



**BNL-79392-2007-CP**

***Solar radiation and induction of DNA damages,  
mutations and skin cancers***

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*Presented at the Solar Radiation and Human Health Symposium*  
Oslo, Norway  
May 10-11, 2007

July 2007

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**Abstract:** An understanding of the effects of sunlight on human skin begins with the effects on DNA and extends to cells, animals and humans. The major DNA photoproducts arising from UVB (280-320 nm) exposures are cyclobutane pyrimidine dimers. If unrepaired, they may kill or mutate cells and result in basal and squamous cell carcinomas. Although UVA (320-400 nm) and visible wavelengths are poorly absorbed by DNA, the existing data indicate clearly that exposures to these wavelengths are responsible, in an animal model, for ~95 % of the incidence of cutaneous malignant melanoma (CMM). Six lines of evidence, to be discussed in detail, support the photosensitizing role of melanin in the induction of this cancer. They are: (1) Melanomas induced in backcross hybrids of small tropical fish of the genus *Xiphophorus*, exposed to wavelengths from 302-547 nm, indicate that ~95% of the cancers induced by exposure to sunlight would arise from UVA + visible wavelengths; (2) The action spectrum for inducing melanin-photosensitized oxidant production is very similar to the spectrum for inducing melanoma; (3) Albino whites and blacks, although very sensitive to sunburn and the sunlight induction of non-CMM, have very low incidences of CMM; (4) The incidence of CMM as a function of latitude is very similar to that of UVA, but not UVB; (5) Use of UVA-exposing sun-tanning parlors by the young increases the incidence rate of CMM and (6) Major mutations observed in CMM are not UVB-induced.

## **Introduction**

The scientific emphasis is on the effects on DNA for historical reasons. The induction of mutations in simple cells (fungi) as a result of ultraviolet (UV) exposures of a range of wavelengths indicated that the wavelength dependence for mutation induction was similar to the absorption spectrum of nucleic acids (1) and subsequent analysis indicated clearly that the DNA in bacteriophage contained all the information for its replication (2). Hence, it was obvious that the analysis of light-induced damage to the DNA bases, or the structure of DNA would be informative and, in the long run, helpful in developing measures to minimize the damages or their effects. The more important, unique base damages identified in UV-exposed DNA, thymine dimers, were first identified in 254 nm exposed frozen solutions of thymine (3). The dimers could be reversed by subsequent exposures to 254 nm (4), or, more efficiently, by shorter wavelengths (5). Dimers were subsequently identified in UV-exposed DNA (6) and shown to be reversed to monomers by treatment with photoreactivating enzyme plus longer wavelengths (7) or by exposures to shorter wavelengths (8). The latter characteristics were used to show that dimers in DNA inactivated the biological activity of the DNA (9). It is of interest that photoreactivating activity has been found in many species (10) including fish and humans (11) but not mice. Dimers have also been found, and are subject to photoreactivation, for all types of pyrimidines (12,13).

## **Direct evidence that the induction of pyrimidine dimers in cellular DNA may lead to cancers (14)**

Isogenic fish were used for the experiment, so as to avoid any possible immunological

effects. The small fish, *poecilia formosa*, are an all female species and cells from one animal are not rejected by another. Thyroid cells from one animal were exposed to UVC (254 nm) and the cells injected into isogenic recipients. The recipients developed thyroid tumors with probabilities that increased with the magnitude of the UV dose. If the UV-exposed cells were subsequently exposed to photoreactivating “Black Light” ( $\lambda > 320$  nm), a negligible fraction of the recipient animals developed tumors, Table 1.

Direct UV-induced damage to DNA follows the absorption spectrum of DNA (Figure 1) (15). The wavelengths responsible for erythema induction in human skin are very similar to the DNA absorption spectrum. The extensive decrease in sunlight intensity at the earth’s surface for wavelengths below UVA is the result of the UV absorption by ozone in the stratosphere.

### **Fish models for sunlight-induced malignant melanoma**

Although there is good evidence that UVB is absorbed by DNA and can give rise to tumors, it was conceivable that UVA could also do so by being absorbed by the dark pigment melanin in melanocytes, the cells of melanoma origin, by virtue of energy transfer to or free-radical attack on cellular DNA. I and my colleagues realized that a possible animal model for light-induced melanoma was certain platyfish-swordtail hybrids (*maculatus x helleri*) of the genus *Xiphophorus* that had been introduced into cancer research in the late 1920s and had been chosen as models for the induction of melanoma by chemical carcinogens and x-rays (16,17). We started our experiments by exposing 5-day old fish (only a few mm long) to filtered sunlamp radiation,  $\lambda > 304$  nm,

for up to 20 consecutive days. Melanomas became visible to the naked eye by 1 month. A similar result was obtained with a single exposure. Significant numbers of melanomas developed within 4 months (18). The genetics of the crosses and the high sensitivity of the hybrids to melanoma induction indicated to us that the UV exposures probably inactivated one, or several tumor suppressor genes in the hybrids. The fish melanomas were similar to mammalian melanomas, as judged by light and electron microscopy. Exposure to visible light, after UV, reduced the melanoma prevalence to background level, indicating that pyrimidine dimers from UVB exposures were a cause of these melanomas.

The small size of the baby fish made them ideal for action spectroscopy. We could expose them to monochromatic wavelengths by placing ~5 fish in a 1 cm spectrophotometer cuvette that could be placed behind the exit slit of a monochromator. We did this (19), and exposed 6-day old fish to wavelengths of 302, 313, 365, 405 or 436 nm, using a range of doses at each wavelength. The fractions of fish with melanomas increased with the dose and we took the initial slope of the dose-response curve as a measure of the sensitivity. If we took the sensitivity at 302 nm to be a value 1.00, the sensitivities for melanoma induction at the other wavelengths, shown in Table 2, are all less than 1.00. They are, however, orders of magnitude greater than the direct damage to DNA shown in Figure 1.

We were able to carry out one more experiment with the fish using an incident green wavelength (547 nm). The results have been summarized (20,21). The sensitivity was about the same as was observed for 405 nm. [Unfortunately, we were not able to repeat

that experiment because our funding from the U.S Department of Energy was cut off.] Figure 2A shows the dose-response data for 547 nm and Figure 2B shows the action spectrum for melanoma induction, the spectrum for human erythema and the midsummer sunlight spectrum on the earth's surface at 41°N latitude. Figure 2C shows the Relative Sunlight Effective Dose-Rates, in Quanta per mm<sup>2</sup>.sec per 5nm, for inducing melanoma and human erythema as functions of wavelength (normalized to 1.0 at 302 nm). The role of UVA is much enhanced in this type of plot because the UVA contains at least 20-fold more photons than UVB. As a result, UVA and visible wavelengths account for ~95% of the melanoma induction in this fish model.

### **Melanin Photosensitization.**

It is clear that the action spectrum for the incidence of malignant melanoma in the fish, because of the high sensitivities (Figure 2B) at wavelengths >320 nm, has a large component indicating photosensitization by melanin. Indeed, melanin has been shown to be involved in UVA induced damage to the DNA of melanoma cells. Electron Spin Resonance was used to detect the light-activated melanin in *Xiphophorus* as a function of the incident wavelengths (303-436 nm). The shape of this spectrum was very similar to the action spectrum for melanoma induction (22), indicating clearly that melanin is indeed a light-induced photosensitizer of melanoma. However, the nature of the resulting change in human DNA is not clear because Xeroderma pigmentosum individuals, defective in nucleotide excision repair, show an incidence of melanoma ~1000 times that of normal individuals at ages <20 yrs (23). Nucleotide excision repair is thought to work

on bulky-types of DNA damages, not simple oxidations of nucleotides.

**Evidence for the role of melanin as a photosensitizer in the induction of human melanoma.**

The direct evidence is the exceedingly low incidence of malignant melanoma in albino, African Blacks (24). The individuals have melanocytes, but they do not make melanin. However, these individuals have an excess of sunburns and non-melanoma skin cancers.

UVB exposures of DNA are known to induce cyclobutane pyrimidine dimers (see Table1). Conventional sunscreens absorb UVB and are extensively used to minimize sunlight induced skin damage. The level of UVB in sunlight is a strong function of latitude, whereas UVA is not. Hence, it is not surprising that the ratios of non-melanoma skin cancer in Australia/Norway is ~an order of magnitude higher than for CMM (25). A more extensive epidemiological survey of white populations from 15-20 countries (26) is shown in Figure 3, indicating the very large differences between melanoma and non-melanoma incidences. The differences between the two types of skin cancer are consistent with the hypothesis that melanomas arise primarily from UVA and visible exposures to melanin, which acts as a photosensitizer to damage the DNA in melanocytes, whereas non-melanomas arise from direct DNA damages (primarily pyrimidine dimers) arising from the UVB exposures of non-melanin containing cells.

**The results of sunscreen use and sun parlor/tanning salon use support the conclusion that melanoma arises primarily from exposures to UVA and longer wavelengths.**

Sunscreens are used to lower the risks of sunburns and were initially designed to absorb the UVB wavelengths in sunlight. Epidemiological studies indicated that their use also significantly lowered the risks of non-melanoma skin cancers. However, in some but not all studies, sunscreens also lowered the risks, in somewhat less than 50 % of the studies, of melanoma incidence (27). In other studies, sunscreens raised the risks of melanoma incidence by an amount depending on the details of the UVB-sunscreen, presumably because of the UVA transmission of the sunscreens used 10-20 years ago (28,29). The ambiguity should disappear, in the not too distant future, because the present sunscreens include components that absorb UVA (29).

Sun parlor/Tanning salon exposures use wavelengths extending well into the UVA. A recent summary of the results of 10 studies came to the conclusion that “ the results indicate a significantly increased risk of cutaneous melanoma subsequent to sunbed/sunlamp exposure” (30).

### **Relevant gene sequences in melanoma.**

Some genes are rather specific to malignant melanoma. Among them are the variants of NRAS and BRAF. In a review of the literature, the variants of ~2000 somatic sequences, had non-UVB changes, i.e. no changes at dipyrimidine sequences, although

BRAF was mutant in 53% and NRAS was mutant in 28% of the melanoma cases studied.

(31). Presumably, the mutant sequences arose from UVA and visible photosensitized reactions.

### **Conclusions.**

The experiments summarized above indicate very clearly that exposures of human melanocytes to UVA and visible wavelengths result in a photosensitization of the DNA by the melanin of the cells, resulting in ~95% of the mutations that result in malignant melanoma.

### **Acknowledgements.**

I am indebted to many collaborators, with me as a co-author, for the results of many of the experiments described and to my wife, Neva Setlow, for her help in the laboratory and her assistance in putting together this manuscript.

The Brookhaven National Laboratory is operated by Brookhaven Science Associates under contract with the U.S. Department of Energy.

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Table 1. The effects of Photoreactivating Light (PRL) of UV-exposed cells\*  
on the appearance of thyroid tumors

Treatment	Fraction of fish with thyroid tumors	
	Gross	Histologic
Experiment 1		
UV (12 J/m <sup>2</sup> )	34/34	29/29
2.5 min PRL + UV	26/26	22/22
5.0 min PRL + UV	48/50	22/23
UV + 5.0 min PRL	1/42	0/6
Experiment 2		
UV (24 J/m <sup>2</sup> )	40/40	10/10
5.0 min PRL + UV	38/40	10/10
UV + 5.0 min PRL	0/22	0/10
Untreated	0/22	0/10

\* Cells from clone 4 animals were exposed with the average 254 nm doses shown.  
PRL was given before or after UV.  
~4.5 x 10<sup>5</sup> cells injected per animal

Table 2. Parameters observed for inducing melanomas in fish

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$\lambda$ , nm	a	b	$k, \text{m}^2/\text{J}$ $\times 10^4$	Relative value of k
302	0.248	0.236	50 (22)	1.00
313	0.235	0.270	8.2 (3.6)	0.16
365	0.242	0.235	19 (11)	0.32
405	0.087	0.410	1.1 (0.4)	0.017
436	0.050	0.427	1.6 (1.1)	0.023

---

The Equation used for the tumor prevalence is

$$\text{Prevalence} = a + b(1 - e^{-kE}) \text{ where } E \text{ is } \text{J}/\text{m}^2.$$

The relative values of k are normalized to 1.00 at 302 nm and are quantum corrected. The numbers in ( ) represent SE.

## Figure Legends

Figure 1. Relative sensitivity for affecting DNA (points, solid curve is very similar to (Ref.15) the absorption spectrum of DNA), human erythema, and the typical sunlight intensities at the surface of the ground all as functions of wavelength.

Figure 2.A. The dose-response curve for melanoma induction in fish from exposures to (Ref. 20) 547 nm.

B. Action spectra for induction of melanoma in fish and human erythema and Sunlight intensities at ground level.

C. The sunlight effective dose, as a function of wavelength, for inducing fish melanoma.

Figure 3. Upper panels: The relative values of UVB and UVA as functions of latitude. (Ref. 26) Lower panels: The smoothed values for the incidence of three types of skin cancer as a function of latitude for 12 different countries. (experimental points are not shown)

FIGURE 1

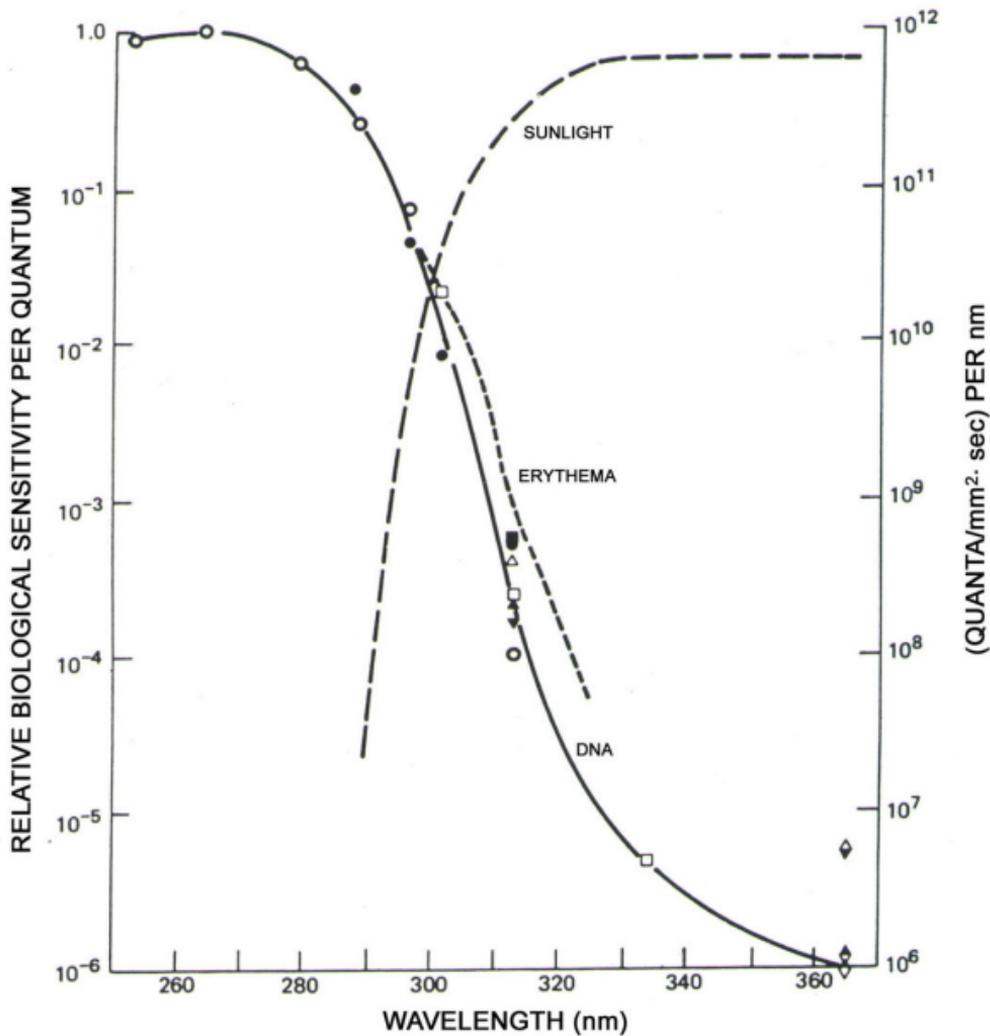
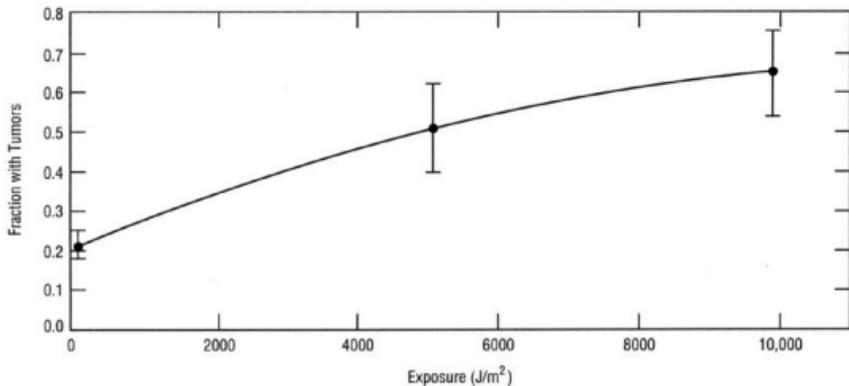
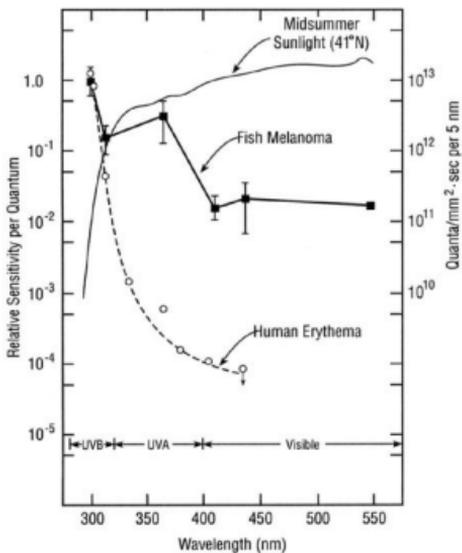


FIGURE 2

A



B



C

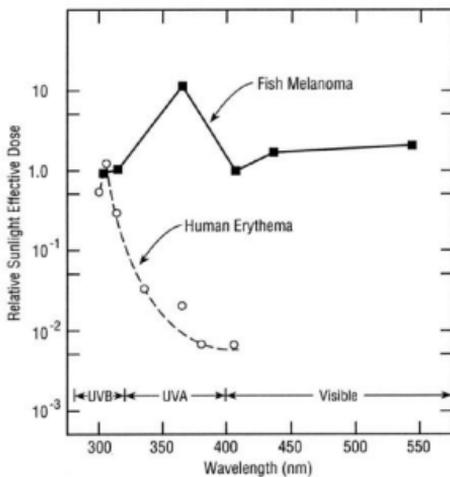


FIGURE 3

