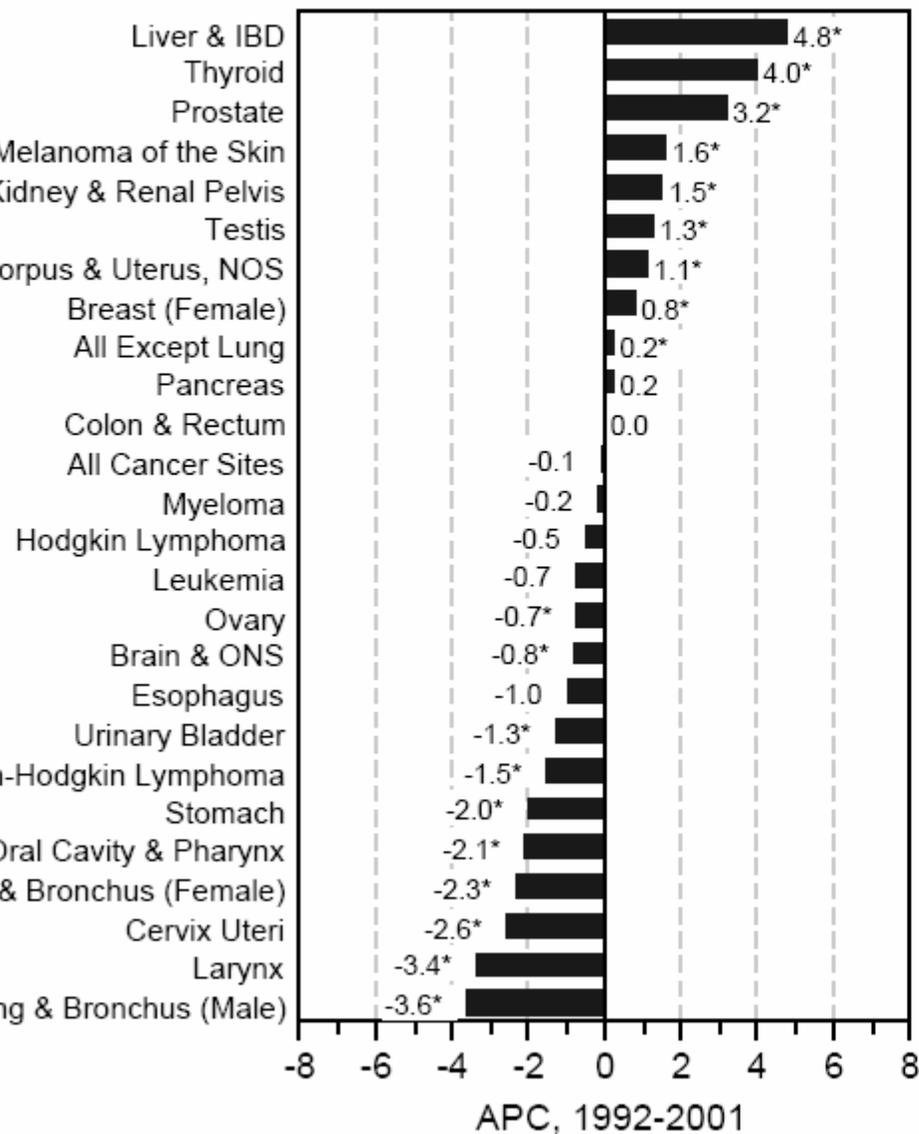


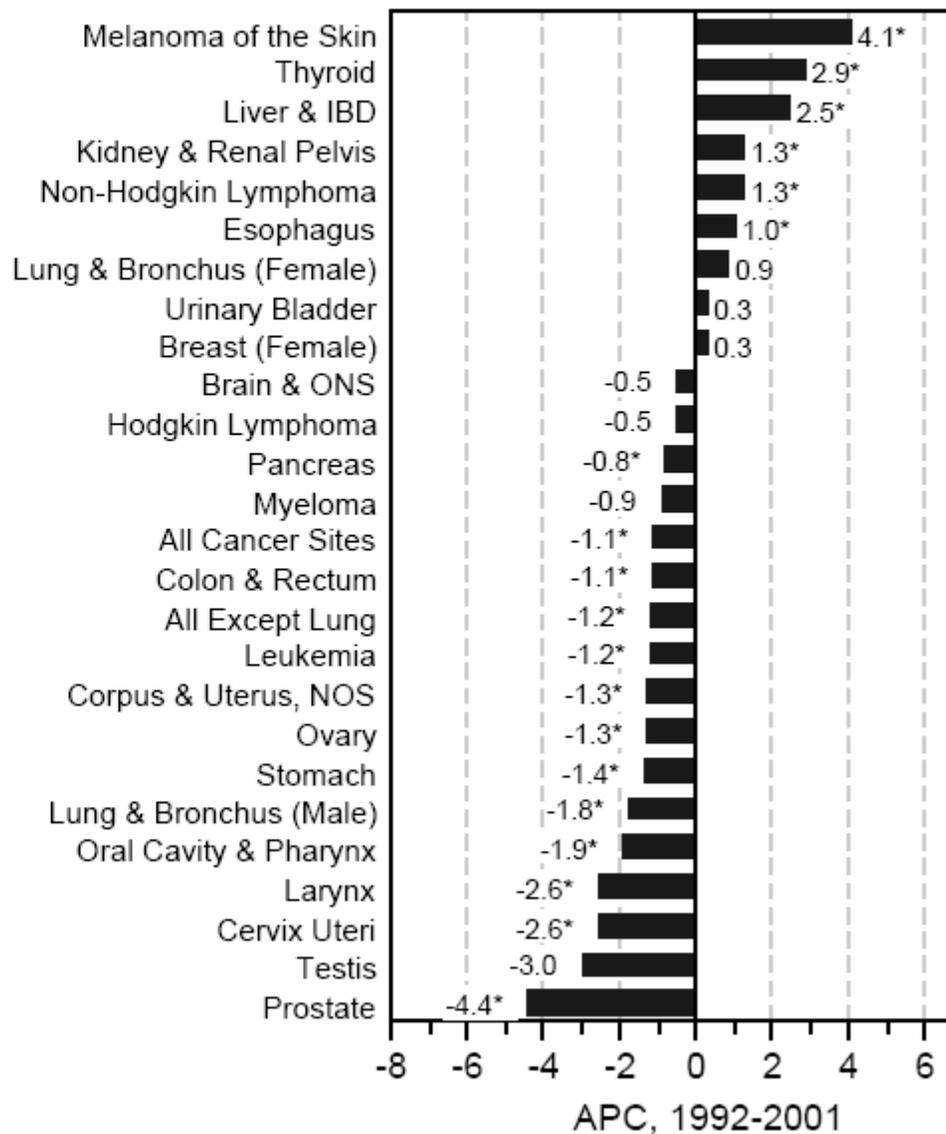
An Action Spectrum for Cutaneous Malignant Melanoma

by Primary Cancer Site 1992-2001

Ages Less Than 65



Ages 65 and Over



TOPICS OF DISCUSSION

- **What is an action spectrum?**
- **Its usefulness & limitations.**
- **How is one constructed?**
- **What is available re: skin cancer?**
- **What is needed?**

ACTION SPECTROSCOPY

- An action spectrum is a wavelength dependence spectrum.
- Its shape can give an indication of the absorption spectrum of an unknown light absorber which initiates the biological reaction.

What Can an *in Vivo* Action Spectrum Do for You?

- Aid in identifying the initial photon-absorbing compound (photoreceptor).
- Be used as a weighting function in calculating “biologically effective doses” important in risk assessment.
- Evaluate the relative effectiveness of different light-emitting sources for inducing a given response.
- Determine RAF’s for stratospheric ozone depletion.

What Are the Limitations of an Action Spectrum?

Screening by other compounds which absorb within the wavelength region of interest.

• **Overlapping of wavelengths by use of wavebands which are too wide.**

• **Incorrect measurement of photon flux (poor calibration).**

Any or all can lead to incorrect interpretation due to distortion of its shape.

A Conundrum !

UVB = 320 nm - 280 nm

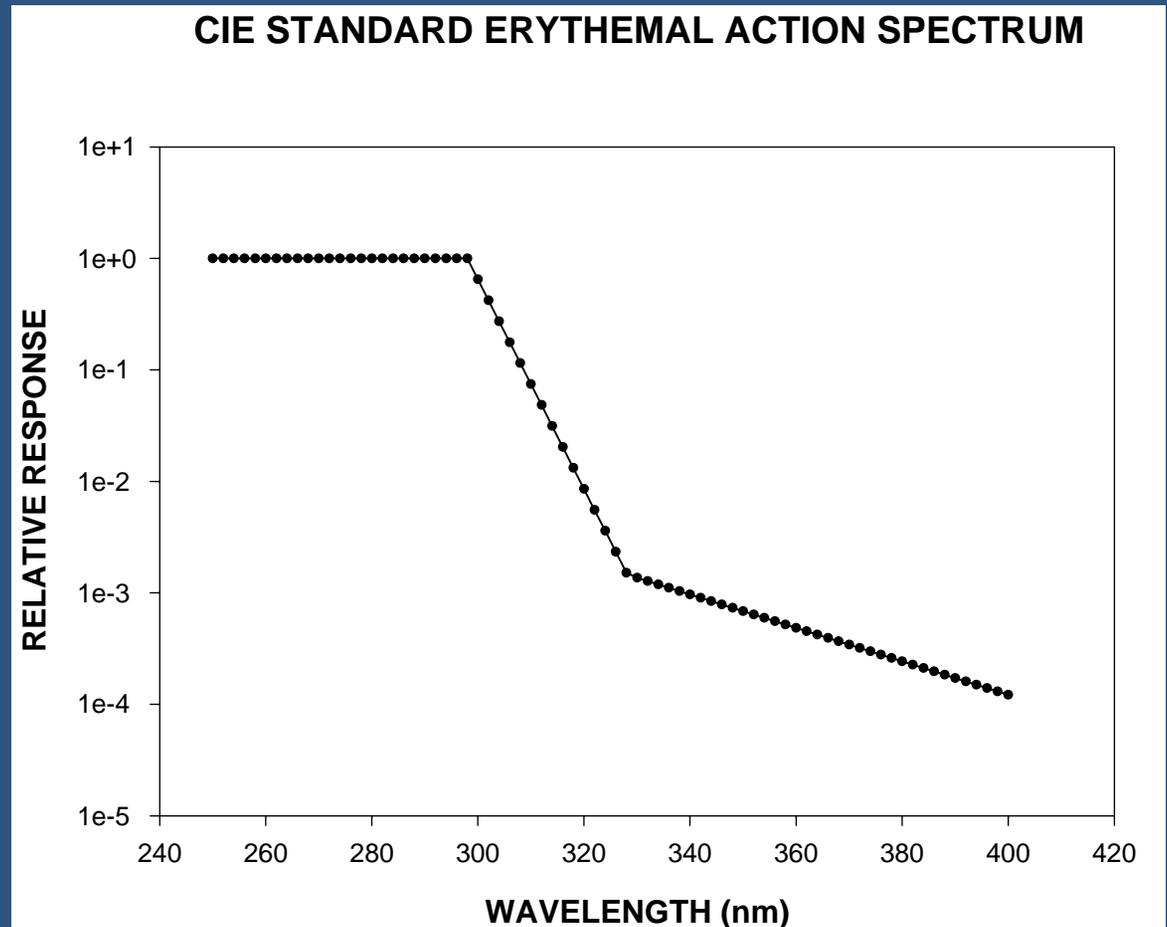
UVB = 320 nm - 290 nm

UVB = 315 nm - 280 nm

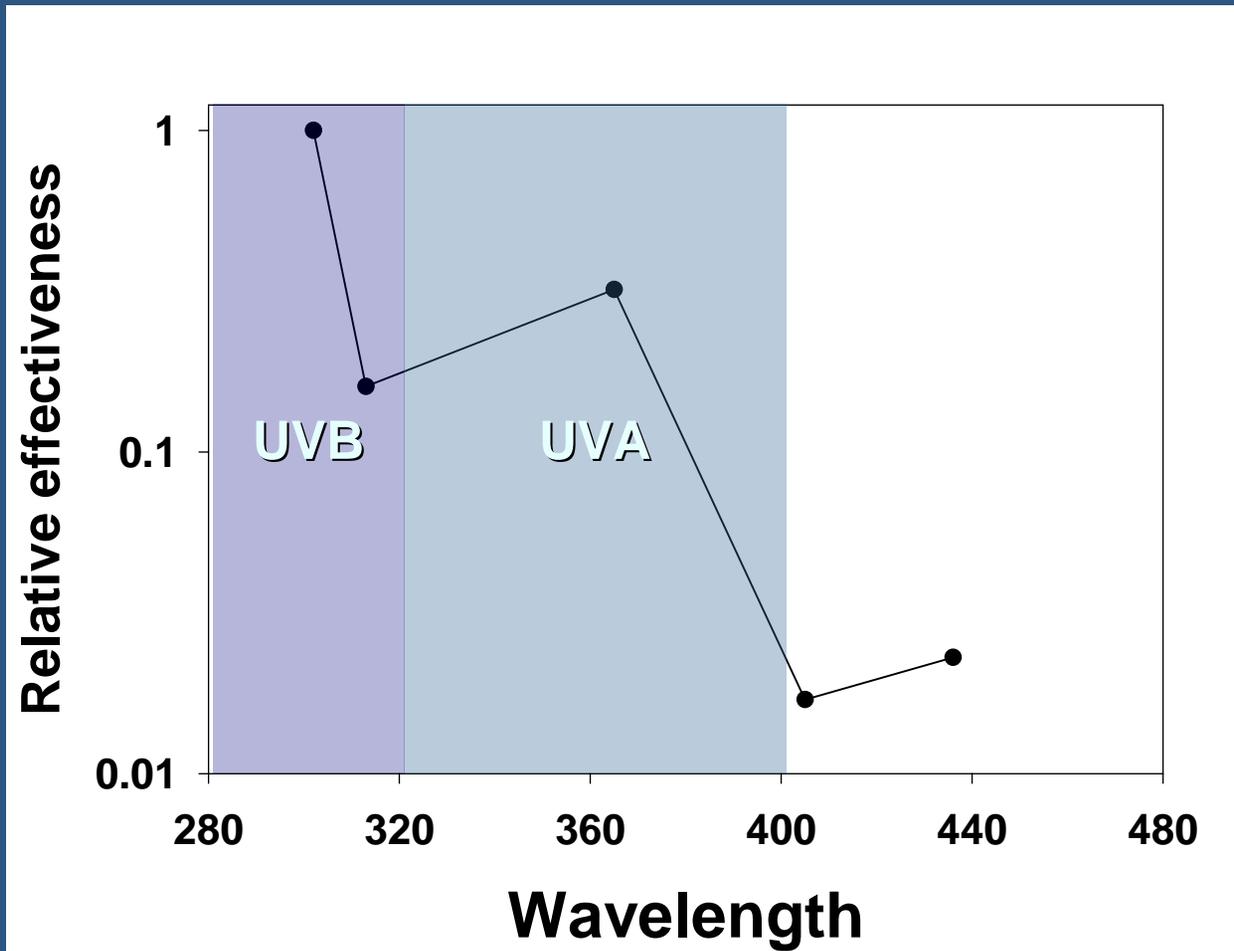
Action Spectra

1. CIE Erythematous - human
2. Melanoma- FISH
3. SCUP Skin Cancer-Mouse
Non-melanoma
4. Pyrimidine Dimer
5. Immune Suppression-Mouse

CIE Standard Sunburn Action Spectrum

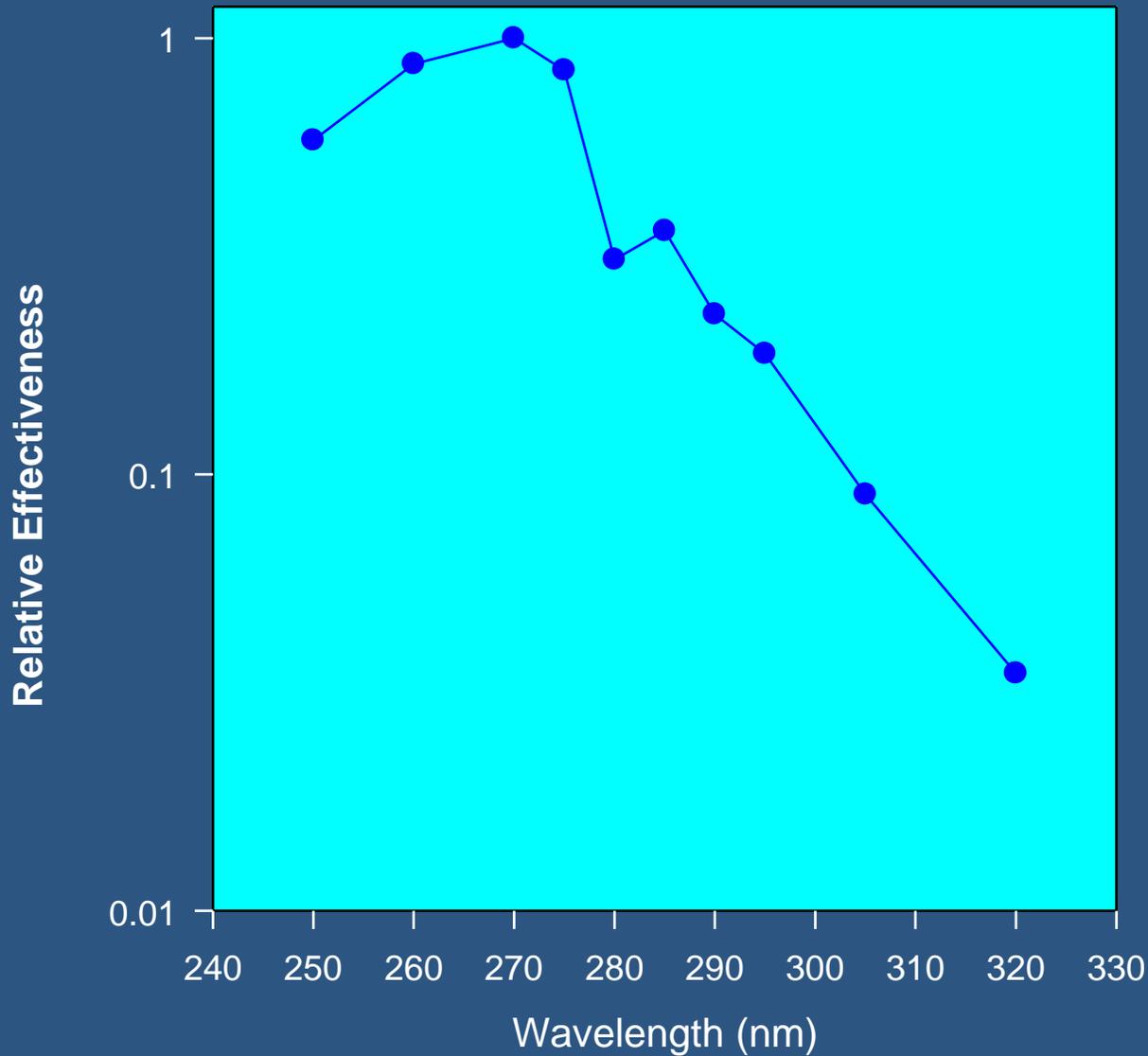


Xiphophorus Fish Melanoma Action Spectrum



Setlow et al Proc Natl Acad Sci U S A. 1993. 90:6666.

Immunosuppression action spectrum (De Fabo & Noonan, 1983)



Action Spectra for Skin Cancer in Animal Models

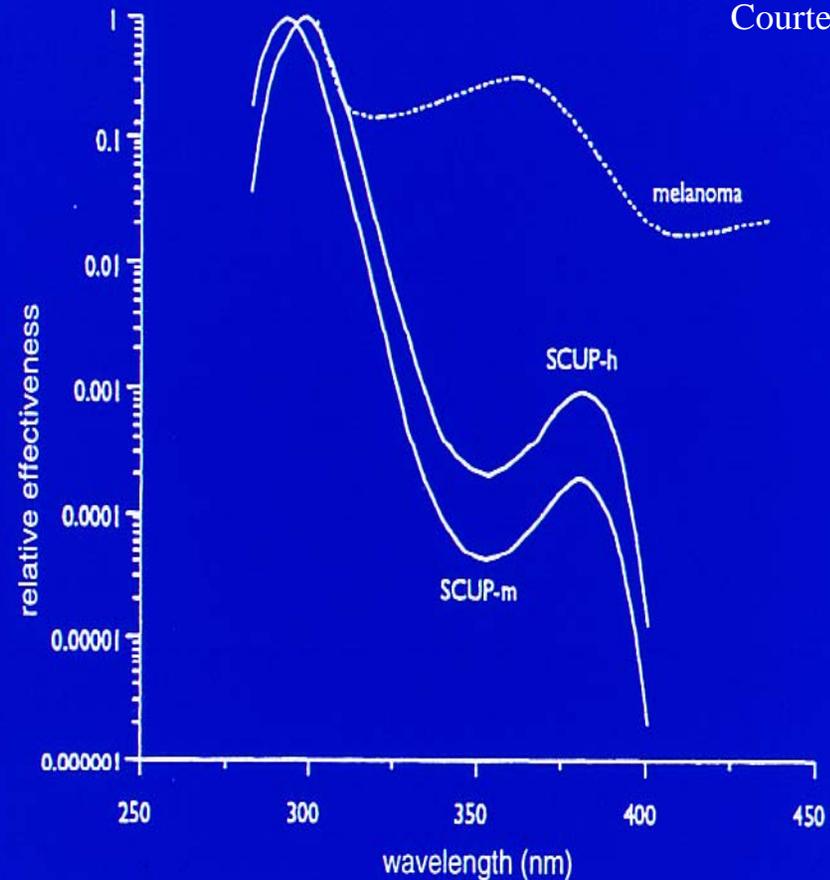
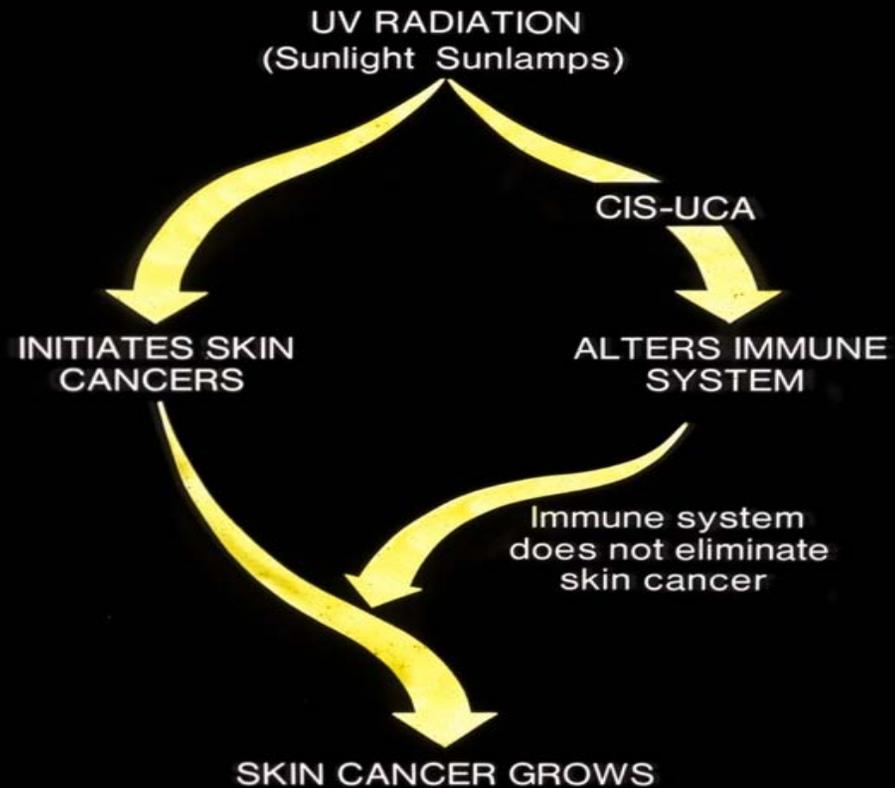


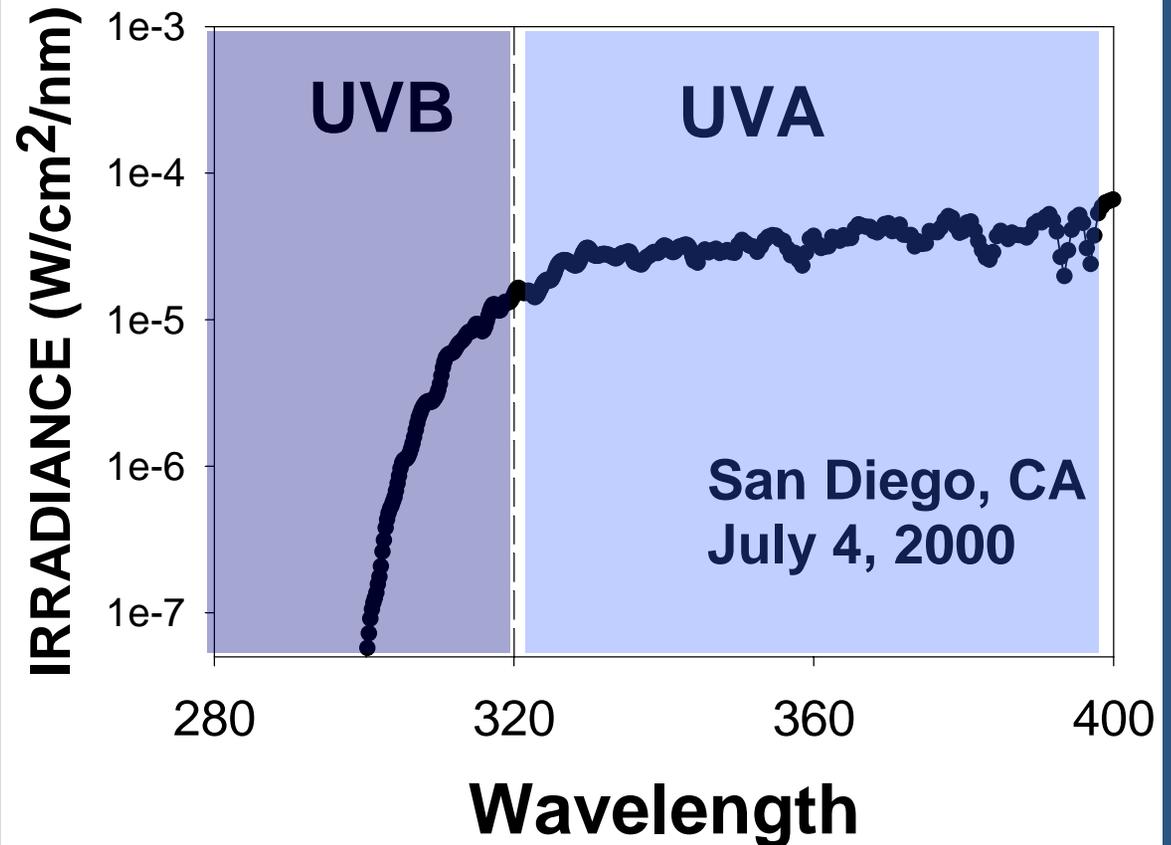
Figure 3. Action spectra for cancer in animal models: Melanoma in fish (89), nonmelanoma skin cancer in mice, SCUP-m (90), and in humans SCUP-h (91).

'Two To Tango'

SCHEME OF SKIN CANCER DEVELOPMENT DERIVED FROM EXPERIMENTAL STUDIES

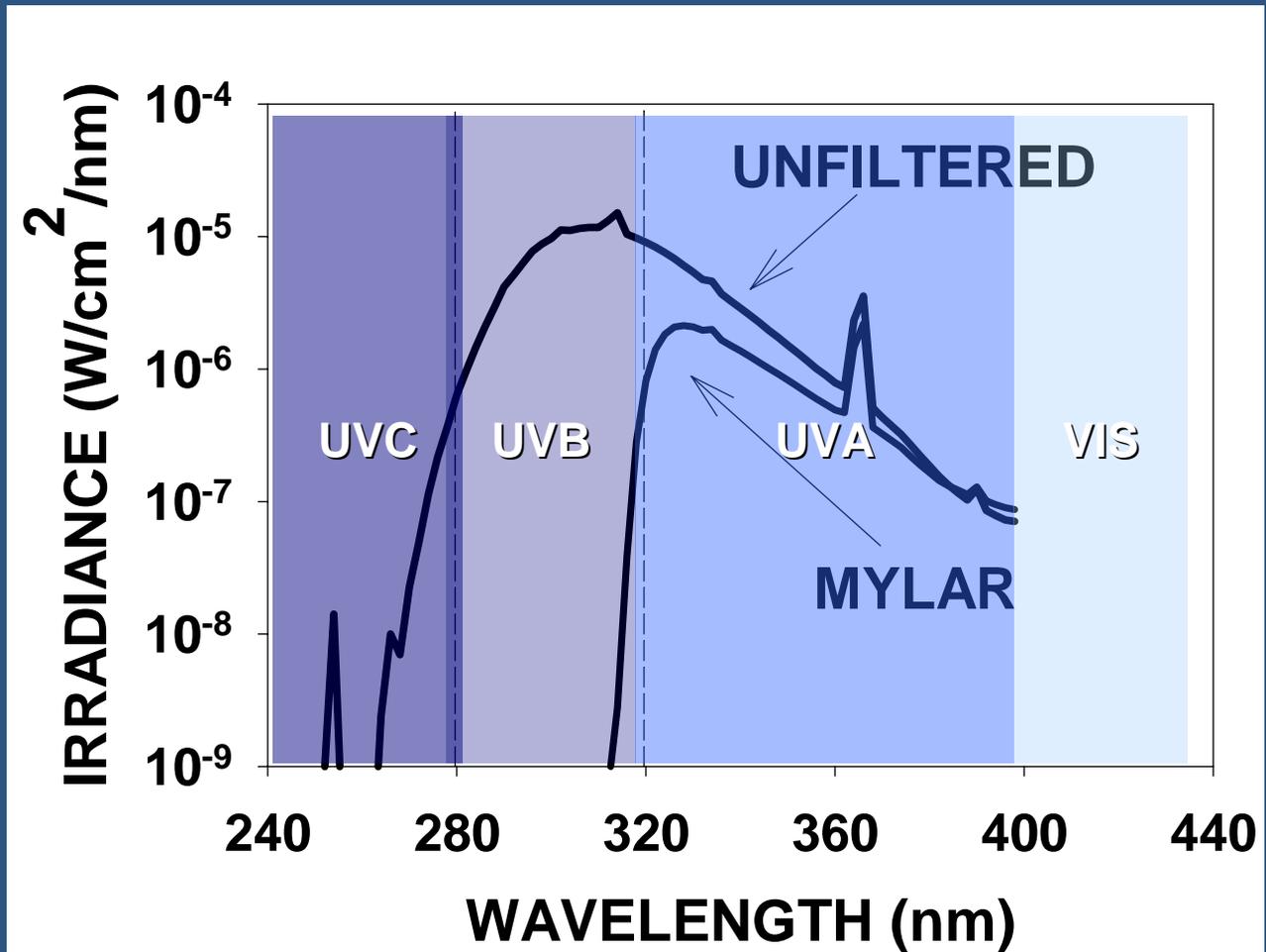


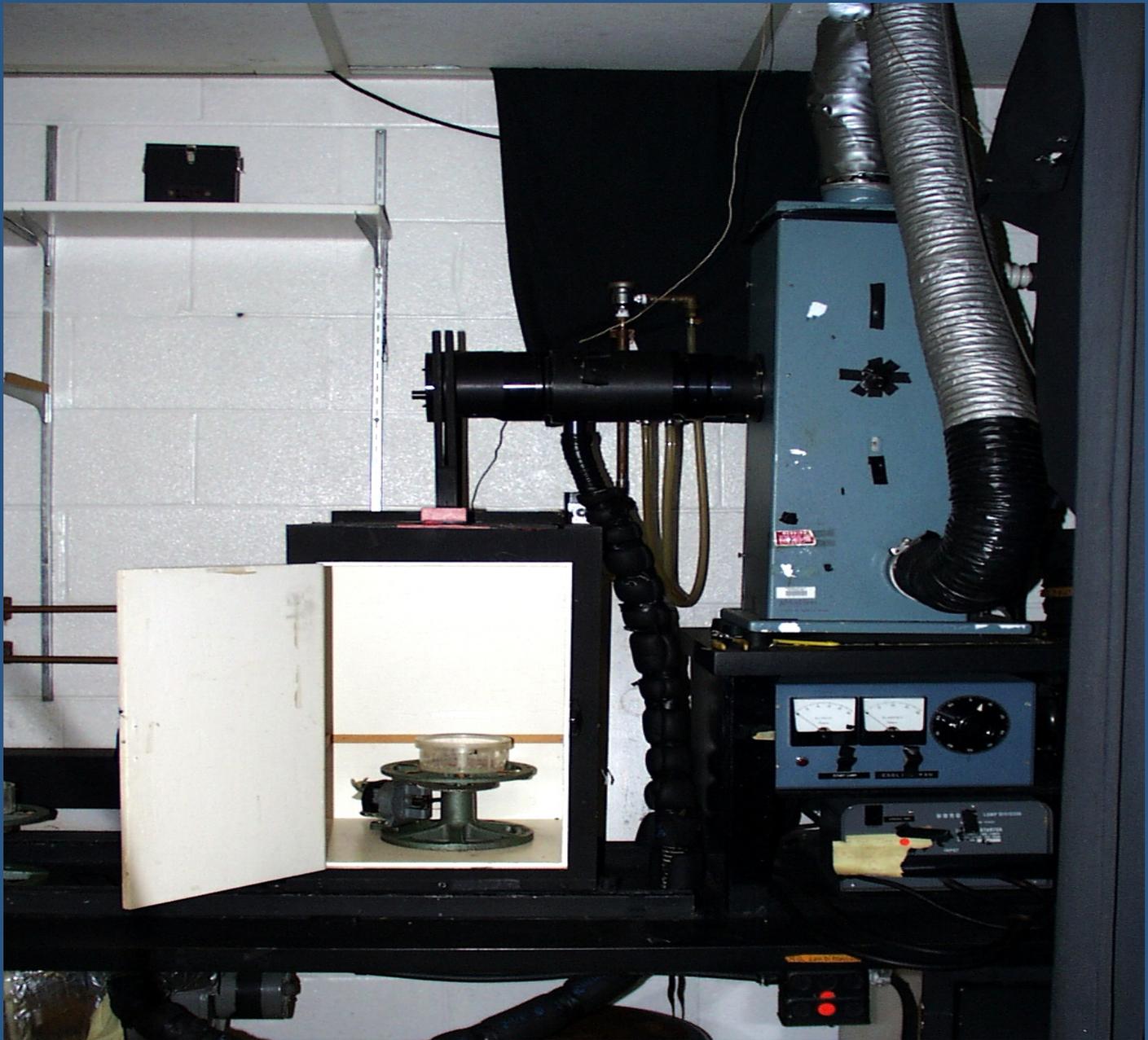
UV in Sunlight



Data courtesy NSF (Polar Programs) & Biospherical Instruments, San Diego, CA.

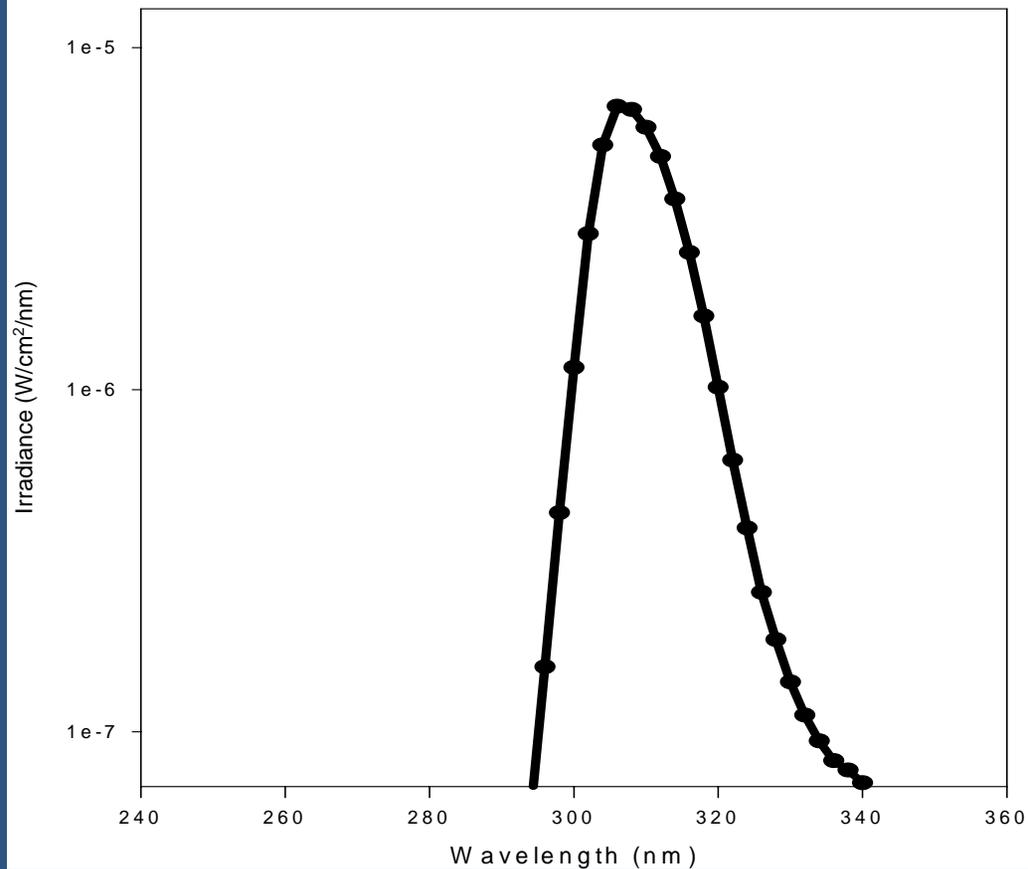
Spectral Output of F40 Sunlamp With and Without Mylar Filter





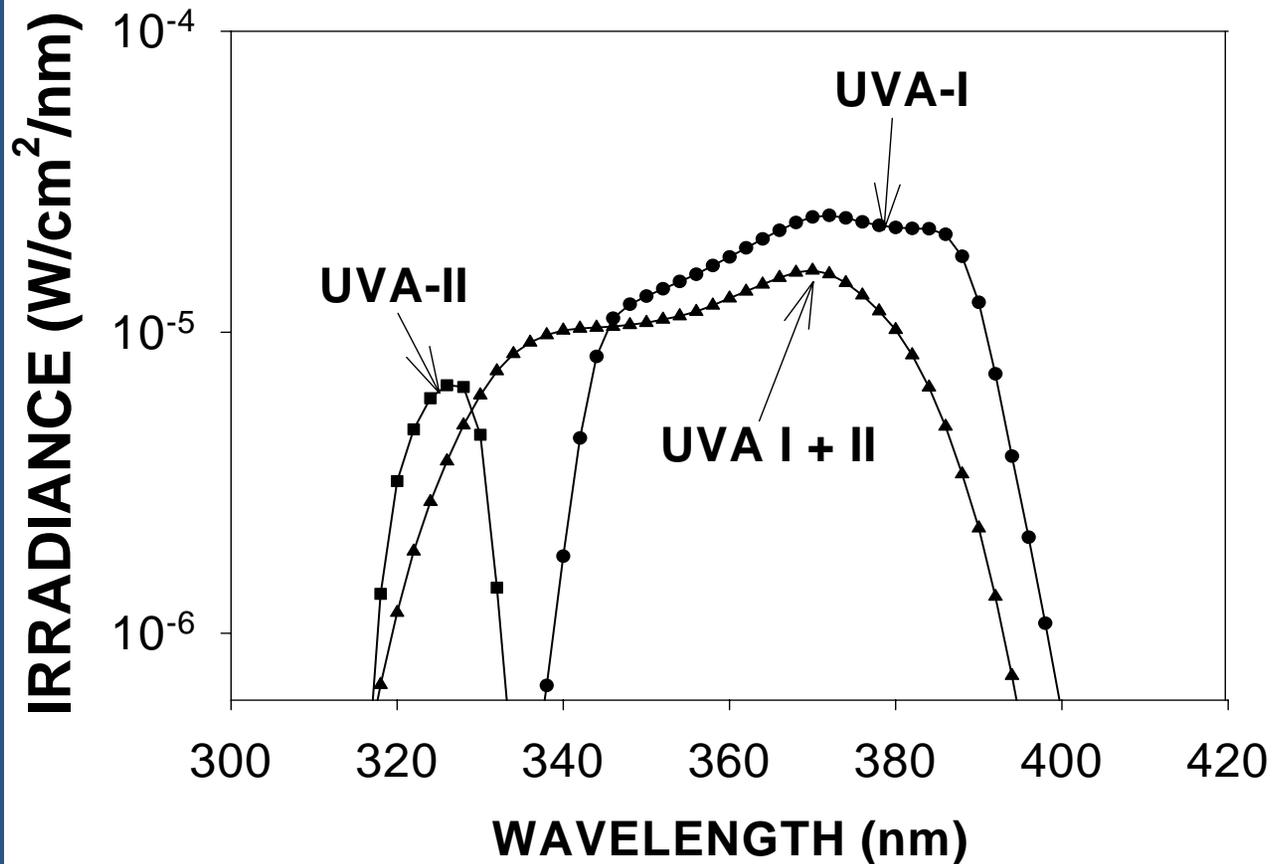
Xenon arc with UVB Filter

Fig. 6. Spectral Irradiance of Xenon Arc through UVB Wide Band Filter



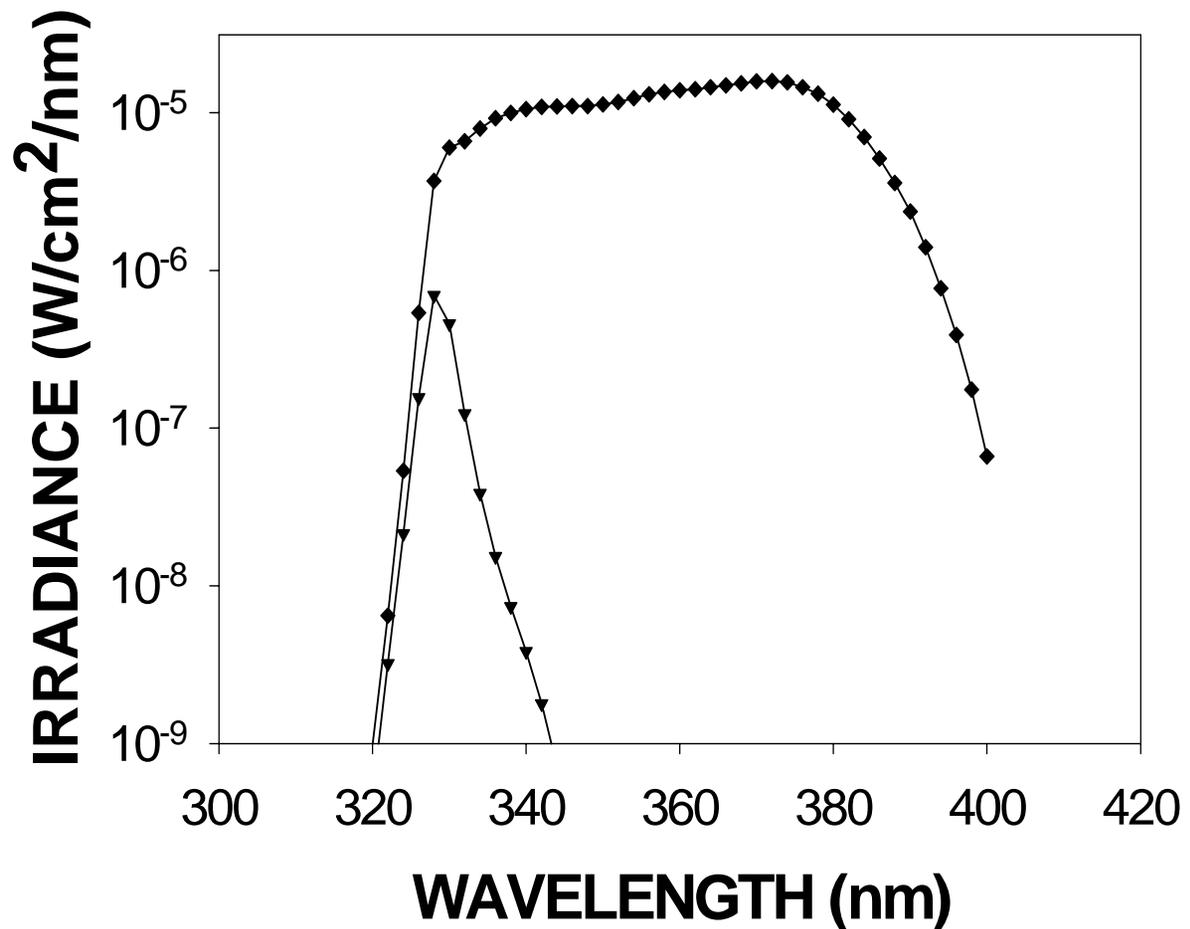
Delivery of UVA-II, UVA-I or UVA I+II

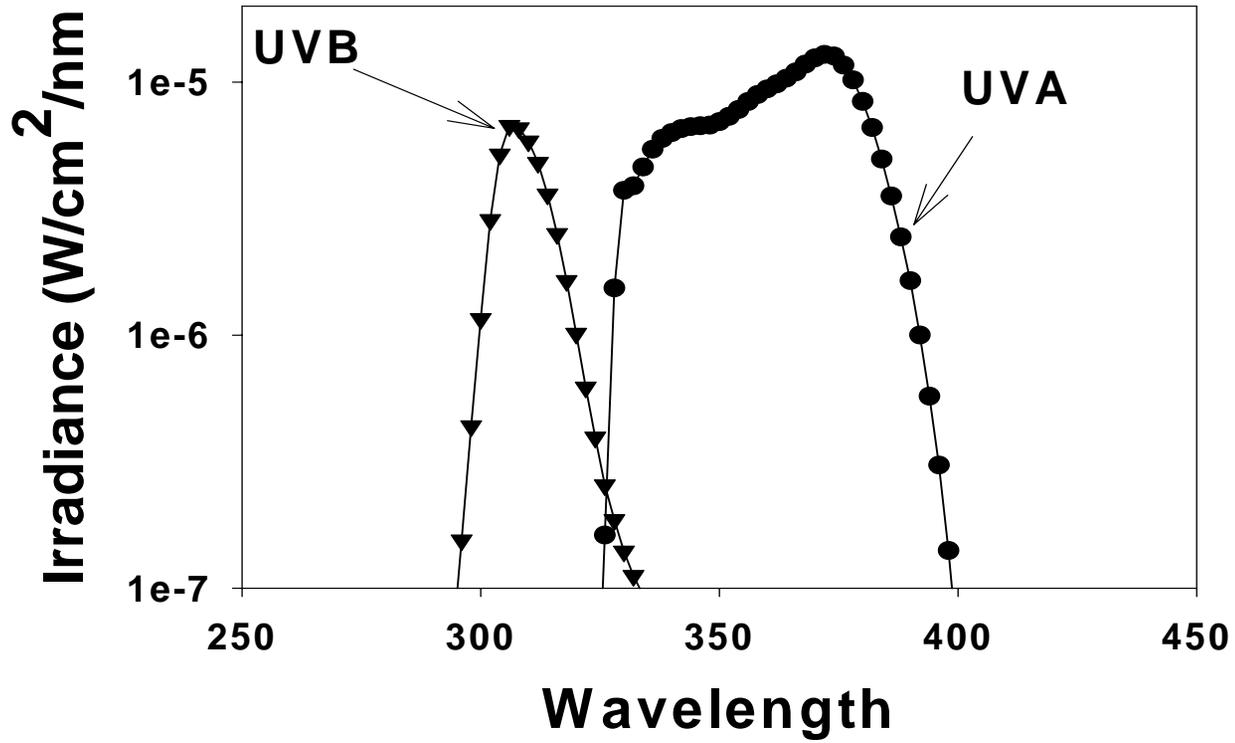
UVA BANDPASS FILTERS COUPLED TO 2.5 kW XENON LAMP



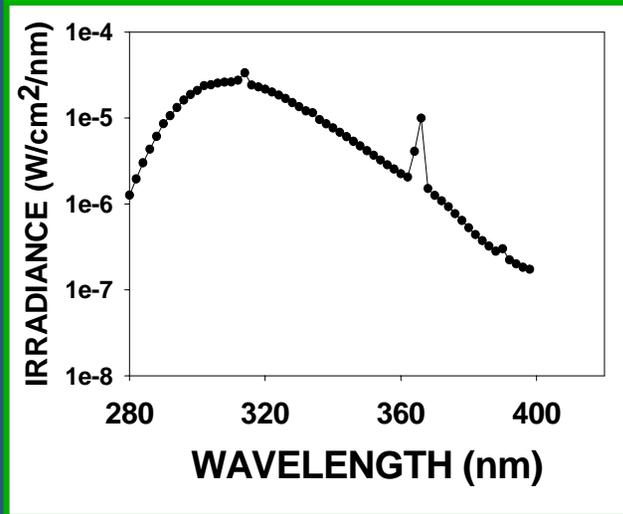
Use Of a Longpass Filter To Remove Contaminant Wavelengths

USE OF A LP 330 nm FILTER TO CUT OFF RADIATION BELOW 320 nm

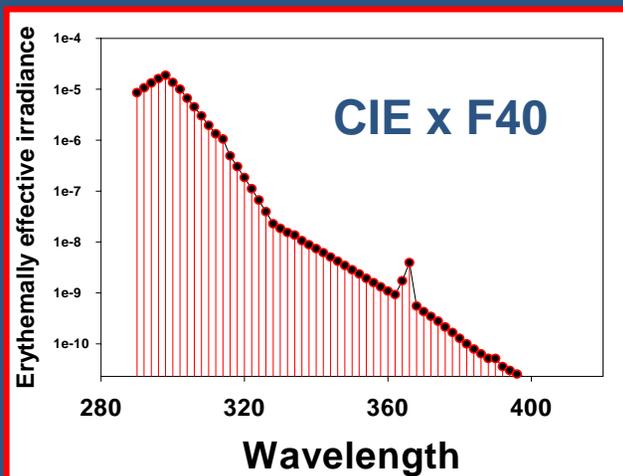
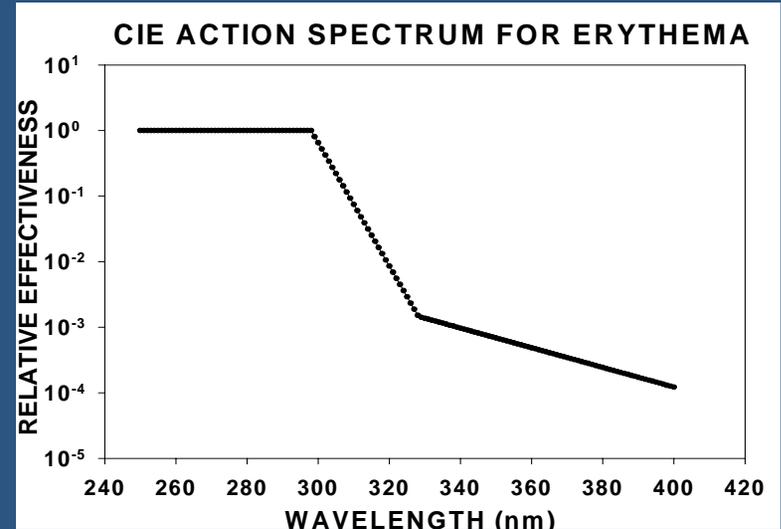




Derivation of Biologically Effective Radiation



X



- Erythemally effective dose = 2300 J/m²
- = 23 standard erythemal doses
- 23 SED received from summer sunlight in ~ 2.5h.

Erythematous (sunburn) dose for melanomagenesis

Neonatal mouse UV dose : 9.57 kJ/m²

Weighted by CIE erythematous action spectrum

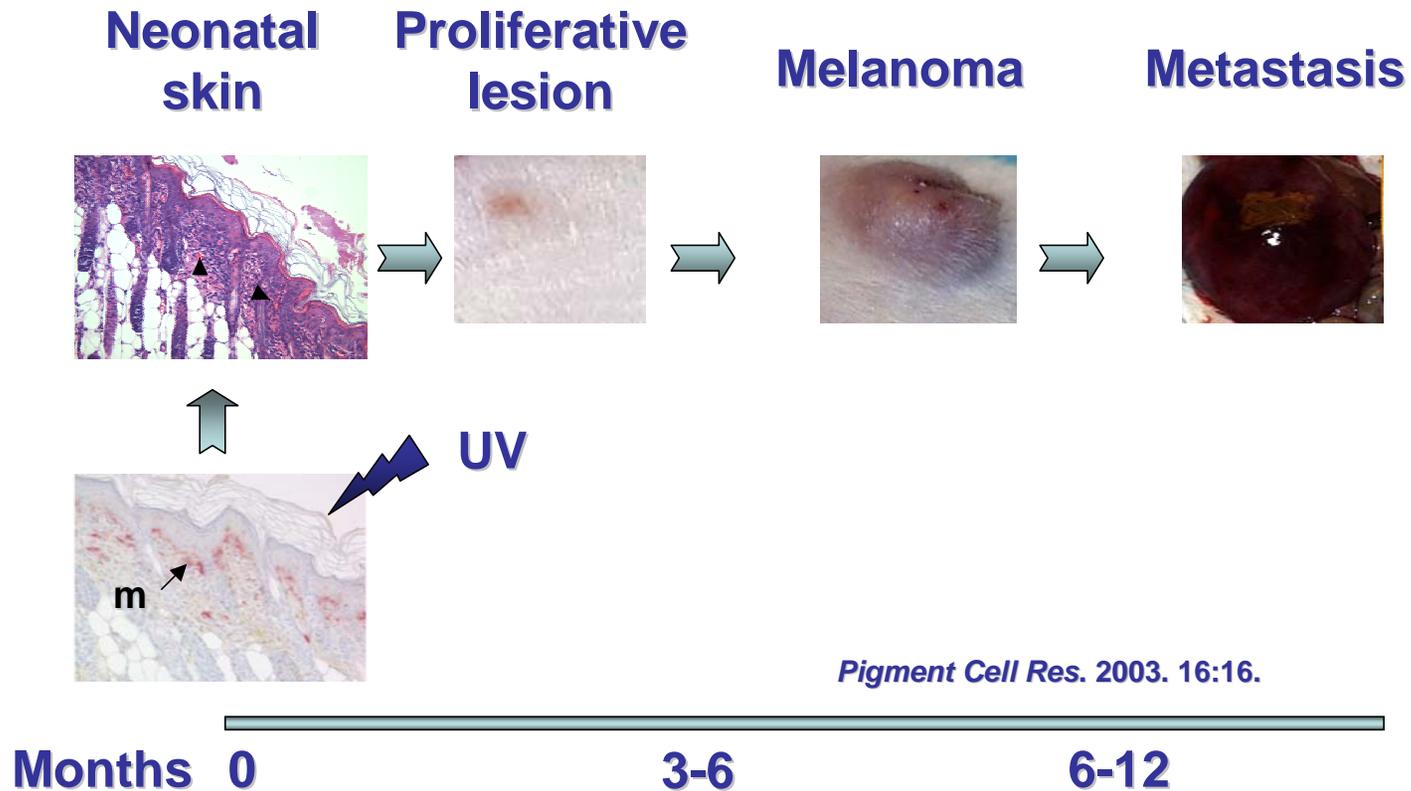
2.3kJ/m² erythemally weighted UV

23 Standard erythematous doses (SED)

**Sunlight monitoring data at 39N, 110W
July 4, 2000**

23 SED received in 2h 40min

HGF/SF transgenic mouse model of UV-induced melanoma



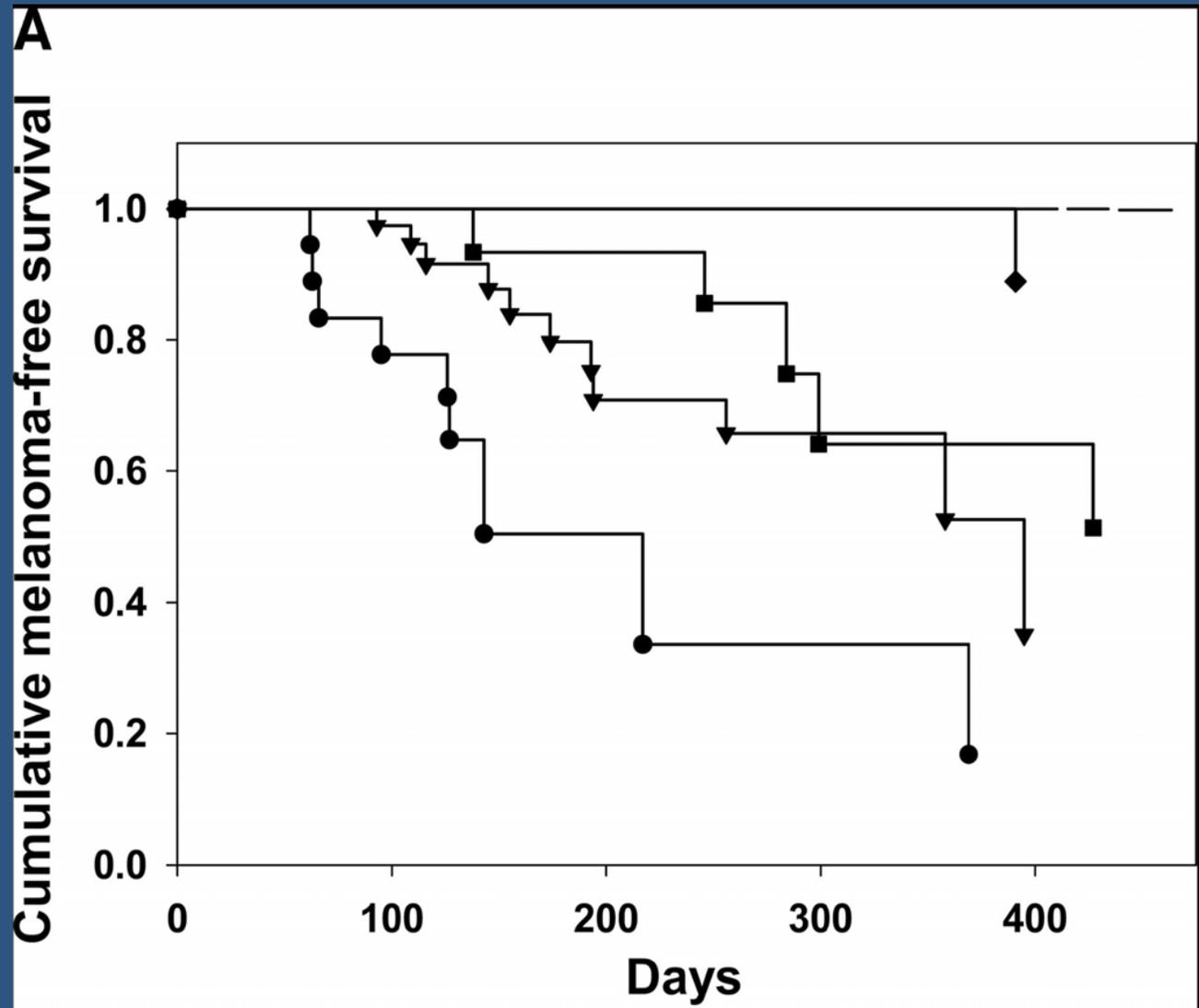


Doses delivered to HGF/SF transgenic mice

| Source | Radiation dose Delivered(kJ/m ²) | | | | SED |
|--------------------------------|--|-----------------|------------------|--------------------|-----|
| | Total 250-800nm | UV 250-400nm | UVA 320-400nm | UVB 280-320nm | |
| UVB filter & Xenon arc | 14.0 | 14.0 | 0.5 | 13.5 | 23 |
| | 4.7 | 4.7 | 0.2 | 4.5 | 7 |
| F40sun lamp (unfiltered) | 14.7 | 9.5 | 3.3 | 6.2 | 23 |
| Solar Simulator | 322.1 | 41.9 | 36.0 | 5.9 | 23 |
| F40 Mylar filtered | 14.1 | 4.0 | 3.8 | 0.2 | 0.1 |
| UVA filter & Xenon arc | 150.0 | 150.0 | 150.0 | 1.5e ⁻⁵ | 1.1 |

Melanoma formation in UV irradiated HGF/SF transgenic mice

| Source | HGF/SF transgenics | No. with melanoma | Median days to melanoma | Mean melanomas per tumor bearer |
|--------------------------|--------------------|-------------------|-------------------------|---------------------------------|
| UVB filter & Xenon arc | 18 | 10 | 127 | 1.7 |
| | 10 | 5 | 132 | 2.0 |
| F40sun lamp (unfiltered) | 42 | 11 | 174 | 1.0 |
| Solar Simulator | 29 | 5 | 284 | 1.2 |
| F40 Mylar filtered | 20 | 1 | - | - |
| UVA filter & Xenon arc | 23 | 0 | - | 0 |
| Nil | 15 | 1 | - | - |

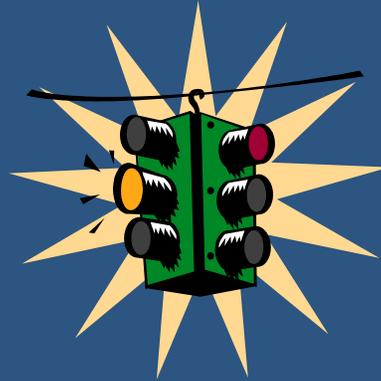


CONCLUSIONS

USING AN HGF/SF TRANSGENIC MOUSE MODEL FOR UV-INDUCED MELANOMA WE FIND:

- **REMOVAL OF UVB (290-320 nm) FROM THE IRRADIATION SOURCE PREVENTED MELANOMA INDUCTION.**
- **EXPOSURE TO UVB ALONE POTENTLY INITIATED MELANOMA.**
- **UVA IRRADIATION WITH A >30-FOLD HIGHER DOSE THAN UVB FAILED TO INITIATE MELANOMA**
- **THE CIE ERYTHEMAL ACTION SPECTRUM IS NOT A GOOD WEIGHTING FUNCTION TO PREDICT MELANOMA RISK IN THIS MODEL**

CAUTION !



Caveats

- **UVA studies described are still on-going.**
- **In previous experiments a second dose of F40 unfiltered radiation, at six weeks of age, caused a multiplicity of melanomas in neonatally irradiated mice. The waveband for this effect is unknown.**

Which Wavelengths Are
Responsible for mammalian
Melanoma?

What are the experiments?

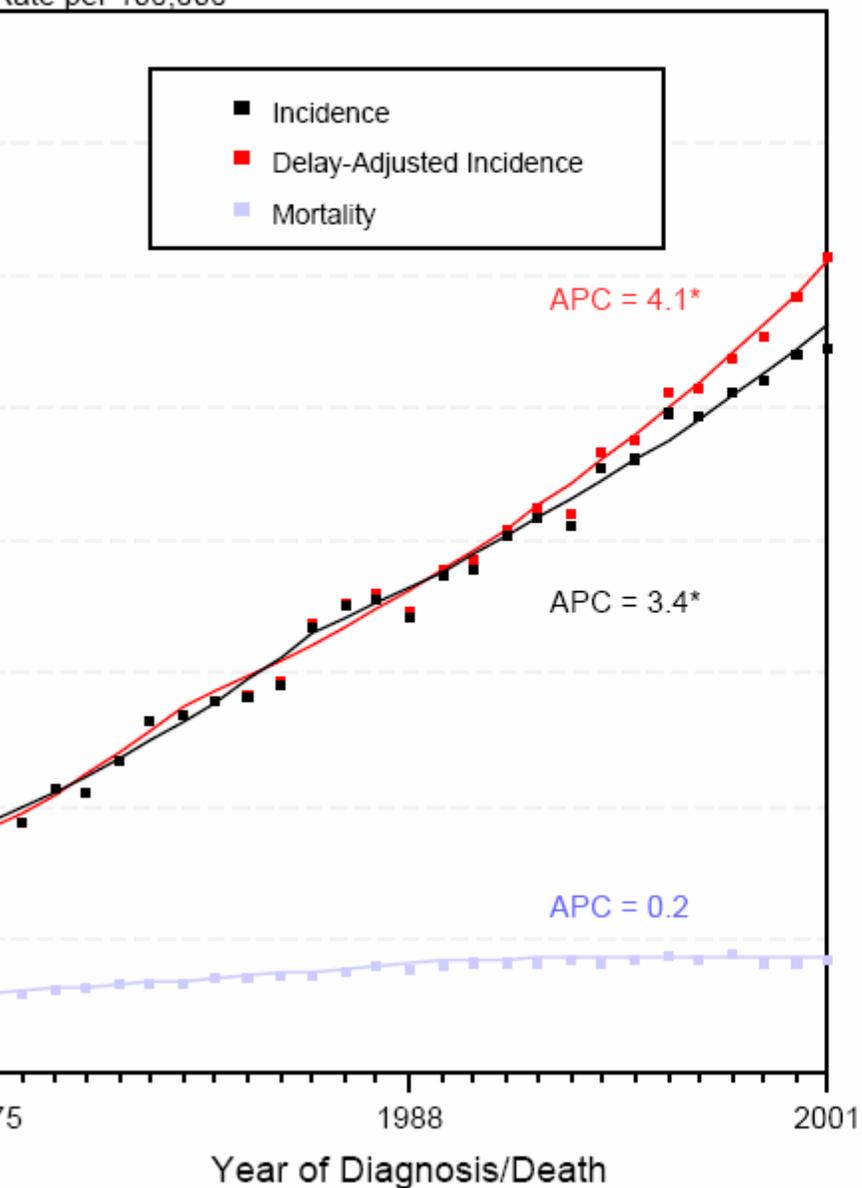
- *An action spectrum for ultraviolet radiation-induced melanoma skin cancer*
- **Why are these experiments important?**

Skin cancer represents more than half of all new cancers in the USA. More than 1 million new cases of skin cancer will be diagnosed in the United States this year.
- About 80 percent of the new skin cancer cases will be basal cell carcinoma, 16 percent are squamous cell carcinoma, and ***4 percent are melanoma.***

Melanoma of the Skin, White, by Sex

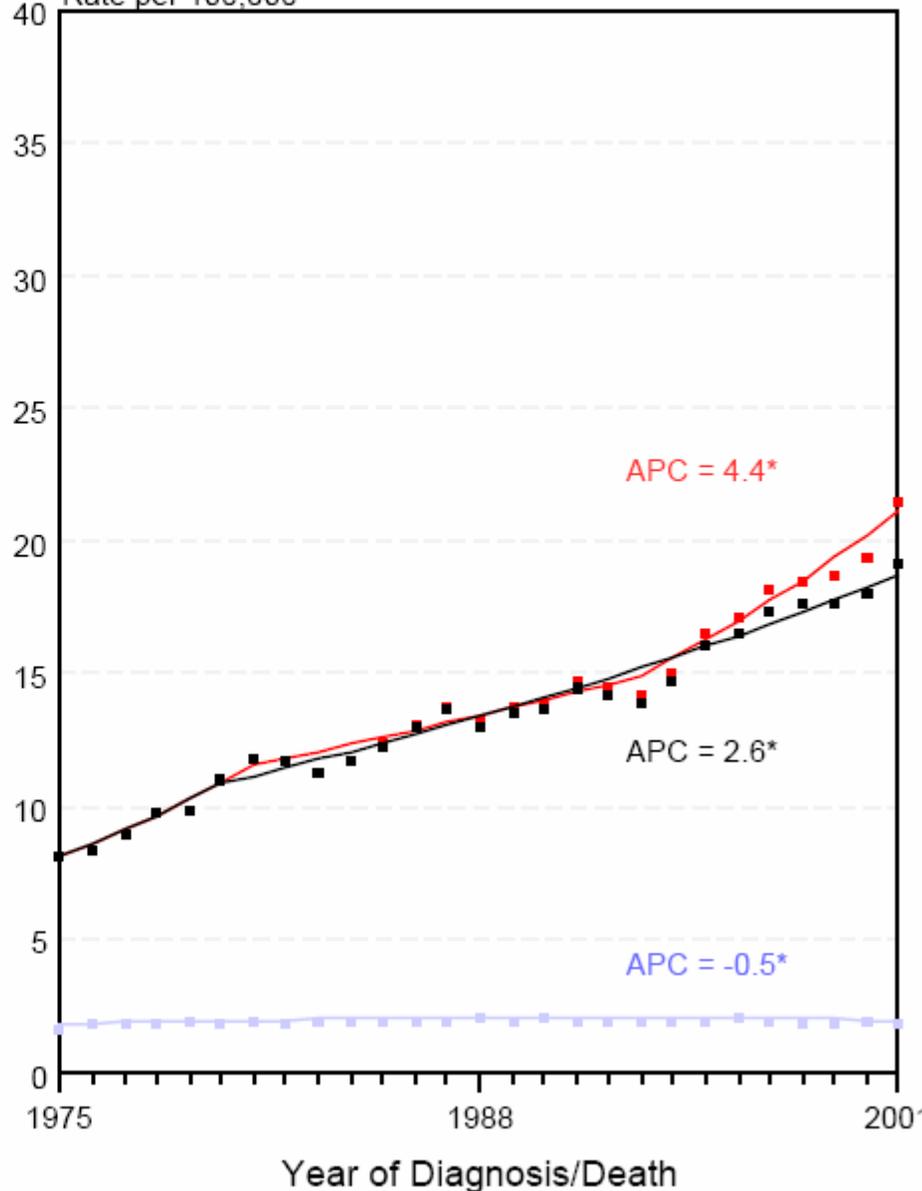
Male

Rate per 100,000



Female

Rate per 100,000



What specific property or properties of the FEL described here are critical for your experiments?

- An essential property of the J-lab FEL is the ability to irradiate a large number of mice across a wide exposure area.
- With the J-lab FEL it will be possible to produce an area of 1 square meter with narrow wavelength resolution of 1 nanometer (nm) throughout the UVB and UVA regions.
- We anticipate being able to irradiate up to 50 or more animals per exposure at a minimum of 3 exposures per wavelength.

OPTICAL OUTPUT PROPERTIES OF THE J-LAB FEL

- A minimum power output of 1 W from the J-Lab FEL, applied over a surface area of 1000 cm² (Irradiance = 10 W/m²) would deliver a maximum melanomagenic dose of UVB of 13.5 kJ/m² in approximately 22.5 min.
- This dose has previously been shown to be highly melanomagenic in this mouse model in our laboratory (Cancer Res. 2004 64(18):6372-6).
- For each exposure the time would be cut down- up to a factor 16 (6 h versus 22.5 min) and the number of mice would be cut down by a factor of 5 (50 versus 10).

What specific property or properties of the FEL described here are critical for your experiments?

Wavelengths will be given in 5 nm steps:

- The UVB (280-320 nm; 9 wavelengths),
- The UVA-2 region (320-400 nm; 4 wavelengths)
- The UVA-1 region (340-400 nm; 6 wavelengths).
- A large number of animals is, therefore, needed for constructing a detailed melanoma action spectrum with a high degree of spectral resolution.

Melanoma action spectrum: a first approximation.

- At 1 wavelength per day (by 3 exposures per wavelength), *total* FELS time would be on the order of 19 days.
- The limiting factor here would be the capacity to deliver 50 neonatal mice per exposure. In reality we may need to produce 100 neonatal mice per exposure since approximately one-half of these would be transgenic HGF/SF mice capable of producing melanoma.
- Synchronous live births of 50 neonatal mice may be doable but synchronous live births for 100 neonatal mice may not be possible. Thus, multiple days per wavelength per dose will, in all likelihood, be needed.

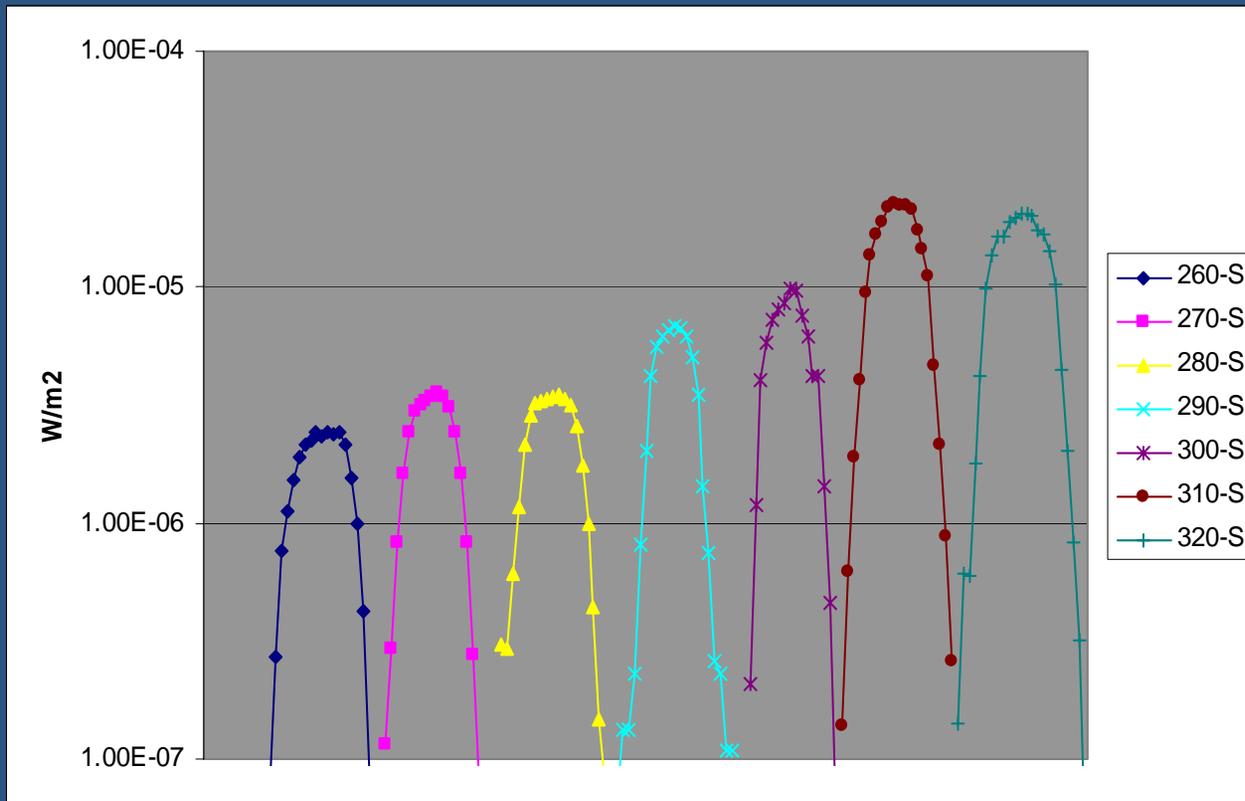
Melanoma action spectrum: a first approximation

- Such an undertaking would require not only substantial FEL time as indicated but logistical support for animal care and maintenance.
- Therefore, access to an animal facility is needed, preferably at or near the FEL.
- Although a substantial amount of time and resources are needed, given the disturbing reports of rising skin cancer rates, melanoma in particular, it would appear that a melanoma action spectrum would be worth the investment.

THANK YOU



TRANSMISSION OF 5nm UVB FILTERS



CONCLUSION (2)

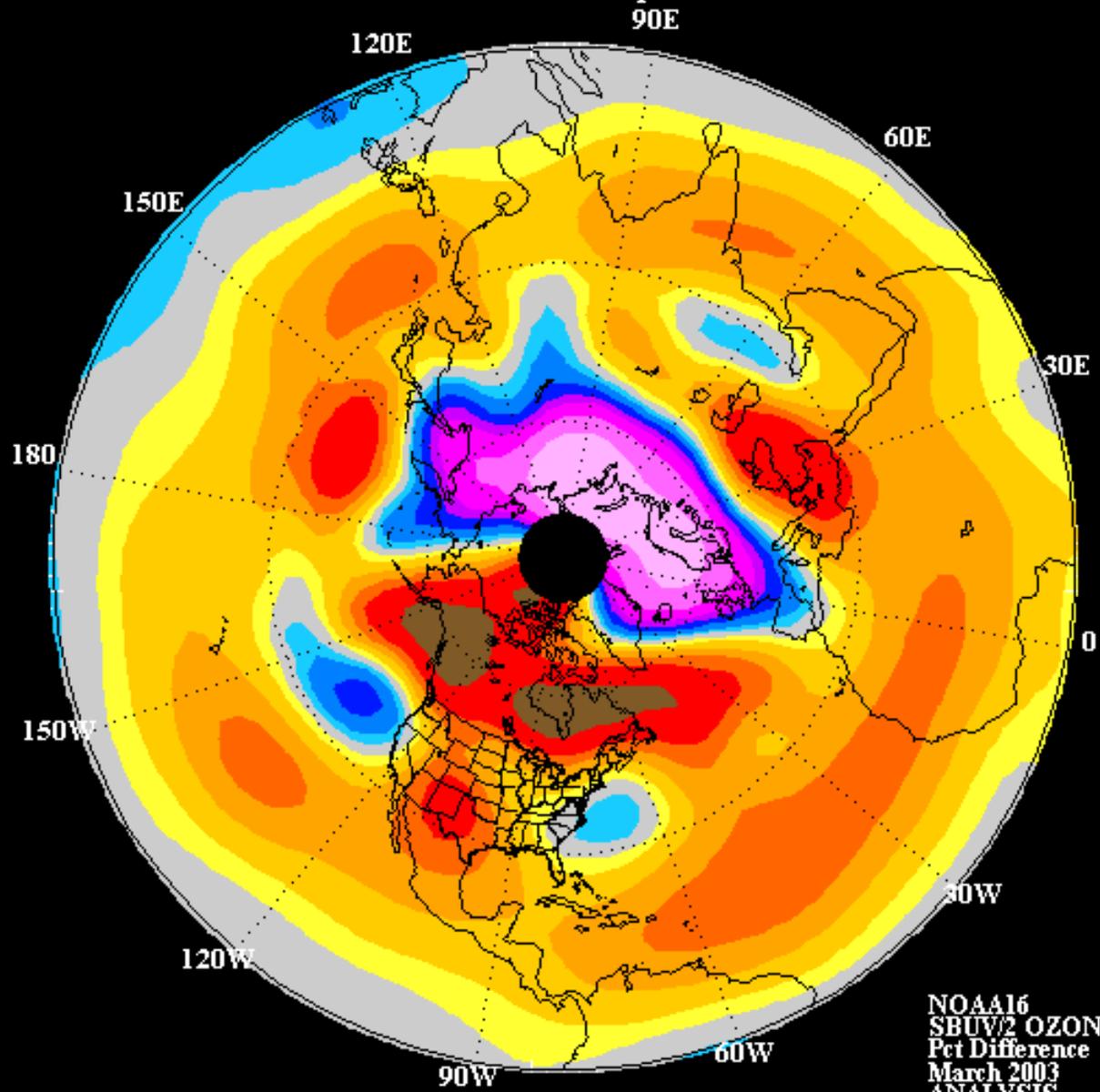
- These data indicate that UVB is likely the major carcinogen for melanoma in sunlight.
- These data cannot unequivocally exclude a potential role in melanoma induction for very high doses of UVA though this is considered unlikely due to an inverse trend seen in tumor-free survival with > doses of UVA (P=0.07).

Conclusions (2)

- **These data indicate that UVB is likely the major carcinogen for melanoma in sunlight.**
- **These data do not exclude a potential role in melanoma induction for high doses of UVA from artificial sources.**

MARCH PERCENT DIFF (2003 - AVG(79-86))

Northern Hemisphere

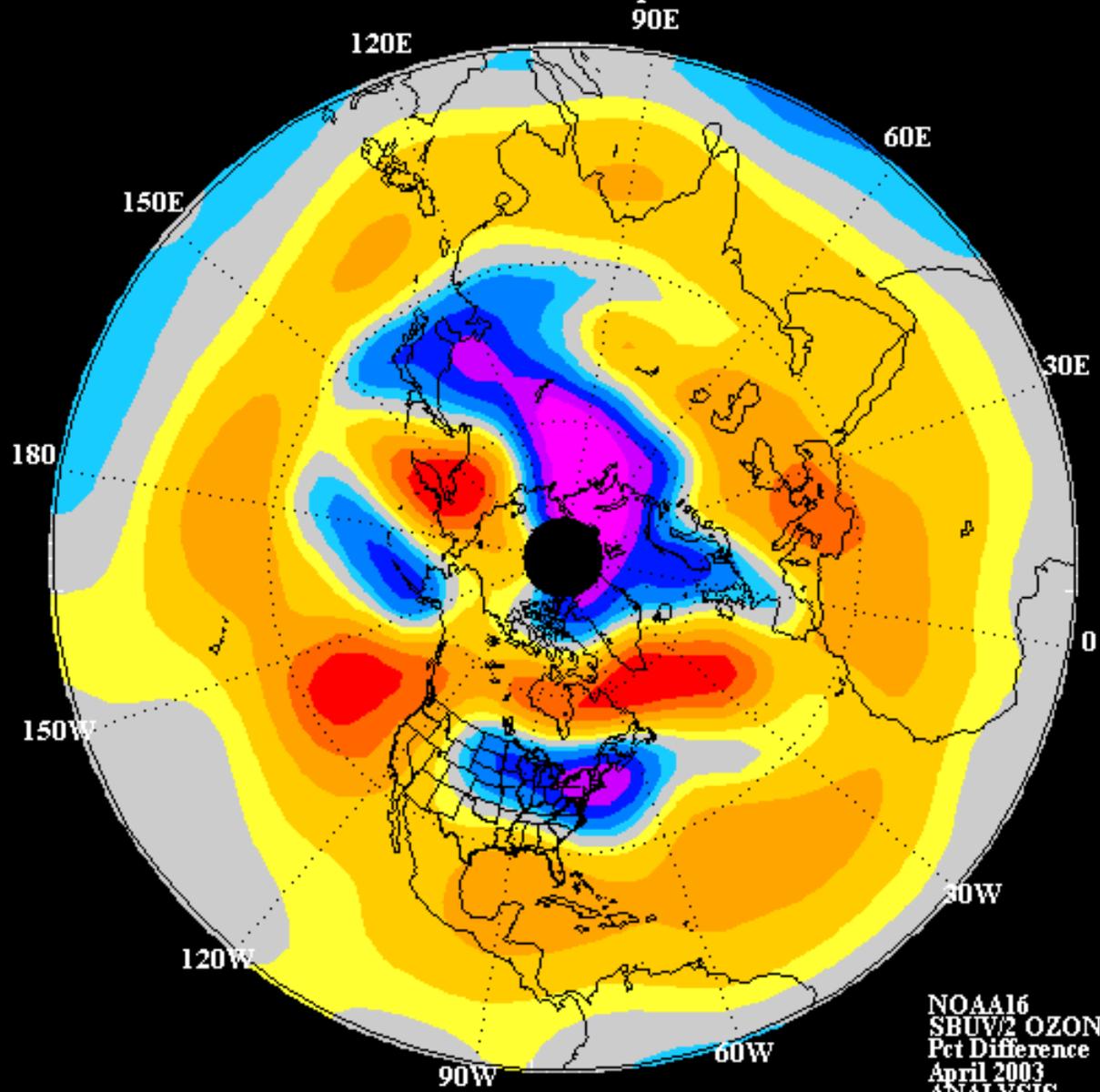


NOAA16
SBUV/2 OZONE
Pct Difference
March 2003
ANALYSIS
No Data Beyond 80N



APRIL PERCENT DIFF (2003 - AVG(79-86))

Northern Hemisphere



NOAA16
SBUV/2 OZONE
Pct Difference
April 2003
ANALYSIS
No Data Beyond 81N







What specific property or properties of the FEL described here are critical for your experiments?

- The J-lab FEL is outstanding in both the spectral breadth and time-average power of the light produced.
- The J-lab FEL will produce high intensity (hundreds to thousands of watts time average power) at wavelengths ranging from the UVC, B, and A, through the infrared and into the THz region (between the IR and microwaves).
- Inactivation cross-sections the UVA spectral region are small and conventional light sources offer a very limited choice of wavelengths.
- With the characteristics described for the J-lab FEL, determination of a high resolution monochromatic action spectrum for melanoma throughout the UVB and UVA is now possible.