

Ultraviolet Radiation–Induced Cataract: Age and Maximum Acceptable Dose

Xiuqin Dong, Marcelo Ayala, Stefan Löfgren, and Per G. Söderberg

PURPOSE. To investigate the effect of age on ultraviolet radiation-B (UVR-B)-induced cataract and to detect the maximum acceptable dose in rats of different age groups.

METHODS. Four age groups of 20 rats each, aged 3, 6, 10, and 18 weeks, were included. Each age group was divided into five UVR-B dose subgroups. The rats were unilaterally exposed to UVR-B ($\lambda_{\max} = 302.6$ nm, $\lambda_{0.5} = 4.5$ nm). The incident dose on the cornea varied between 0 and 8 kJ/m². One week after exposure, the rats were killed, both lenses were extracted, the intensity of forward light-scattering was measured, and photographs were taken. The sensitivity of the lens to UVR-B was estimated as the maximum acceptable dose.

RESULTS. The maximum acceptable dose for 3-, 6-, 10-, and 18-week-old rats was estimated to be 1.4, 2.7, 4.3 and 5.2 kJ/m², respectively.

CONCLUSIONS. Young rats were more sensitive to UVR-B than old ones. Age should be considered when estimating the risk for UVR-B-induced cataract. (*Invest Ophthalmol Vis Sci.* 2003;44:1150–1154) DOI:10.1167/iovs.02-0541

In the present study, the impact of age on ultraviolet radiation-B (UVR-B)-induced cataract was investigated.

Sunlight is the principal source of ultraviolet radiation (UVR) for most of the world's population. Depletion of the stratospheric ozone increases the intensity of UVR. UVR is considered one of the major risk factors for cataract.^{1–4} Several studies have shown that sunlight increases the risk for cortical cataract.^{5–7} A correlation between cortical cataract and exposure to UVR was demonstrated in the Chesapeake Bay Study.⁸

Effects of UVR may be analyzed from different perspectives (e.g., at molecular, cellular, tissue, individual, population, and ecosystem levels).⁹ UVR damages the lens by disturbing cell proliferation in the lens epithelium,¹⁰ by altering kinetic properties of enzymes in the energy metabolism,¹¹ by increasing insoluble and decreasing soluble protein,^{12,13} by inducing unscheduled DNA synthesis,¹⁴ and by disturbing the sodium potassium balance and thereby the water balance in the lens.^{15,16}

One of the major difficulties in epidemiologic studies has been quantification of exposure to UVR from the sun. In addition to intensity of sunlight, the ocular dose depends on other factors, such as the amount of time spent outdoors, the

environment, the use of ocular protection, and the use of hats.^{3,4,17–19}

Ocular sensitivity versus wavelength^{20–23} and exposure time²⁴ for UVR-induced cataract have been studied experimentally in animals. In pigmented rabbits, Pitts et al.²² determined the threshold dose for UVR in the most toxic wavelength region, around 300 nm, to be 1.5 kJ/m² for transient and 5 kJ/m² for permanent lens damage. Deduction from animal data to the human situation is always questionable. However, it is the only option for development of empirically based safety recommendations for avoidance of cataract after exposure to UVR. With such knowledge, appropriate public health measures could be taken.²⁵

The public health significance of UVR-induced cataract is substantial. A possible thinning of the stratospheric ozone would probably translate into a large number of cataract cases worldwide. Safety limits for avoidance of UVR-induced cataract, established by animal models will help to develop appropriate public health measures.

Safety limits for UVR-B-induced cataract have been based on a dichotomous dose–response model, assuming that the outcome of UVR-B exposure is limited to a binary response: cataract/no cataract.²² In those studies, cataract was measured qualitatively by slit lamp, with a grading scale. However, it has recently been shown with quantitative measurements of cataract that UVR-B-induced cataract has a continuous dose–response function.²⁶ For this reason, a new concept, maximum acceptable dose (MAD) for avoidance of UVR-B cataract, was developed for estimation of UVR-B toxicity in the lens (Fig. 1).²⁷

Based on the dose–response function, MAD is defined as the dose corresponding to a limit for pathologic forward light-scattering. The limit for pathologic forward light-scattering is settled arbitrarily, based on the frequency distribution of light-scattering in normal unexposed lenses. The limit is defined so that a certain fraction (α), of normal unexposed lenses scatter light in the forward direction to an intensity above the limit. The magnitude of the fraction is a parameter that has to be settled and is given as an index to $MAD_{1-\alpha}$.

The purpose of the present study was to determine the dependence of MAD on age.

MATERIALS AND METHODS

The experimental animal was the albino Sprague-Dawley outbred rat. All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Animals were exposed to UVR-B in vivo, kept a week after exposure, and then killed for measurement of forward light-scattering.

Experimental Devices

The radiation from a high-pressure mercury lamp (HBO 200 W; Osram, Munich, Germany) was collimated, passed through a water filter and then a double monochromator ($\lambda_{\max} = 302.6$ nm, $\lambda_{0.5} = 4.5$ nm), and finally projected on the cornea of the exposed eye.²⁸ Irradiance was measured with a thermopile (model 7101; Oriol, Stratford, CT) in the corneal plane. The thermopile had been calibrated to a standard established by the U.S. National Bureau of Standards. The radiant expo-

From the St. Erik's Eye Hospital, Karolinska Institute, Stockholm, Sweden.

Supported by grants from the Crown Princess Margaretas Foundation (KMA), the China Scholarship Council, and Carmen & Bertil Regners Research Foundation.

Submitted for publication June 5, 2002; revised September 9 and September 18, 2002; accepted September 23, 2002.

Disclosure: X. Dong, None; M. Ayala, None; S. Löfgren, None; P.G. Söderberg, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Xiuqin Dong, Research Department, St. Erik's Eye Hospital, Karolinska Institutet, Polhemsgatan 50, S-112 82 Stockholm, Sweden; xiuqin.dong@ste.ki.se.

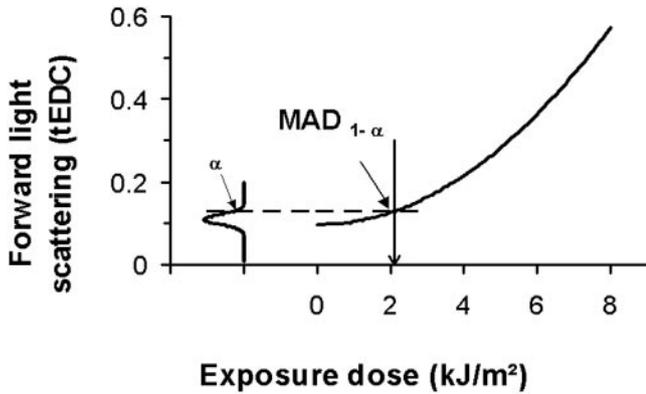


FIGURE 1. Maximum acceptable dose concept, according to Michael.²⁷ *Left function:* frequency distribution for nonexposed control lenses, where α (arrow) is the probability for a nonexposed control lenses to be classified as pathologic. *Dashed line:* limit between physiological and pathologic light-scattering. *Right function:* dose-response function. *Arrow:* Maximum acceptable dose ($MAD_{1-\alpha}$).

sure varied between 0.25 to 8 kJ/m² in the corneal plane, depending on the age group.

The amount of cataract was quantified as forward light-scattering. The intensity of forward light-scattering was measured with a light-dissemination meter.²⁹ This instrument uses the principle of dark-field illumination. The object to be measured is transilluminated from below at an angle of 45° against the horizontal plane of the object. Above the object, light is collected and focused on a photodiode. If the light impinging on the object to be measured at 45° is directly transmitted, the transmitted light is lost outside the collecting optic. If there is forward light-scattering in the object, a fraction of that light is focused on the photodiode and gives rise to a signal. The instrument was calibrated with a standard lipid emulsion of diazepam (Diazemuls; KabiVitrum, Stockholm, Sweden) and the primary unit of intensity of forward light-scattering was therefore expressed as transformed equivalent diazepam concentration (tEDC).²⁹

Experimental Procedure

Ten minutes preceding the exposure, the animals were anesthetized with a mixture of 95 mg/kg ketamine and 14 mg/kg xylazine, injected intraperitoneally, as recommended by Wixson et al.³⁰ Five minutes after the injection, mydriatic tropicamide was instilled in both eyes. After another 5 minutes, one eye of each animal was exposed to a narrow beam of UVR-B that cover only the cornea and the eyelids. The exposure time was 15 minutes.²⁴

One week after exposure, the animals were killed with an overdose of carbon dioxide. The eyes were enucleated, and both lenses were extracted and placed in balanced salt solution (BSS; Alcon, Ft. Worth, TX). Remnants of the ciliary body were removed from the lens equator under a microscope. The side (left or right) of the exposed eyes was masked to the measurement operator. Photographs were taken of each lens against a dark background with a white grid. Finally, the intensity of forward light-scattering was measured.

Experimental Design

The experimental design is given in Figure 2. Four groups of twenty rats aged 3, 6, 10, and 18 weeks were used. Each group was subdivided into five dose subgroups of four rats. The subgroups were assigned to 0, 2^{1/2}, 2⁰, 2¹, and 2² expected $MAD_{0.975}$. The expected $MAD_{0.975}$ was, based on a pilot experiment, 0.75, 1, 1.5, or 2 kJ/m² for 3-, 6-, 10-, and 18-week-old rats, respectively. The exposure time was 15 minutes. The intensity was 0 to 0.9 mW/cm² at the corneal level. In each rat, one eye was exposed to UVR-B while the contralateral eye was kept unexposed. Side exposed to UVR-B as well as radiant exposure received was alternated in a controlled fashion throughout the experiment to avoid

bias. The intensity of forward light-scattering was measured three times in each lens.

MAD Estimation

The intensity of forward light-scattering (y), as a function of UVR-B dose received (x) in all the rats within an age group were fitted to a second-order polynomial omitting the first order term as described by Michael et al.²⁷

$$y = a + kx^2 \tag{1}$$

MAD was estimated as outlined in the introduction (Fig. 1). The mean (μ) and the SD (σ) for normal nonexposed control lenses were estimated in each age group from the readings of the intensity of forward light-scattering for the contralateral nonexposed lenses. The limit between normal and pathologic light-scattering was then calculated from the standardized normal distribution setting the risk for wrong classification of a normal lens as pathologic to 2.5%

$$y_{\text{limit}} = \hat{\mu} + 1.96 \hat{\sigma} \tag{2}$$

Finally, the MAD (in this case, thus, $MAD_{0.975}$) was read on the dose-response function as the dose corresponding to the limit between pathologic and normal light-scattering.

RESULTS

One lens in the dose subgroup of rats receiving 0.25 kJ/m² in the 3-week age group was damaged when the remnants of the ciliary body was removed from the equator. This lens was excluded from the study.

Examination of backscattered light in the stereomicroscope demonstrated that 3-week-old rats, after receiving 2 kJ/m², exhibited development of more cataract than the other age groups after receiving similar doses (Fig. 3).

In the lenses exposed from the 3-week-old rats, the anterior pole appeared rough and hazy, and the lens equator showed dense opacities. In the lenses exposed from the 6- and 10-week-old rats, there were light opacities at the equator (Fig. 3, arrow). The backscattered light from the lenses from the 18-week-old rats was impossible to differentiate macroscopically from that of the lenses not exposed to UVR-B.

The images in Figure 3 also illustrate the growth of the lens with age. The distance between the white wires was 0.79 mm. The mean diameter of lenses from 3-week-old rats was 3.6 mm; from 6-week-old rats, 4.0 mm; from 10-week-old rats, 4.4 mm; and from 18-week-old rats, 4.6 mm.

The intensity of forward light-scattering measured in the different age groups after 2 kJ/m² UVR-B is given in Figure 4. The regression line for each age group was estimated based on equation 1. The inset in Figure 5 shows individual data and regression line for the 6-week-old group. The dose-response functions for UVR-B-induced cataract for the four age groups examined indicated that lenses in young rats had a higher level of light-scattering than old rats at the same dose of UVR-B (Fig. 5). Each curve in Figure 5 is the linear regression fit of the experimental data obtained by equation 1.

| Age groups | Dose subgroups | Rats | Measurement |
|------------|---|------|-------------|
| 3 weeks | — 0, 0.25, 0.75, 1, 2 kJ/m ² | 1 | 1 |
| 6 weeks | — 0, 0.5, 1, 2, 4 kJ/m ² | : | 2 |
| 10 weeks | — 0, 0.75, 1.5, 3, 6 kJ/m ² | : | 3 |
| 18 weeks | — 0, 1, 2, 4, 8 kJ/m ² | 20 | |

FIGURE 2. Experimental design.

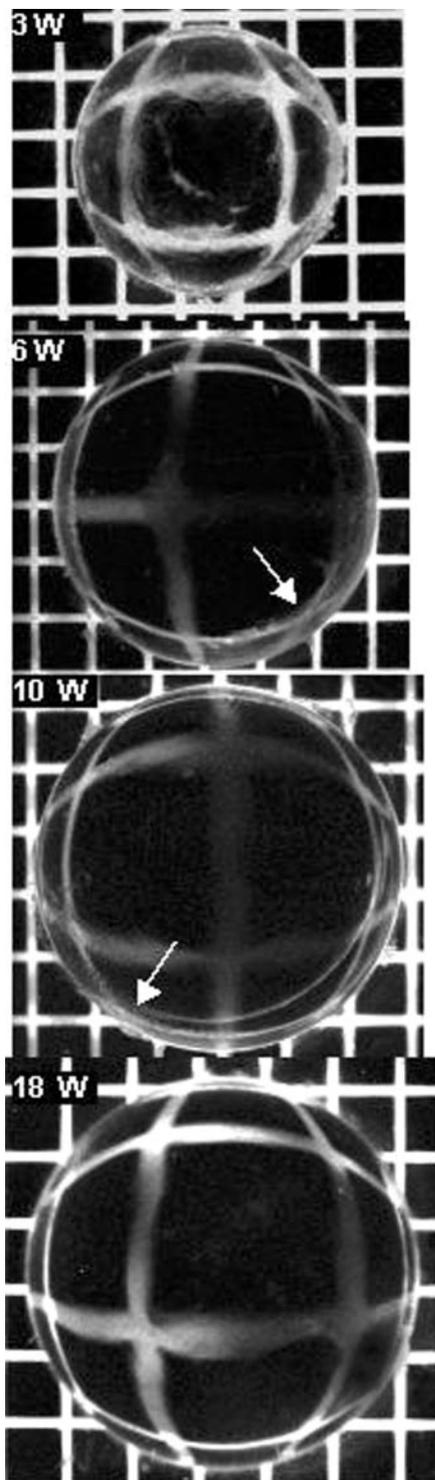


FIGURE 3. Backscattering of light from isolated rat lenses 1 week after in vivo exposure to UVR-B; 3, 6, and 18 weeks: 2 kJ/m²; 10 weeks: 1.5 kJ/m². (There was no 2 kJ/m²-exposure in the 10-week age group; the closest dose was 1.5 kJ/m².)

Because, for each age group the experimental error increased with increasing dose, the estimation of the regression line is valid but the estimation of the residual variance is mathematically invalid. For this reason, the regression line, but not its confidence intervals, is given in Figure 5.

The homogeneity of the variation of light-scattering for contralateral nonexposed lenses among age groups, was tested

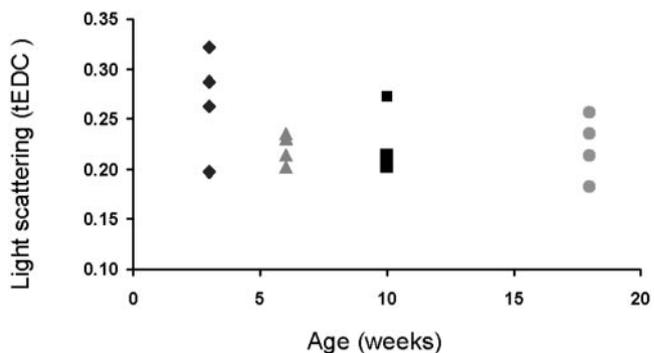


FIGURE 4. Light-scattering measurements for rats of different ages after 2 kJ/m² UVR-B: (◆) 3 weeks of age; (▲) 6 weeks of age; (■) 10 weeks of age; (●) 18 weeks of age.

with Bartlett's test. The result showed that there is a statistically significant difference among age groups ($P < 0.05$). Therefore, the frequency distribution for light-scattering in normal contralateral nonexposed lenses was estimated separately for each age group. MAD_{0.975} was estimated to be 1.4, 2.7, 4.3, and 5.2 kJ/m² for the 3-, 6-, 10- and 18-week-old rat groups, respectively (Fig. 6). It should be noted that MAD_{0.975} was almost four times lower for 3-week-old rats than for 18-week-old rats.

One week after exposure to UVR-B, corneal damage increased with increasing radiant exposure. Most rats exhibited corneal edema and opacities. However, 3-week-old rats had more severe corneal damage than older rats after the same radiant exposure. Anterior chamber hemorrhage (Fig. 7, arrowhead) was found after 2 kJ/m² UVR-B in the group of 3-week-old rats but not in any other age group.

DISCUSSION

In the current study, the age dependence of sensitivity of the ocular rat lens to in vivo exposure to UVR-B was determined. Sprague-Dawley rats were selected for this study because they are available at uniform size in large numbers and they provide a good model to gather primary empiric information that can serve as a basis for further studies. It should be pointed out, however, that the rat as an experimental model for damage from optical radiation has several limitations: It is nocturnal and the dimensions of the eye tissues are very different from

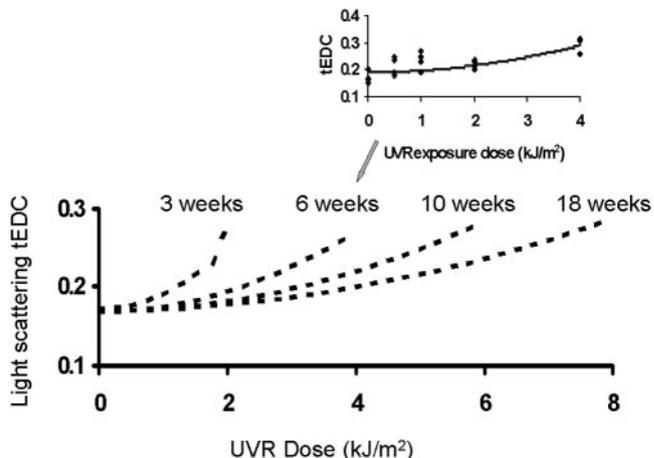


FIGURE 5. Dose-response functions for UVR-B-induced cataract as a function of rat age.

the human. The rat eye has a thinner cornea than the human eye, and therefore, due to higher transmittance is expected to have a higher sensitivity to UVR-B. Data from more species are required before the details of the age aspect can be conclusively settled and interpreted to human exposure.

Empiric data on toxicity of UVR-B in the human lens can be derived only from in vitro experiments on isolated human lenses. In vitro experiments, however, have the drawback of the lack of co-reactivity with surrounding tissues. Therefore, it is necessary to perform in vivo experiments in animals to derive an empiric foundation for safety standards. We believe that, in general, the significance of an examined aspect of safety increases if the aspect can be demonstrated in several species. In the current study, age was an important variable for prediction of UVR-B toxicity in the rat lens after a close-to-threshold dose.

When risk is estimated for damage to the eye from optical radiation, energy application (intensity, exposure time and exposure pattern) and spectral radiance are essential. The currently accepted strategy for risk estimation is based on adding the individual spectral components of a source while weighting each spectral component with its relative biological efficiency according to the action spectrum. In the present study, we chose to use UVR-B in the 300-nm wavelength region and exposure for 15 minutes, because the lens has a maximum sensitivity in this wave band^{22,23} and this exposure time.²⁴ However, we would like to point out that for the highest dose used in present experiment was 8 kJ/m^2 , for 15 minutes exposure, the intensity was 0.9 mW/cm^2 at the corneal level. This intensity was 10 to 100 times higher than the expected irradiation of the human cornea in sunlight.

The current safety limits estimation for UVR-B-induced cataract was based on slit lamp observations and assuming a binary dose-response model.²² MAD was introduced based on the objective observation and is based on the notion that UVR-B-induced cataract has a continuous dose-response model.²⁶ There are some limitations for estimation of MAD, because the mean and the SD for normal nonexposed control lenses were estimated from limited samples of contralateral nonexposed eyes ($n = 20$), the probability for wrong classification of a normal lens as pathologic is in the strict statistical sense not exactly known. However, the strategy for estimation of MAD is a practical objective method for estimation of UVR-B toxicity and provides useful information on the relative toxicity for the ages measured.

In the present study, $\text{MAD}_{0.975}$ was estimated to be 2.7 kJ/m^2 in 6-week-old rats. This closely agrees to the previous estimates of MAD in 6-week-old rats.²³

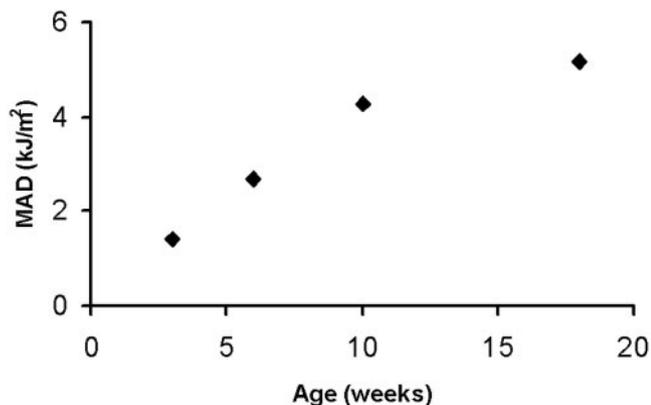


FIGURE 6. Age dependence of toxicity of in vivo UVR-B exposure in rat. Toxicity is given as maximum acceptable dose ($\text{MAD}_{0.975}$).

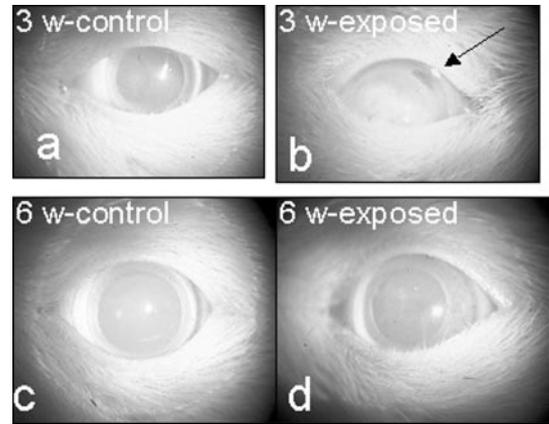


FIGURE 7. Rat anterior segment after exposure to UVR-B in the 300-nm wavelength region. (a) Three-week-old rat eye without exposure. (b) Three-week-old rat eye 1 week after exposure to 2 kJ/m^2 UVR-B. (c) Six-week-old rat without exposure. (d) Six-week-old rat 1 week after exposure to 2 kJ/m^2 UVR-B.

The higher sensitivity to UVR-B presently found in young individuals may be due to a thinner cornea that thus transmits more UVR-B. This is supported by measurements of the transmittance of the cornea at 300 nm in mouse (81%), rat (76%), rabbit (71%), and man (63%).³¹ Further, it was shown that UVR-B at 300 nm penetrates only approximately 0.5 mm in the lens (intensity = $1/e^2$ of original).³² It may be that the zone penetrated includes a biologically more important part of the young than of the old lens.

Michael et al.,³³ found that there is frequent occurrence of TUNEL-positive cells in the germinative zone after exposure to UVR-B in the 300-nm wavelength region. The high rate of cell division in the germinative zone in the young lens may render the young lens more sensitive to UVR-B-triggered DNA fragmentation. Further, the young lens requires more protein synthesis that includes a biologically more important part of the young than of the older lens.^{12,34} Sidney Lerman³⁵ exposed young (first decade) and old (seventh decade) normal human lenses to low level ($<0.1 \text{ kJ/cm}^2$) broad band UVR-B (300–400 nm) and found that the γ -crystallins were significantly affected by UVR-B in young lenses, whereas the aged lens proteins appeared to be relatively unaffected by this degree of UVR-B exposure.

Today, age is not considered in toxicity estimates for avoidance of cataract after exposure to UVR-B. The present finding that MAD for avoidance of UVR-B-induced cataract strongly depends on age (Fig. 6) implicates that, in the future, age should be considered in safety estimations for avoidance of cataract from UVR-B. We also feel that until better data are available, the current data should be considered in toxicity estimates for avoidance of UVR-B cataract after exposure to the sun as well as to artificial sources.

References

- Slaney DH. Eye protection techniques for bright light. *Ophthalmology*. 1983;90:937–944.
- Slaney DH. Physical factors in cataractogenesis: ambient ultraviolet radiation and temperature. *Invest Ophthalmol Vis Sci*. 1986;27:781–790.
- Slaney DH. UV radiation ocular exposure dosimetry. *J Photochem Photobiol B*. 1995;31:69–77.
- Rosenthal FS, Phoon C, Bakalian AE. The ocular dose of ultraviolet radiation to outdoor workers. *Invest Ophthalmol Vis Sci*. 1988;29:649–656.

5. Graziosi P, Rosmini F, Bonacin M, et al. Location and severity of cortical opacities in different regions of the lens in age-related cataract. *Invest Ophthalmol Vis Sci.* 1996;37:1698-1703.
6. West SK, Duncan DD, Munoz B, et al. Sunlight exposure and risk of lens opacities in a population-based study: The Salisbury Eye Evaluation Project. *JAMA.* 1998;280:714-718.
7. Schein OD, West S, Munoz B, et al. Cortical lenticular opacification: distribution and location in a longitudinal study. *Invest Ophthalmol Vis Sci.* 1994;35:363-366.
8. Taylor HR, West SK, Rosenthal FS, et al. Effect of ultraviolet radiation on cataract formation. *N Engl J Med.* 1988;319:1429-1433.
9. Meyer-Rochow VB. Risks, especially for the eye, emanating from the rise of solar UV-radiation in the Arctic and Antarctic regions. *Int J Circumpol Health.* 2000;59:38-51.
10. Li WC, Spector A. Lens epithelial cell apoptosis is an early event in the development of UVB-induced cataract. *Free Radic Biol Med.* 1996;20:301-311.
11. Andley UP, Song Z, Mitchell DL. DNA repair and survival in human lens epithelial cells with extended lifespan. *Curr Eye Res.* 1999;18:224-230.
12. Zigman S, Griess G, Yulo T, Schultz J. Ocular protein alterations by near UV light. *Exp Eye Res.* 1973;15:255-264.
13. Zigman S, Schultz J, Yulo T. Possible roles of near UV light in the cataractous process. *Exp Eye Res.* 1973;15:201-208.
14. Söderberg PG, Philipson BT, Lindström B. Unscheduled DNA synthesis in lens epithelium after in vivo exposure to UV radiation in the 300 nm wavelength region. *Acta Ophthalmol (Copenh).* 1986;64:162-168.
15. Söderberg PG. Na and K in the lens after exposure to radiation in the 300 nm wave length region. *J Photochem Photobiol B.* 1991;8:279-294.
16. Söderberg PG, Chen E. Determination of Na and K in the rat lens by atomic absorption spectrophotometry. *Acta Ophthalmol (Copenh).* 1989;67:582-592.
17. Sliney DH. UV radiation ocular exposure dosimetry [review]. *Doc Ophthalmol.* 1994;3-4:243-254.
18. Pitts DG. The ocular ultraviolet action spectrum and protection criteria. *Health Phys.* 1973;25:559-566.
19. Rosenthal FS, Safran M, Taylor HR. The ocular dose of ultraviolet radiation from sunlight exposure. *Photochem Photobiol.* 1985;42:163-171.
20. Verhoeff FH, Bell L, Walker CB. The pathological effects of radiant energy upon the eye. *Proc Am Acad Art Sci USA.* 1915/16;51:629-818.
21. Bachem A. Ophthalmic ultraviolet action spectra. *Am J Ophthalmol.* 1956;41:969-975.
22. Pitts DG, Cullen AP, Hacker PD. Ocular effects of ultraviolet radiation from 295 to 365 nm. *Invest Ophthalmol Vis Sci.* 1977;16:932-939.
23. Merriam J, Lofgren S, Michael R, et al. An Action Spectrum for UV-B Radiation and the Rat Lens. *Invest Ophthalmol Vis Sci.* 2000;41:2642-2647.
24. Ayala MN, Michael R, Söderberg PG. Influence of exposure time for UV radiation-induced cataract. *Invest Ophthalmol Vis Sci.* 2000;41:3539-3543.
25. McCarty CA, Taylor HR. Recent developments in vision research: light damage in cataract. *Invest Ophthalmol Vis Sci.* 1996;37:1720-1723.
26. Michael R, Söderberg PG, Chen E. Dose-response function for lens forward light scattering after in vivo exposure to ultraviolet radiation. *Graefes Arch Clin Exp Ophthalmol* 1998;236:625-629.
27. Michael R. Development and repair of cataract induced by ultraviolet radiation. *Ophthalmic Res.* 2000;32(suppl 1):1-44.
28. Söderberg PG. Experimental cataract induced by ultraviolet radiation. *Acta Ophthalmol (Copenh).* 1990;68:1-77.
29. Söderberg PG, Chen E. An objective and rapid method for the determination of light dissemination in the lens. *Acta Ophthalmol (Copenh).* 1990;68:44-52.
30. Wixson SK, White WJ, Hughes HC. A comparison of pentobarbital, fentanyl-droperidol, ketamine-diazepam anesthesia in adult male rats. *Lab Anim Sci.* 1987;37:726-730.
31. Dillon J, Zheng L, Merriam JC, Gaillard ER. The optical properties of the anterior segment of the eye: implications for cortical cataract. *Exp Eye Res.* 1999;68:785-795.
32. Löfgren S. Lens lactate dehydrogenase inactivation after UV-B irradiation: an in vivo measure of UVR-B penetration. *Invest Ophthalmol Vis Sci.* 2001;42:1833-1836.
33. Michael R, Vrensen G, van Marle J, Gan L, Söderberg PG. Apoptosis in the rat lens after in vivo threshold dose ultraviolet irradiation. *Invest Ophthalmol Vis Sci.* 1998;13:2681-2687.
34. Zigman S, Yulo T, Schultz J. Cataract induction in mice exposed to near UV light. *Ophthalmic Res.* 1974;6:259-270.
35. Lerman S, Megaw JM, Moran MN. Further studies of UV radiation on the human lens. *Ophthalmic Res.* 1985;17:354-364.