brings about photosynthesis, and acts as a natural disinfectant. However, it is also one of the most dangerous environmental hazards because acute exposure to sunlight causes cutaneous inflammation, sunburn and immune suppression, while chronic exposure causes skin cancer. Various bioeffects of solar radiation are largely attributed to solar UV. UV is a well-known mutagen and carcinogen. It induces carcinogenic somatic mutations by causing DNA photolesions at dipyrimidine sites. In addition, the inflammation and immune suppression induced by UV also play a crucial role in developing skin cancers (1). Both these beneficial and harmful aspects originate from the same UV characteristic of inducing DNA lesions: both the disinfection and carcinogenic potencies depend on the efficiency of causing DNA lesions. Therefore, knowing the subcellular location of the DNA becomes pertinent to understanding whether a given UV wavelength can be absorbed by it. Although rapidly increasing, the existing literature on this aspect of short-wavelength UV-C is lacking. In this review, we summarize the state-of-the-art knowledge regarding UV-C including far UV-C, to emphasize existing knowledge and future directions. We discuss the bio-impact of UV, with a major focus on shorter wavelength UV-C emitted by artificial devices. In addition, we discuss the disinfection efficacy and acute and chronic bio-effects of the safe-far UV-C wavelengths within the UV-C spectrum.

DNA LESIONS CAUSED BY UV

UV-B is responsible for sunburns, immune suppression and photocarcinogenesis in humans. And, 254 nm UV-C emitted from germicidal lamps is used for disinfection and its influence on

*Corresponding author email: chikako@med.kobe-u.ac.jp (Chikako Nishigori)
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human health is crucial. Therefore, previous studies on DNA photolesions have focused on UV-B and 254 nm UV-C.

Absorption of UV-C (100–280 nm) or UV-B (280–315 nm) by DNA excites the nucleobases, resulting in the formation of covalently linked dimeric photoproducts—called cyclobutane pyrimidine dimers (CPD) and pyrimidine pyrimidone (6–4) photoproducts ((6–4)PP)—at adjacent dipyrimidine sites (called both together as "dipyrimidine photoproducts"). Whereas, UV-A (315–400 nm) and visible lights tend to participate in the formation of reactive oxygen species (ROS) in the presence of photosensitizers and indirectly produce oxidative DNA lesions, UV-B produces dipyrimidine photoproducts through direct excitation and oxidative pathways. Radiation biologists have shown that the DNA absorption spectra is concordant with the action spectra for killing *Escherichia coli* or induction of mutation to fungal spores. Thus, DNA is the target molecule for these UV-mediated effects. Further, CPD is the major class of UV-induced DNA lesions involved in the cytotoxicity and mutagenesis (2). Accumulation of DNA lesions in response to repeated and prolonged exposures to sunlight results in skin carcinogenesis.

As mentioned above, among UV-induced DNA photolesions, CPD is the major molecule involved in cytotoxicity and mutagenesis. ROS cause various biological effects by the redox-signaling pathway and produce oxidative DNA lesions that also play a role in carcinogenesis (3). The guanine base in genomic DNA is highly susceptible to oxidative stress, as it possesses the lowest oxidation potential of all nucleobases; 8-oxyguanosine (8-oxoG) is a sensitive marker of oxidative DNA damage.

On the other hand, in human skin cells in culture and *in vivo*, exposure to UV-A produces CPDs at higher yield than 8-oxoG (4), thereby implying that UV-A may interact with photosensitizers in the body to produce CPD. Hydrochlorothiazide (HCT, a commonly used diuretic medicine) significantly enhances UV-A-mediated thymine dimer (T<>Ts) production in an oxygen-independent manner (5), indicating that excited HCT molecules function as UV-A absorbing chromophores that transfer energy to adjacent pyrimidines, resulting in the formation of T<>Ts. As such, photosensitizers similar to HCT might facilitate UV-A-mediated production of T<>Ts in human skin. These data suggest that studies on DNA photolesions should consider the location, surrounding molecules and mechanisms that could directly/indirectly help mediate the effect of UV on target DNA; moreover, one should be aware of photosensitizers in the DNA environment.

DNA LESIONS CAUSED BY SHORTER WAVELENGTH UV-C AND ITS BIOLOGICAL IMPACT

In the late 1990s, a new type of UV irradiation equipment, namely, dielectric barrier discharge excilamp, having emission wavelengths in the range 170–350 nm, was developed using the transition of excited dimers/complexes of rare gas halides (6). Recently, many devices emitting UV-C other than 254 nm, including far UV-C, have been invented and introduced into medical and industrial use. Despite the recent increase in research on shorter wavelength, UV-C knowledge on use of excilamps in medical (for disinfection of surgical sites, water sterilization) or other occupational settings is still lacking. More studies detailing the shorter wavelength UV-C characteristics are desirable.

DNA has two absorption peaks in the UV-C spectra: 260 nm and 200 nm. Absorption of UV by DNA substantially decreases at longer UV wavelengths (UV-B and UV-A). The lowest absorption is observed between the two peaks at 240 nm. Below 200 nm, UV absorption by DNA once decreases slightly toward 180 nm and then increases toward further shorter wavelength (Fig. 1, dotted line). Matsunaga *et al*. (7) measured the action spectra for the induction of thymine dimers and (6–4) PP in DNA using 150–300 nm UV and monoclonal antibodies against thymine dimers. Both types of DNA photolesions are efficiently produced by 280–180 nm UV-C; 260 nm UV which is known to be the most efficient at inducing dipyrimidine photoproducts falls within this range. In addition, 200 nm UV is also efficient at inducing dipyrimidine photoproducts. The action spectra for the formation of thymine dimers and (6–4)PP were similar for 180–300 nm UV. However, wavelengths <160 nm produced 9-fold higher thymine dimers than (6–4)PP (Fig. 1).

The extent of UV-induced cytotoxicity and mutagenicity (in mammalian cells or microorganisms) mainly depends on the amount of DNA photolesions produced. The next crucial determinant is the penetrative ability of the UV, *i.e*. whether the UV can reach the target DNA, in the hosts/bacteria/viruses. As expected, this depends on the size of the host cell and location and environment of the microorganisms.

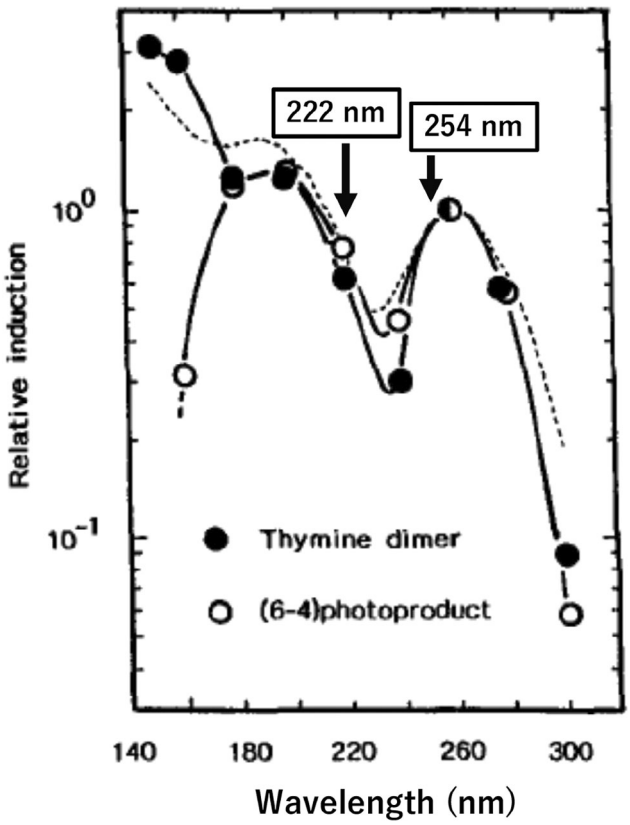


Figure 1. Action spectra for the induction of thymine dimers and (6–4)-photoproducts in the UV region from 150 nm to 300 nm. Efficacy for formation of dipyrimidine photoproducts is highest at approximately 260 nm and decreases at shorter and longer wavelengths. However, the dimer formation at 222 nm and 254 nm are not significantly different, indicating similar efficiency in forming DNA lesions in microorganisms. Modified from Ref. (7).

DNA PHOTOLESIONS CAUSED BY 193 NM UV AND ITS BIOLOGICAL IMPACTS

Kochevar *et al*. investigated the amount of cellular DNA lesions produced and the biological impact of irradiating cells with 193 nm UV using several methods, including endonuclease sensitive sites (ESS) assay, measuring unscheduled DNA synthesis (UDS), and colony formation assay. They quantified pyrimidine dimer formation, UDS ability and number of surviving colonies. They found that higher fluence of 193 nm UV is required to produce outcomes similar to 254 nm UV, *i.e*. 193 nm UV is strongly absorbed by proteins in the cellular constituents before reaching nuclear DNA (8). Although mostly dependent on the constituent amino acids, the absorption spectra of most proteins peak around 190 nm wavelength (9). A dose of 1 J m^{-2} of 254 nm UV produces 17 ESS/Mb in normal human fibroblasts (NHFs), whereas the same dose of 193 nm UV produces 0.2 ESS/Mb. They also studied the effect of the cell shape on UV-induced ESS numbers using NHFs and Chinese hamster ovary (CHO) cells. The ESS numbers in response to 254 nm UV exposure was similar between the two cell types, whereas CHO cells had less than half the number of ESS found in NHF in response to 193 nm exposure. This discrepancy in ESS numbers between the two cell types can be explained by the differences in the distances of the nucleus from the cell membrane. In the round CHO cells the cellular membrane to nucleus distance is approximately 1.09 nm (at center), while in NHFs it is 0.52 nm; thus, 254 nm UV penetrates deeper into the cell compared with 193 nm UV. CHO cells have lower ESS numbers by 193 nm UV than 254 nm UV, because of three reasons: (1) the thickness of their cytoplasm, (2) the lower penetrance of 193 nm due to the physical reasons (shorter wavelength UV reaches shorter) and (3) higher absorption of 193 nm UV by proteins in the cytoplasm between the cell membrane and nucleus. On the other hand, 254 nm UV is capable of reaching the nucleus; its absorption by protein was noted to be low. Although 193 nm UV causes less photo lesions, these results and earlier reports prove that DNA absorbs 193 nm UV and 193 nm UV can penetrate the cellular membranes, *in vitro*, to reach the DNA (Fig. 1).

Results from colony formation assays suggest that the formation of dipyrimidine photoproducts partly play a role in 193 nm UV-induced cytotoxicity. Cells exposed to 193 nm UV in patients with xeroderma pigmentosum (XP, a nucleotide excision repair disorder), had approximately 4× lower D_0 values (dose which gives 37% survival rate) than normal cells. If the lethality can be solely attributed to the formation of dipyrimidine photoproducts, the ratio of D_{37} values should be similar to that of aforementioned ESS ratio (0.2 *vs* 17), but this was not the case. Therefore, although 193 nm UV-induced cytotoxicity results from unrepaired dipyrimidine photoproducts, they are not solely responsible for its induction of cytotoxicity.

DNA PHOTOLESIONS CAUSED BY 207 NM UV AND ITS BIOLOGICAL IMPACT

Recently, lamps emitting 207 nm and 222 nm UV have been developed and shown to be harmless to murine skin. Buonanno *et al*. compared the epidermal damage caused by equivalent doses (1.57 k J m^{-2}) of 207 nm and 254 nm UV in SKH hairless mice; four relevant cellular and molecular damage endpoints were evaluated 48 h after UV irradiation. They used excimer lamps emitting monoenergetic 207 nm UV (based on Kr-Br gas mixture) with a custom band pass filter to remove essentially all but the dominant 207 nm wavelength (10). They found that epidermal CPD positive cells and (6–4)PP-positive cells were scarce after irradiation with 207 nm UV. However, after irradiation with 254 nm UV, CPD- and (6–4)PP-positive cells accounted for 50% and 30% of epidermal cells, respectively. In addition, while epidermal thickness and positive staining for Ki-67 were similar between 207 nm UV and sham irradiations, they were significantly increased with 254 nm UV irradiation.

DNA PHOTOLESIONS CAUSED BY 222 NM UV-C AND ITS BIOLOGICAL IMPACT

Further, to narrow-down the wavelength range that is harmful only to microorganisms but the host tissue, they extended the study to 222 nm UV-C. They used a krypton-chlorine (KrCl) excimer lamp with a custom bandpass filter to remove essentially all but the dominant 222 nm wavelength; the lamp emitted principally 222 nm UV. They found that UV fluence giving the level of 4–5 log inactivation of microorganisms, which was around 0.6–1 kJ m^{-2} at 222 nm UV, did not produce CPD in the epidermis, while 254 nm UV did (11). Moreover, Narita *et al*. (12), confirmed that 10 daily exposures to 222 nm UV at a dose of 4.5 kJ m^{-2} (at a time) did not produce CPDs in the epidermis of hairless mice, suggesting there to be no carcinogenic consequences with repetitive irradiation. Exposure to 222 nm UV did not induce acute corneal damage in rats (13).

EFFECT OF LONG-TERM EXPOSURE TO FAR UV-C

The most critical effect of 254 nm UV in humans and animals is skin carcinogenicity caused by genotoxicity (14,15). One of main causes of UV-induced skin tumors is the formation of the highly mutagenic DNA lesions called dipyrimidine photoproducts, which are carcinogenic when left unrepaired (16,17). Photocarcinogenic effects of 222 nm UV was tested by repetitive and long-term irradiation of hairless mice with 222 nm UV, using a protocol with a 100% skin-tumor incidence rate in wild-type mice (18,19). The UV source was a KrCl excimer lamp with a filter to remove nearly all wavelengths except 222 nm wavelength. The energy of irradiation was 100%, 0.13% and 0.04% for the wavelength ranges 200–230 nm, 235–280 nm and 280–320 nm, respectively.

To evaluate the safety of the UV sources we tried to obtain direct evidence in addition to the CPD formation, which have been measured in earlier studies (10–12), an XP mouse model with DNA repair disorder was employed to precisely evaluate the risk of 222 nm UV in a sensitive way. Patients with XP are characterized by multiple, early onset malignant skin tumors in sun-exposed areas (20,21). They have >10 000-fold increased risk of non-melanoma skin cancer and a >2000-fold increased risk of melanoma before the age of 20 years (22). Likewise, the XP model mice used in the study were extremely hypersensitive to UV and highly susceptible to UV-induced skin carcinogenesis (21,23). CPD formation was recognized only in the uppermost layer of epidermis of XP model mice even at doses as high as 10 kJ m^{-2}. Tumors were absent in both XP model and wild-type

mice given repetitive irradiation with 222 nm-UVC over a course of 15 weeks, followed by the 10 weeks of follow-up observations. Further, this irradiation protocol did not significantly affect the stratum corneum of mice. Furthermore, inflammatory reactions, such as erythema and ear swelling, were absent in both wild type and XP model mice following 222 nm-UVC exposure (24). In the same study, 222 nm UV-irradiated mice were examined for various chronic ophthalmic effects. Irrespective of *Xpa*-genotype, these mice did not show significant changes in corneal and retinal tissues throughout the period of examinations. Welch *et al*. (25) also reported that skin tumors were absent in wild-type hairless albino mice, even after 66 weeks of chronic irradiation with 222 nm UV.

EFFECT OF EXPOSURE TO 222 NM UV ON PLANTS

Ohtake *et al*. (26), reported that 222 nm UV caused greater damage to the guard and epidermal cells of *Arabidopsis* plant than 254 nm UV. They used a 222 nm KrCl excimer lamp, which emits 235–265 nm wavelengths UV with the integrating intensity of 9.0%. Nevertheless, they deduced that the lower growth rate of *Arabidopsis* plant caused by the exposure to the 222 nm KrCl excimer lamp than exposure to 254 nm-germicidal lamp was due to 222 nm UV component rather than 235–265 nm UV spectrum of the lamp. They probably deduced from the facts that *E. coli* are more susceptible to 254 nm UV than to 222 nm UV, whereas P1 bacteriophages were more sensitive to 222 nm UV than to 254 nm UV, which is consistent with data reported elsewhere (27). They suggested that severe mitochondrial damage caused by 222 nm UV could lead to such effects. These results imply that the susceptibility of the living beings to UV depends on the cell-organelle critical for its survival/growth, the chromophore, and other vicinity molecules (proteins, amino acids, lipids, or nucleic acids) capable of absorbing the given UV radiation.

POTENCY OF SHORTER WAVELENGTH UV-C ON INACTIVATION OF MICROORGANISMS

Genotoxicity, owing to which UV inactivates microorganisms, is attributed to UV absorption by nucleic acids and is mainly related to its potency to produce dipyrimidine photoproducts. Data regarding UV action spectra for lethality of *E. coli* are concordant with that of the UV action spectrum of CPD formation. Taylor *et al*. (28), using repair-deficient bacterial mutants showed that efficiency of 222 nm UV in inactivating bacteria and its spores can be attributed to its DNA photolesion (especially pyrimidine dimers) inducing ability.

As described previously, 222 nm UV produces CPD in the uppermost stratum corneum layer (to which microorganisms usually adhere) in host (mice). Consequently, shorter wavelength UV can be used to inactivate microorganisms on stratum corneum surface, while it is less harmful to the hosts; it penetrates bacterial/viral nuclei (present $\lesssim$1 μm from cell membrane), but it cannot reach epidermal cell nuclei (present $\gtrsim$10 μm from cell membrane) of the host. Buonanno *et al*. (11) showed that 222 nm UV emitted by a filtered KrCl excimer lamp can kill methicillin-resistant *Staphylococcus aureus* (MRSA) as efficiently as 254 nm UV emitted by germicidal UV lamp. However, they previously showed that 254 nm UV also efficiently kills human cells (10).

Narita *et al*. showed that the efficiency of UV in inactivating microorganisms varies between species. Some microorganisms, including *Bacillus cereus, Clostridium sporogenes, and Clostridioides difficile*, are more susceptible to 222 nm UV, while others, such as *Aspergillus niger* spores and *Trichophyton rubrum* spores, are more susceptible to 254 nm UV. The 222 nm and 254 nm UV were comparable in their ability to inactivate viruses. Although both 222 nm UV and 254 nm UV were not so effective in disinfecting *Feline calicivirus* (FCV), they could inactivate influenza A viruses (29). Further, Buonanno *et al*. (30), showed that 222 nm UV safely inactivates airborne human coronaviruses. Although pyrimidine dimers are the primary photoproducts of UV-C exposed DNA, Setlow *et al*. found that *Bacillus subtilis* spores have a unique photochemistry. UV-induced photolysis of DNA in the spores does not produce detectable thymine dimers, but it produces 5-thyimyl-5,6-dihydrothyine, a unique spore photoproduct (SP) (31). Fukui *et al*. (32), reported that irradiating healthy volunteers (n = 20) with 222 nm UV at the sterilizing dose of 0.5–5 kJ m^{-2} is safe and effective in disinfecting skin surface, suggesting that 222 nm UV can be utilized in the future as a disinfectant in surgical fields. Wang *et al*. reported a 2 log reduction in *Bacillus subtilis* spore count on irradiation with 172 nm, 222 nm and 254 nm UV at 8.7, 0.22 and 0.4 kJ m^{-2} fluence, respectively. 222 nm UV was much more efficient, whereas 172 nm UV is less efficient, than 254 nm UV in killing *B. subtilis* (33). The disinfection efficacy of the vacuum-UV (VUV, 100–200 nm) in aqueous environment is attributed to the various reactive oxygen species (ROS)—primarily comprised hydroxy radicals, and other ROS, including hydroperoxyl radicals, hydrogen peroxide and superoxide radicals—produced by UV-induced photolysis of water (33); involvement of inactivation by ozone might be considered. However, the involvement of ozone seems to be negligible in the disinfection process mediated by the 220 nm UV because UV absorption by oxygen peaks at 150 nm UV with almost no absorption near 220 nm UV. Far UV-C mediated inactivation of microorganisms is summarized elsewhere (34). Recently, a report indicated that 222 nm UV is less efficient than the 254 nm UV in inactivating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in human saliva (35). Further, the required dose for inactivating SARS-CoV-2 in saliva is 30 times higher than that required to inactivate it in saline solution (PBS). Further investigation will be required to search for the optimal ways to inactivate microorganisms. The optimal wavelength and method would depend on type of the microorganism and its local environment.

EVALUATION OF MINIMUM RESPONSE BY SHORTER WAVELENGTH UVC IN MICE SKIN

Invention and development of excimer lamps emitting shorter wavelength UV has drawn attention of environmental and medical researchers, and the amount of literature on its safety and usefulness is increasing (10–12,24,25,32,33). Previously, to study the shorter wavelength UV-C safety threshold for use in occupational environments, biological responses and DNA lesions caused by irradiation with shorter wavelength UV-C were examined *in vivo* by evaluating cutaneous inflammatory responses to UV of

207 nm, 222 nm, 235 nm and 254 nm wavelengths (36). At first a qualitatively and quantitatively appropriate measurement strategy was investigated to evaluate the biological reactions, and minimal perceptible response dose (MPRD) was applied for the purpose. MPRD is determined by visual inspection of any subtle cutaneous response, such as erythema, edema, and scale which could be observed or perceived. Erythema was scarcely observed following irradiation with 207 nm and 222 nm UV, but it was visible after 254 nm UV irradiation. However, edema and scale formation were evident after shorter wavelength UV-C irradiations. The MPRD for 207 nm, 222 nm and 235 nm was determined to be >15, 15 and 2.0 kJ m^{-2}, respectively (36) (Table 1). We proposed that action spectra of MED/MPRD are correlated with the functions of the action spectra of CPD formation multiply their reciprocal of transmittance through stratum corneum. Tentative action spectra for a given MED/MPRD was simulated by factoring in the reciprocal of reported action spectra of CPD formation multiply their reciprocal of transmittance through stratum corneum (in humans and mice). Estimation of MED by this manner, namely, action spectra of CPD formation multiply their transmittance through stratum corneum, fit well with the reported figures of MED at each wavelength, in the UVB range (especially at 300 nm or higher UV). At the shorter wavelength UV-C range, erythema is not elicited by UV-C, but only edema is elicited, followed by scale formation. The curve for estimated MED/MPRD action spectra was concordant with the figures of MPRD mentioned above; MPRD for 207 nm, 222 nm and 235 nm to be >15, 15 and 2.0 kJ m^{-2}, respectively. Though CPD was not formed in the mouse epidermal cells except stratum corneum by irradiation with shorter wavelength UV-C, edema was observed. This indicates that the biological response to shorter wavelengths UV-C is different. Although the mechanisms are unknown, MPRD could be used as indicators for assessing safety at the shorter wavelength UV-C (Fig. 3).

PROTECTION SYSTEM AGAINST UV IN LIVING ANIMALS

Having evolved under the sun, humans cannot avoid sun exposure. Therefore, various sorts of physiological protections exist to protect them from solar UV insults, such as the following. (1) DNA repair systems: dipyrimidine photoproducts are repaired by nucleotide excision repair system (37). If the dipyrimidine photoproducts remain in the replication cycle, they are replaced with A–A by translesion synthesis, eventually resulting in correction of T–T dimers. If photolesions are too large to be repaired, apoptosis is elicited to prevent induction of mutation. This step manifests as "Sunburn cells"/apoptotic keratinocytes. In a sense, genotoxicity is prevented by the biological apoptotic response. Thus, "sunburn" could be a warning sign to prevent genotoxicity. In the UVB range, wherein the UV-induced erythema is prominent, action spectrum of erythema is closely correlated with the action spectrum of photocarcinogenesis. (2) Presence of stratum corneum whose major constituent is keratin, with abundant cystin (absorbance maxima around 210 nm): keratin is produced by keratinocytes in epidermis and forms stratum corneum, which functions as a skin barrier from environments. Low penetrance of shorter wavelength UV is a physical characteristic that is dependent on the wavelength (the longer the wavelength, the farther the UV penetration is). In addition, one of the reasons for the aforementioned negligible epidermal injury in mice after

Table 1. Dose-dependent UV characteristics on biological effect regarding DNA lesions, epidermal injuries and inflammation.

		DNA absorption	Protein absorption	Cellular CPD(ESS)	CPD in epidermis MoAb	Epidermal Hyperplasia	Inflammatory cells	Edema/scale	Erythema
172 nm (33)	Xe_2 excilamp	+	++			NT(not tested)	NT	NT	NT
193 nm (10)	ArF eximer laser	+++	+++	16 at 120 J m^{-2}	NT	NT	NT	NT	NT
207 nm (12,35)	KrBr excimer lamp with filter	++	++		None at 15 k J m^{-2}	− at 15 kJ m^{-2}	None at 15 k J m^{-2}	+ >15 kJ m^{-2}	none
222 nm (11,12,24,36)	Kr-Cl excimer lamp with filter	++	+		− at 1.5 kJ m^{-2} − at 4.50 k J m^{-2} + upper-most at 5 k J m^{-2}	− at 15 kJ m^{-2}	none	+ >15 kJ m^{-2}	none
235 nm (12,36)	Xe lamp with filter/ monochro-meter set 235 nm	+	slightly	at basal cells at 2 kJ m^{-2}	+ at 2.0 kJ m^{-2}	Slightly increased at 2.0 kJ m^{-2} + at basal cell at 2 kJ m^{-2}	NT	+ >2 kJ m^{-2}	+ faintly at 2 kJ m^{-2}
254 nm (11,12,24)	Conventional germicidal lamp	++	Very slightly	17 at 1 J m^{-2}	+ at 250 J m^{-2}	++ at 1.57 kJ m^{-2} ++ at 750 J m^{-2} ++ at 1 k J m^{-2}	Significantly increased at 1.57 kJ m^{-2}	+	present
311 nm (18)						++ at 370 J m^{-2}	+ at 370 J m^{-2}	+ at 370 J m^{-2}	+ at 370 J m^{-2}

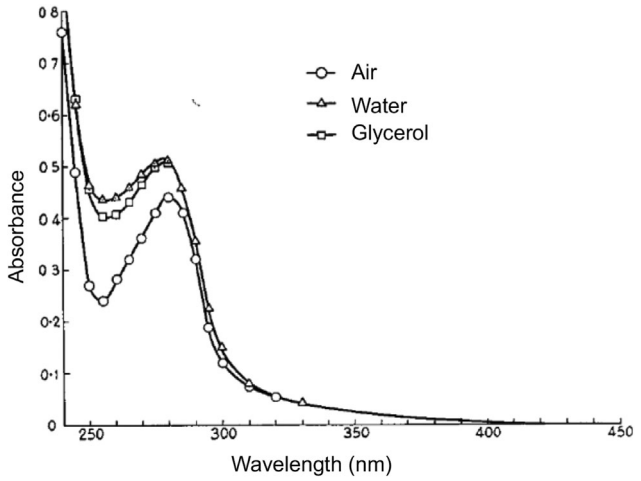


Figure 2. Keratin absorbs far UV-C to a greater extent than 280 nm. The graph indicates UV absorption spectra of solid keratin in the different eluents. A peak absorbance is observed around 280 nm, it decreases around 250 nm, and substantially increases at the wavelength <250 nm (Ref. 38).

irradiation with 207 nm and 222 nm UV could be the absorption of UV by keratin (Fig. 2) (38). Figure 2 depicts the absorbance of keratin in the different eluents. In literature, keratin absorption spectra are available for wavelengths above 220 nm. The two absorbance maxima of keratin are approximately 280 nm and 240 nm and under (with a substantially high absorption peak below 250 nm); it is the lowest at approximately 250 nm.

ESTIMATING ACTION SPECTRA OF UV-INDUCED PHOTOCARCINOGENESIS

Two key factors determine the occurrence of UV-induced skin cancer: (1) the efficacy of the given UV wavelength to induce pyrimidine dimer formation, and (2) the transmittance of the UV to the basal cells in the epidermis, where cancer stem cells reside. Figure 1 depicts the action spectrum of CPD formation (7). Previously, we have shown that the diffuse transmission of UV (235–360 nm) through commercially available human stratum corneum (approximately 20 μm thickness) is wavelength-dependent (36); for UV with wavelengths between 200 nm and 235 nm, transmittance was linearly correlated with wavelength. The transmittance of UV through human stratum corneum is shown by green line (Fig. 4a). At wavelengths <240 nm, UV transmittance decreases by approximately two to three orders. This substantial decrease of transmittance reciprocally correlates with the substantial increase in UV-absorbance by keratin at wavelengths <240 nm (Fig. 2). The target cells for skin cancer development by photocarcinogenesis, are the basal cells at the bottom of epidermis—cancer stem cells originate here through mutations. Keratinocytes, which are abundant of keratin fibers, constitute 90% of the epidermis. Therefore, we postulate that the transmittance of UV through the epidermis could be substituted by a function of the transmittance through 80–90 μm stratum corneum (shown in light blue line, Fig. 4a).

From these results, it is safe to state that the wavelength-dependent photocarcinogenesis at shorter wavelength UV-C is a function of the transmittance to the epidermal basal cells multiplied by the action spectrum of CPD (shown by purple line; Fig. 4b). Calculated action spectra of human and mouse are shown by blue and red line in Fig. 4b, which is consistent with the reported data that the maximum action spectrum of UV-induced carcinogenesis in animal experiments falls in the UV-B (293 nm) range (39). The estimated photocarcinogenic action spectrum at shorter wavelengths indicates that photocarcinogenesis risk due to chronic exposure to 222 nm UV is about 7 log smaller compared to that due to exposure to 300 nm or 254 nm UV.

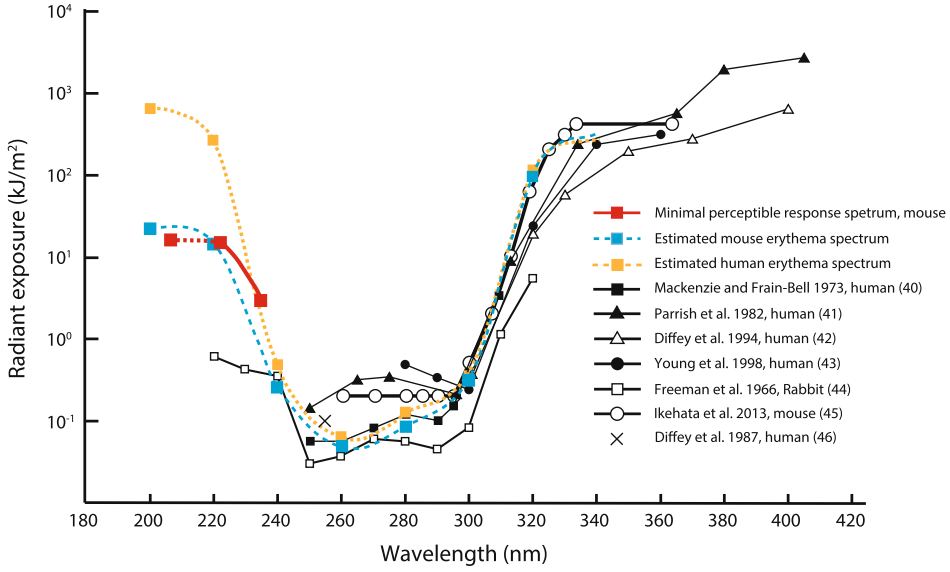


Figure 3. Juxtaposition of observed MED/MPRD action spectrum on estimated MED/MPRD action spectra including shorter Wavelength UV-C range. Dose-dependent mammalian MED values from literature are plotted (Refs. 40–46). Further, dose-dependent MED/MPRD in mice and human was simulated using the function of reciprocal of action spectra of CPD formation multiplies the reciprocal of stratum corneum transmittance values (represented by dotted lines). Simulated MED/MPRD fitted well with measured MED in the UV-B range and with MPRD in the shorter wavelength UV-C range.

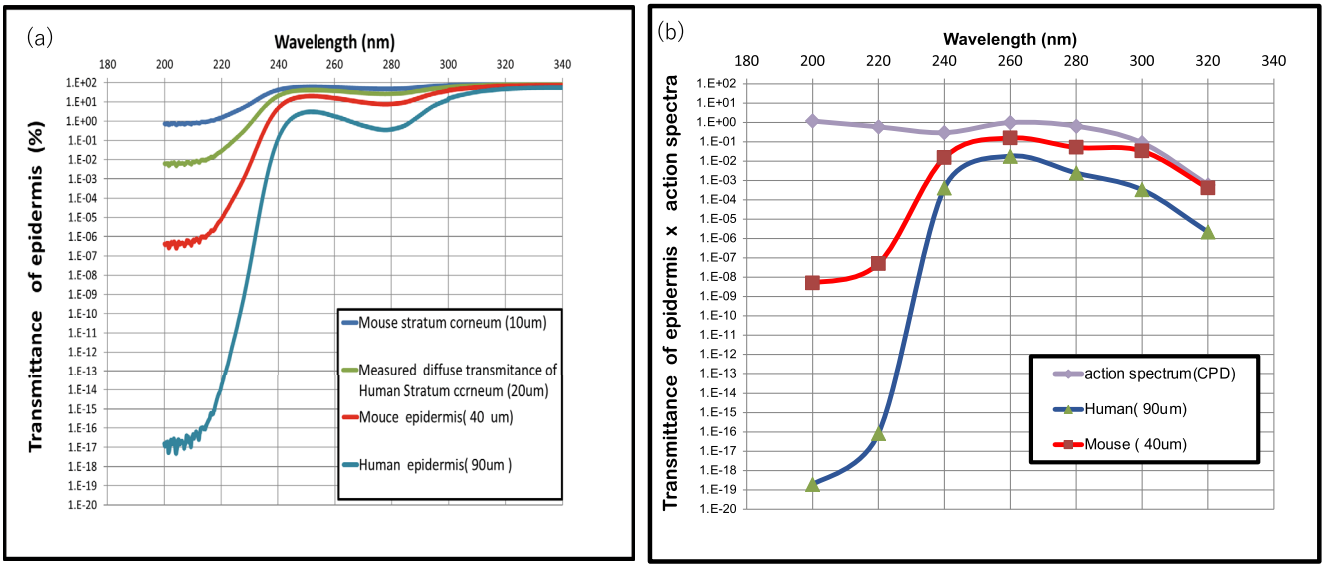


Figure 4. Wavelength-dependent transmittance of stratum corneum (a) and action spectrum of pyrimidine dimer formation and estimated action spectrum of photocarcinogenesis (b). (a) Transmittance of human stratum corneum (thickness 20 μm) are indicated by green line. The significant decrease of transmittance at wavelength shorter than 240 nm is reciprocally correlated with the significant increase in keratin absorbance (Fig. 2). Since keratinocytes, major constituents of epidermis are filled with keratin fibers, wavelength-dependent transmittance through epidermis was estimated as a thickness of epidermis (90 μm for human and 40 μm for mice) and indicated by light blue line and red line, respectively. (b) The action spectra of photocarcinogenesis were estimated for mice (red line) and humans (blue line) as a function of action spectrum of CPD formation (purple line) multiplies wavelength-dependent transmittance of the stratum corneum shown in (a).

FUTURE DIRECTION: BALANCING RISK AND USEFULNESS

Emergence of new devices emitting far UV-C is useful for various purposes. UV is one of the best ways to safely disinfect because it inactivates microorganisms immediately without leaving chemical substance. However, UV can cause acute and chronic injuries to the eyes and skin. The most critical outcome of chronic exposure to UV is the development of skin cancers. The mechanism underlying sterilizing potency and skin carcinogenic potency of UV irradiation is formation of DNA photolesions. Penetration depth of 222 nm UV is too shallow to reach cellular DNA of the host, but it can reach the bacterial/viral/fungal DNA existing on the surface of the host skin. Shorter wavelength UV-C are less genotoxic to mammalian skin and eyes, compared with 254 nm UV, in addition to being useful for inactivation of microorganisms on the skin surface. However, we must take into consideration that the inactivation efficacy of UV substantially depends on the structure and size of the microorganisms, DNA localization and molecules in the environment being irradiated.

Available literature data on the shorter wavelength UV-C characteristics are summarized in Table 1. Absence of epidermal CPD staining after far UV-C exposure confirms its safety. Studies on UV-induced photoreaction are mostly limited to CPD formation. However, 193 nm UV was lethal to cells at a much dose than 254 nm UV, based on the colony formation assay, *i.e.* 193 nm irradiation is cytotoxic (8). Cellular UV cytotoxic assay by colony formation has not been studied using 222 nm UV. Investigating details of various shorter wavelength UV-C-induced mechanisms, such as formation of DNA lesion (by different pathways), photolysis, interaction of UV with protein, and effect on mitochondrial DNA, are required to understand the scope of biological impact of shorter wavelengths UV-C.

Further, the inactivation efficiency and its mechanisms are not equal among all microorganisms. Therefore, UV treatment must be individually standardized for different microorganisms, taking into account their size, structure, and local environment and the physical characteristics of the given UV wavelength. The filter characteristics and resulting spectrum used in these experiments must be described in detail. Studies on the biological effects of the 200-235 nm components of the UV spectra should be encouraged to deepen our understanding to help balancing their usefulness and risk. Improved knowledge of these new UV-C emitting devices will inform people on their appropriate and optimal use.

REFERENCES

1. Nishigori, C. (2015) Current concept of Photocarcinogenesis. *Photomed. Photobiol. Sci.* **14**, 1713–1721.
2. Elison, M. J. and J. D. Childs (1981) Pyrimidine dimers induced in Escherichia coli DNA by ultraviolet radiation present in sunlight. *Photochem. Photobiol.* **34**, 465–469.
3. Kunisada, M., K. Sakumi, Y. Tominaga, A. Budiyanto, M. Ueda, M. Ichihashi, Y. Nakabeppu and C. Nishigori (2005) 8-Oxoguanine formation induced by chronic ultraviolet B exposure makes ogg1 knockout mice susceptible to skin carcinogenesis. *Cancer Res.* **65**, 6006–6010.
4. Mouret, S., C. Baudouin, M. Charveron, A. Favier, J. Cadet and T. Douki (2006) Cyclobutane pyrimidine dimers are predominant DNA lesions in whole human skin exposed to UVA radiation. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 13765–13770.
5. Kunisada, M., T. Masaki, R. Ono, H. Morinaga, E. Nakano, F. Yogianti, K. Okunishi, H. Sugiyama and C. Nishigori (2013) Hydrochlorothiazide enhances UVA-induced DNA damage. *Photochem. Photobiol.* **89**, 649–654.
6. Sosnin, E. A., T. Oppenländer and V. Tarasenko (2006) Application of capacitive and barrier discharge excilamps in photoscience. *J. Photochem. Photobiol. C* **7**, 145–163.
7. Matsunaga, T., K. Hieda and O. Nikaido (1991) Wavelength dependent formation of thymine dimers and (6–4) photoproducts in DNA

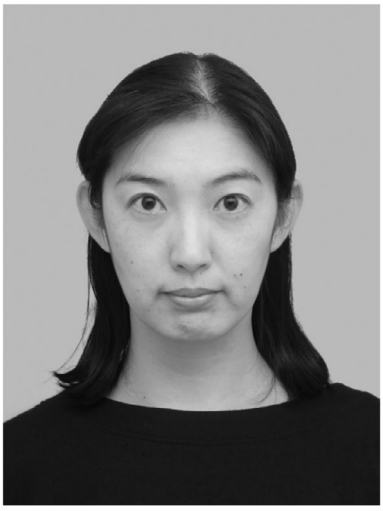
by monochromatic ultraviolet light ranging from 150 to 365 nm. *Photochem. Photobiol.* **54**, 403–410.
8. Kochevar, I. E., A. A. Walsh, H. Green, M. Sherwood, A. Shih and B. Sutherland (1991) DNA damage induced by 193-nm radiation in mammalian cells. *Cancer Res.* **51**, 288–293.
9. Goldfarb, R., L. J. Saidel and E. Mosovich (1951) The ultraviolet absorption spectra of proteins. *J. Biol. Chem.* **193**, 397–404.
10. Buonanno, M., M. Stanislauskas, B. Ponnaiya, A. Bigelow, G. Randers-Pehrson, Y. Xu, I. Shuryak, L. Smilenov, D. Owens and D. Brenner (2016) 207-nm UV light-a promising tool for safe low-cost reduction of surgical site infections. II: In-vivo safety studies. *PLoS One.* **11**, e0138418.
11. Buonanno, M., B. Ponnaiya, D. Welch, M. Stanislauskas, G. Randers-Pehrson, L. Smilenov, F. Lowy, D. Owens and D. Brenner (2017) Germicidal efficacy and mammalian skin safety of 222-nm UV light. *Radiat. Res.* **187**, 483–491.
12. Narita, K., K. Asano, Y. Morimoto, T. Igarashi and A. Nakane (2018) Chronic irradiation with 222-nm UVC light induces neither DNA damage nor epidermal lesions in mouse skin, even at high doses. *PLoS One* **13**, e0201259.
13. Kaidzu, S., K. Sugihara, M. Sasaki, A. Nishiaki, T. Igarashi and M. Tanito (2019) Evaluation of acute corneal damage induced by 222-nm and 254-nm ultraviolet light in Sprague-Dawley rats. *Free Radic. Res*, **53**, 611-617.
14. Forbes, P. D. and F. Urbach (1975) Experimental modification of photocarcinogenesis. I. Fluorescent whitening agents and short-wave UVR. *Food Cosmet. Toxicol.* **13**, 335–337.
15. Sterenborg, H. J., S. C. van der Putte and J. C. van der Leun (1988) The dose-response relationship of tumorigenesis by ultraviolet radiation of 254 nm. *Photochem. Photobiol.* **47**, 245–253.
16. Ikehata, H. and T. Ono (2011) The mechanisms of UV mutagenesis. *J. Radiat. Res.* **52**, 115–125.
17. Pfeifer, G. P. and A. Besaratinia (2012) UV wavelength-dependent DNA damage and human non-melanoma and melanoma skin cancer. *Photochem. Photobiol. Sci.* **11**, 90–97.
18. Kunisada, M., H. Kumimoto, K. Ishizaki, K. Sakumi, Y. Nakabeppu and C. Nishigori (2007) Narrow-band UVB induces more carcinogenic skin tumors than broad-band UVB through the formation of cyclobutane pyrimidine dimer. *J. Invest. Dermatol.* **127**, 2865–2871.
19. Yogianti, F., M. Kunisada, E. Nakano, R. Ono, K. Sakumi, S. Oka, Y. Nakabeppu and C. Nishigori (2014) Inhibitory effects of dietary spirulina platensis on UVB-induced kin inflammatory responses and carcinogenesis. *J. Invest. Dermatol.* **134**, 2610–2619.
20. Nishigori, C., E. Nakano, T. Masaki, R. Ono, S. Takeuchi, M. Tsujimoto and T. Ueda (2019) Characteristics of Xeroderma Pigmentosum in Japan: Lessons from two clinical surveys and measures for patient care. *Photochem. Photobiol.* **95**, 140–153.
21. Kunisada, M., C. Hosaka, C. Takemori, E. Nakano and C. Nishigori (2017) CXCL1 inhibition regulates UVB-induced skin inflammation and tumorigenesis in Xpa-deficient mice. *J. Invest. Dermatol.* **137**, 1975–1983.
22. Bradford, P. T., A. M. Goldstein, D. Tamura, S. Khan, T. Ueda, J. Boyle, K.-S. Oh, K. Imoto, H. Inui, S.-I. Moriwaki, S. Emmert, K. Pike, A. Raziuddin, T. Plona, J. Digiovanna, M. Tucker and K. Kraemer (2011) Cancer and neurologic degeneration in xeroderma pigmentosum: Long term follow-up characterises the role of DNA repair. *J. Med. Genet.* **48**, 168–176.
23. Nakane, H., S. Takeuchi, S. Yuba, M. Saijo, Y. Nakatsu, H. Murai, Y. Nakatsuru, T. Ishikawa, S. Hirota and Y. Kitamura (1995) High incidence of ultraviolet-B-or chemical-carcinogen-induced skin tumours in mice lacking the xeroderma pigmentosum group a gene. *Nature* **377**, 165–168.
24. Yamano, N., M. Kunisada, S. Kaidzu, K. Sugihara, A. Nishiaki-Sawada, H. Ohashi, A. Yoshioka, T. Igarashi, A. Ohira, M. Tanito and C. Nishigori (2020) Long-term effects of 222 nm ultraviolet radiation C sterilizing lamps on mice susceptible to ultraviolet radiation. *Photochem. Photobiol.* **96**, 853–862.
25. Welch, D., J. N. Kleiman, P. Aden, C. Kuryla, M. Buonanno, B. Ponnaiya, X. Wu and D. J. Brenner (2022) No evidence of induced skin cancer or other skin abnormalities after long-term (66 week) chronic exposure to 222 nm far-UVC radiation. *Photochem. Photobiol.* https://doi.org/10.1111/php.13656
26. Otake, M., K. O. Yoshiyama, H. Yamaguchi and J. Hidema (2021) 222 nm ultraviolet radiation C causes more severe damage to guard cells and epidermal cells of *Arabidopsis* plants than does 254 nm ultraviolet radiation. *Photochem. Photobiol. Sci.* **20**, 1675–1683.
27. Bucheli-Witschel, M., C. Bassin and T. Egli (2010) UV-V inactivation in Escherichia coli is affected by growth conditions preceding irradiation, in particular by the specific growth rate. *J. Appl. Microbiol.* **109**, 1733–1744.
28. Taylor, W., E. Cmillwei, L. Craft, G. Korza, M. R. Granados, J. Peterson, R. Szczpaniak, S. Weller, R. Moeller, T. Douki, W. Mok and P. Setlow (2020) DNA damage kills bacterial spores and cells exposed to 222-nm nanometer UV radiation. *Appl. Environ. Microbiol.* **86**, 1–14.
29. Narita, K., K. Asano, K. Naito, H. Ohashi, M. Sasaki, Y. Morimoto, T. Igarashi and A. Nakane (2020) Ultraviolet C light with wavelength of 222 nm inactivates a wide spectrum of microbial pathogens. *J. Hosp. Infect.* **105**, 459–467.
30. Buonanno, M., D. Welch, I. Shuryak and D. J. Brenner (2020) Far-UVC light (222 nm) efficiently and safely inactivates airborne human coronaviruses. *Sci. Rep.* **10**, 102585.
31. Setlow, P. (2001) Resistance of spores of *bacillus* species to ultraviolet light. *Environ. Mol. Mut.* **38**, 97–104.
32. Fukui, T., T. Niikura, T. Oda, Y. Kumabe, H. Ohashi, M. Sasaki, T. Igarashi, M. Kunisada, N. Yamano, K. Oe, T. Matsumoto, T. Matsushita, S. Hayashi, C. Nishigori and R. Kuroda (2020) Exploratory clinical trial on the safety and bactericidal effect of 222-nm ultraviolet C irradiation in healthy humans. *Plos One* **15**, e0235948.
33. Wang, D., T. Oppenländer, M. El-Din and J. Bolton (2010) Comparison of the disinfection effects of vacuum-UV (VUV) and UV light on *Bacillus subtilis* spores in aqueous suspensions at 172, 222 and 254 nm. *Photochem. Photobiol.* **86**, 176–181.
34. Blatchley, E. R., III, D. J. Brenner, H. Claus, T. Cowan, K. Linden, Y. Liu, T. Mao, S.-J. Park, P. Piper, R. Simons and D. Sliney (2022) Far UV-C radiation: An emerging tool for pandemic control. *Crit. Rev. Env. Sci. Tec.* 1–21. https://doi.org/10.1080/10643389.2022.2084315. Online ahead of print.
35. Sesti-Costa, R., C. V. Z. Negrão, J. Shimizu, A. Nagai, R. Tavares, D. Adamoski, W. Costa, M. A. Fontoura, T. da Silva, A. de Barros, A. Girasole, M. de Carvalho, V. C. Teixeira, A. L. B. Ambrosio, F. Granja, J. Proença-Módena, R. Marques and S. M. G. Dias (2022) UV 254 nm is more efficient than UV 222 nm in inactivating SARS-CoV-2 present in human saliva. *Photodiagnosis Photodyn. Ther.* **39**, 103015. https://doi.org/10.1016/j.pdpdt.2022.103015
36. Yamano, N., M. Kunisada, A. Nishiaki-Sawada, H. Ohashi, T. Igarashi and C. Nishigori (2021) Evaluation of acute reaction on mouse skin irradiated with 222 and 235 nm UV-C. *Photochem. Photobiol.* **97**, 770–777.
37. Nishigori, C. and K. Sugasawa (2019) *DNA Repair Disorders*. Springer Nature, Singapore.
38. Bendit, E. G. and D. Ross (1961) A technique for obtaining the ultraviolet absorption spectrum of solid keratin. *Appl. Spec.* **15**, 103–105.
39. de Gruijl, F. R. (1995) Action spectrum for photocarcinogenesis. *Recent Results Cancer Res.* **139**, 21–30.
40. MacKenzie, L. A. and W. Frain-Bell (1973) The construction and development of a grating monochromator and its application to the study of the reaction of the skin to light. *Br. J. Dermatol.* **89**, 251–264.
41. Parrish, J. A., K. Jaenicke and R. Anderson (1982) Erythema and melanogenesis action spectra of normal human skin. *Photochem. Photobiol.* **36**, 187–191.
42. Diffey, B. L. (1994) Observed and predicted minimal erythema doses: A comparative study. *Photochem. Photobiol.* **60**, 380–382.
43. Young, A. R., C. Chadwick, G. Harrison, O. Nikaido, J. Ramsden and C. Potten (1998) The similarity of action spectra for thymine dimers in human epidermis and erythema suggests that DNA is the chromophore for erythema. *J. Invest. Dermatol.* **111**, 982–988.
44. Freeman, R. G., D. Owens, J. Knox and H. Hudson (1966) Relative energy requirements for an erythemal response of skin to monochromatic wave lengths of ultraviolet present in the solar spectrum. *J. Invest. Dermatol.* **47**, 586–592.
45. Ikehata, H., S. Higashi, S. Nakamura, Y. Daigaku, Y. Furusawa, Y. Kamei, M. Watanabe, K. Yamamto, K. Hieda, N. Munakata and T. Ono (2013) Action spectrum analysis of UVR genotoxicity for skin. *J. Invest. Dermatol.* **133**, 180–1856.
46. Diffey, B. L. and P. M. Fan (1987) The color of UVB and UVC erythema—How red is pink? *Br. J. Dermatol.* **116**, 459–461.

AUTHOR BIOGRAPHIES

Chikako Nishigori is a Director of Japanese Red Cross Hyogo Blood Center/Visiting Professor of Dermatology at Kobe University. She was trained as a dermatologist at Kyoto University and received her Ph.D. there. She joined Prof. Kripke's laboratory at MD Anderson Cancer Center. Her major topics of interest are photodermatosis, photobiology, skin cancers and pigmentary disorders. She served as president of Japanese Society for Photomedicine and Photobiology (2013–2018) and President of Japanese Pigment Cell Research (2014–2016) and board of Japanese Dermatological Association (2016–2022). She has received "Edna Roe Lectureship 2014" from the International Union of Photobiology for her achievements on photobiology.

Nozomi Yamano, received her M.D. at Yamaguchi University Faculty of Medicine and Health Sciences in 2014, and her Ph.D. at Kobe University Graduate School of Medicine in 2022. She started research on the reaction after 222 nm UVC irradiation using an animal model of xeroderma pigmentosum group A, *Xpa*-knockout mice and hairless mice in the graduate school. She is currently an assistant professor at a division of dermatology, at Kobe University Graduate School of Medicine and evaluate the methods of shading from UVA and UVB.

Makoto Kunisada, M.D. and Ph.D. was graduated from Kobe University, Faculty of Medicine, in 1998 and has been a dermatologist. Currently he is an Associate Professor of Division of Dermatology, Kobe University Graduate School of Medicine, Japan. His specialty is Skin photocarcinogenesis, Genodermatosis, Molecular biology. He studied at Graduate School of Kobe University (2003–2006). His thesis project was ultraviolet radiation-induced skin carcinogenesis. During 2006–2009, he joined Dr. Shcresinger's Laboratory, Laboratory of Genetics, Human Genetics Section, NIA/NIH-IRP, Baltimore MD, USA as a Visiting Fellow, and studied the development gene of mice sweat glands.



Aiko Nishiaki-Sawada reserved the associate degree of electric engineering from National Institute of Technology, Akashi College in 1999, and the B.S. from Kobe University of Mercantile Marine in 2001. After graduating, she joined Ushio Inc. She engaged research and development of xenon lamp for endoscopes and fiber laser until 2016. Since 2017, she has been investigating the Safety and efficacy of 222 nm UVC.

Hiroyuki Ohashi got his Ph.D. in protein evolutionary engineering research from the University of Tokyo in 2007. He worked for the Department of Biochemistry at the University of Cambridge as an assistant professor in the UK. In recent years, he has been conducting research on the safety of Far UV-C and infection control with 222 nm in Ushio Inc. He is a Deputy General Manager and a Leader of CARE222 Project.

Tatsushi Igarashi was born in Hokkaido in 1954. In 1979, he received a MS degree in analytical chemistry from Muroran Institute of Technology. In 1979 he engaged in research on heavy metal pollution in the ocean at California Institute of Technology. Joined Ushio Inc. in 1980, developed an excimer lamp in 1993, an ArF excimer laser in 2000, and an EUV light source as an executive officer and CTO in 2013. 2015–2022. His research interests include the safety of 222 nm bactericidal effect and reliable 222 nm lamps. He is Senior Technical Advisor of Ushio Inc.