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Safety Limit Estimation for Cataract induced by Ultraviolet Radiation

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To My family

with love

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Preface

This thesis is based on research reported in the following studies, which are referred to in the text by their Roman numerals:

- I. Dong X, Ayala M, Löfgren S, Söderberg P. Ultraviolet radiation–induced cataract: age and maximum acceptable dose. *Invest Ophthalmol Vis Sci*. 2003;44:1150-4.
- II. Dong X, Löfgren S, Ayala M, Söderberg P. Maximum tolerable dose for avoidance of cataract induced by ultraviolet radiation–B for 18 to 60 week old rats. *Exp Eye Res*. 2005;80:561-6.
- III. Dong X. Age-dependent sensitivity to UVR-B: cataract development after in vitro UVR-B exposure. *manuscript*.
- IV. Dong X, Söderberg P, Ayala M, Löfgren S. The effect of exposure time on maximum acceptable dose for avoidance of ultraviolet radiation–induced cataract. *Ophthalmic Res*. 2005;37:197-201.
- V. Dong X, Löfgren S, Ayala M, Söderberg P. Maximum tolerable dose for avoidance of cataract after repeated exposure to ultraviolet radiation. *Submitted*.

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List Of Abbreviations

ARVO	The Association for Research and in Vision and Ophthalmology
ATP	Adenosine-tri-phosphate
CIE	Commission Internationale de l'Éclairage
ED ₅₀	Effective dose in 50% of exposed subjects
EDC	Equivalent Diazemuls Concentration
FWHM	Full width at half maximum
ICNIRP	International Commission for Non-Ionizing Radiation Protection
IR	Infrared radiation
MAD	Maximum acceptable dose
MTD	Maximum tolerable dose
ROS	Reactive oxygen species
SMHI	Swedish Meteorological and Hydrological Institute
tEDC	transformed Equivalent Diazemuls Concentration
UVI	Ultraviolet radiation Index (from 0 to > 11)
UVR	Ultraviolet radiation (wavelength in 100-400 nm)
UVR-A	Ultraviolet radiation type A (wavelength in 315-400 nm)
UVR-B	Ultraviolet radiation type B (wavelength in 280-315 nm)
UVR-C	Ultraviolet radiation type C (wavelength in 100-280 nm)

Summary

Background: Ultraviolet radiation (UVR) is considered one of the major risk factors for age-related cataract. UVR-induced cataract follows a continuous dose-response function. The concepts maximum acceptable dose (MAD) and its improvement, maximum tolerable dose (MTD), were developed for estimation of threshold dose for avoidance of UVR-induced cataract. The present report details the results of our research investigating the impact of age, exposure time, and interexposure interval for double exposures on threshold dose.

Methods: Lenses from female Sprague-Dawley outbred rats were exposed to UVR type B (UVR-B). The radiation from a high-pressure mercury arc lamp was collimated, passed through a water filter and a double monochromator ($\lambda_{\max} = 300 \text{ nm}$), and projected in vivo on the cornea or in vitro on the anterior surface of lens. One week after in vivo UVR-B exposure, extracted lenses were photographed and forward light scattering was measured. After in vitro UVR-B exposure, both exposed and nonexposed lenses were cultured. The intensity of forward light scattering was measured daily during week 1 and every second day during weeks 2 and 3. Morphologic changes were documented.

Results: The MAD for 3-week-old rats was almost 3 times lower than that for 18-week-old rats (study I). Rats aged 18 to 60 weeks had a stable MTD of approximately 5 kJ/m^2 (study II). The data from rats aged 3 to 60 weeks were used to derive a general expression for threshold dose (MTD) as a function of rat life span. It was shown that MTD increases with increasing age in the first third of the life and then remains stable for the remaining two thirds of the life span. The study of lenses exposed in vitro to UVR-B proved that the sensitivity difference between young and old rats does not depend on age-related anatomic differences (study III). With varying exposure times (7.5, 15, 30, 60, or 120 minutes), it was found that there was a lowest threshold dose at 15 minutes of UVR-B exposure (study IV). When varying intervals between 2 repeated exposures at a constant UVR-B dose, it was found that the 1 day interexposure interval was associated with a lower threshold dose than the interexposure intervals 6 hours, and 1, 3, 9, and 30 days (study V).

Key words: Ultraviolet radiation (UVR), Sprague-Dawley rat, cataract, light scattering, dose-response function, maximum acceptable dose (MAD), maximum tolerable dose (MTD), in vivo, in vitro, age.

1. Introduction

1.1 Ultraviolet Radiation

Ultraviolet radiation (UVR) refers to wavelengths from 1 to 400 nm. The waveband 1 to 100 nm is usually referred to as far UVR or vacuum UVR. According to the Commission Internationale de l'Éclairage (CIE), UVR in the waveband 100 to 400 nm can be divided into types A, B, and C (UVR-A, 315-400 nm; UVR-B, 280-315 nm; UVR-C, 100-280 nm). The visible light ranges from 400 to 760 nm.

The division of UVR across the optical spectrum is based roughly on its transmission in common media. Whereas UVR-C is efficiently blocked by glass and the atmosphere, UVR-B is transmitted by the atmosphere but is strongly blocked by glass; UVR-A is strongly transmitted by both glass and the atmosphere. In the living eye, the cornea has a maximum sensitivity for photokeratitis in the UVR-C waveband,¹ and the lens has a maximum sensitivity for cataract in the UVR-B waveband.^{2,3} The UVR-A waveband requires massive radiation exposure to produce damage to the cornea and lens of the eye. The visible light is efficient in exciting the visual pigments in the photoreceptors and is responsible for initiating vision.

1.1.1 Ozone and UVR

Natural radiation from the sun remains the most common source of UVR for most of the population. The spectral radiance of the sun depends on the sun temperature as described by the Planck radiation law. The sunlight humans receive is filtered by the atmosphere and consists of approximately 13% UVR, 44% visible light, and 43% infrared radiation (IR).⁴ Although IR causes the skin to feel hot during sunbathing, it is UVR that is responsible for the photochemical response to sunburn.

The thickness of the atmospheric ozone is closely related to the intensity of solar UVR-B on the earth. The ozone found in the earth's atmosphere is formed by an interaction between oxygen molecules (O_2), composed of 2 atoms of oxygen,

and UVR. When an oxygen molecule absorbs UVR, the oxygen molecule breaks apart into single atoms of oxygen (**Equation 1**)



These single atoms of oxygen are very reactive, and a single atom combines with a molecule of oxygen to form ozone (O_3), which is composed of 3 atoms of oxygen (**Equation 2**).



Although the ozone layer is spread out between 10 and 50 km in the stratosphere, it is only 3 mm thick when compressed to ground level pressure. It efficiently absorbs UVR to about 310 nm (**Figure 1**).

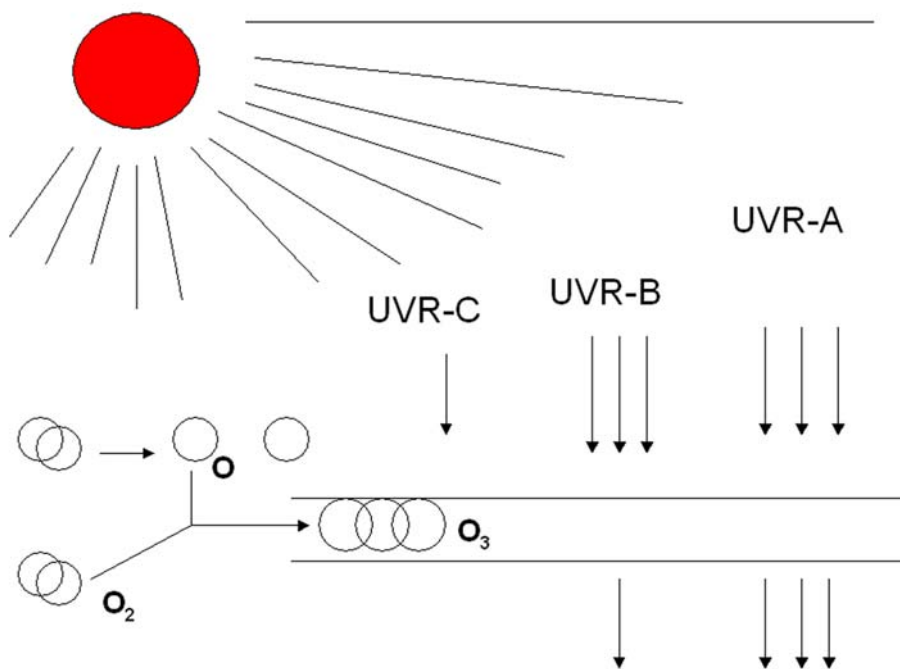


Figure 1. Formation and function of ozone.

However, approximately 10% UVR-B reaches ground level and may have deleterious effects on cells. UVR-A is less affected by the atmosphere. Therefore, UVR from the sun reaching the earth's surface is largely composed of UVR-A plus a small component of UVR-B.

The importance of the recent changes in the ozone layer and its effect on the UVR portion of the solar spectrum have been studied carefully over the past decade.⁵ A gradual decrease of stratospheric ozone has been measured in temperate and polar climate zones over the last two decades (e.g., 3%–6% per decade at mid-latitudes).^{6, 7} It was estimated that a sustained 1 % decrease in ozone, would result in between 100 000 and 150 000 additional cataract cases annually in the world.⁸

1.1.2 Earth surface UVR dose

The intensity of UVR reaching the surface of the earth depends on a variety of atmospheric factors, of which stratospheric ozone is the most important. Other atmospheric conditions such as boundary layer aerosol, clouds, and boundary layer ozone can also have a significant impact on the amount of UVR reaching the ground.

The length of the path of sunlight varies with time of day, season and latitude. Other factors such as air pollution, landscape and ground reflection also affect the UVR dose on the earth surface. Approximately 60% of effective UVR falls on the Earth between the hours of 10:00 AM and 2:00 PM. (**Figure 2**).

The intensity of UVR in a region on the surface of the earth can be described as UV index (UVI). A joint recommendation of the World Health Organization (WHO), World Meteorological Organization (WMO), United Nations Environment Programme (UNEP), International Commission on Non-Ionizing Radiation Protection (ICNIRP) defines UVI. The UV Index provides a daily forecast of the expected risk of overexposure to the sun. The index ranges from zero upward, where low indicates a minimal risk of overexposure and ≥ 11 means an extreme risk. The higher the UVI, the greater the potential for damage to the skin and eye, and the less time it takes for harm to occur. The interpretation of UVI is given in **Table 1**.

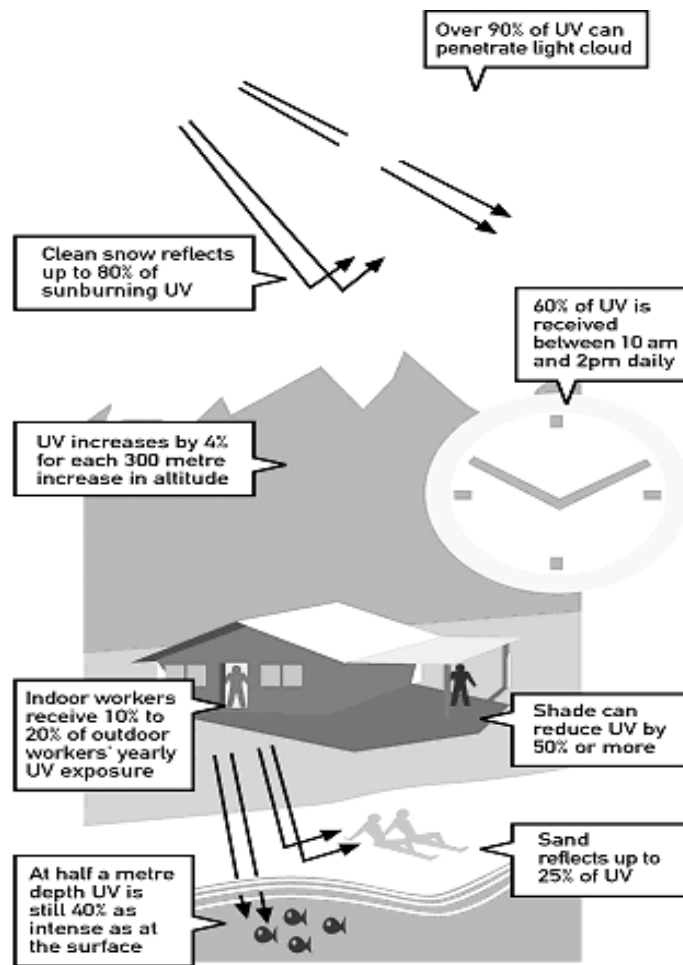


Figure 2. UVR on the earth.

Table 1. UV Index

UVI	Quantity of UVR	Need for protection	Safe exposure time
0–2	Low	No protection	Whole day
3–5	Moderate	Protection	1–2 hr
6–7	High	Protection	30–60 min
8–10	Very high	Extra protection	15–30 min
≥11	Extreme	Extra protection	5–15 min

As an example, according to the Swedish Meteorologic and Hydrological Institute (SMHI) and the Swedish Radiation Protection Authority (SSI), the UVI in Sweden is about 4 to 8 at noon during the summer, and about 0 to 2 at noon in winter.

1.1.3 Intraocular UVR dose

One of the major problems in epidemiologic studies on the relation between UVR and cataract is accurate estimation of ocular exposure to UVR. Many factors besides the intensity of UVR overhead affect intraocular UVR. It has been shown that ground reflections from freshly fallen snow can be as high as 88%, compared with only about 0.8% to 1.6% from green mountain grassland.⁹ The amount of time spent outdoors, as well as the use of hats and ocular protection, affects ocular exposure.¹⁰⁻¹³ Later studies have suggested that the degree of eyelid opening can have a significant effect on the amount of UVR reaching the eye.¹⁴

Corneal attenuation of UVR protects intraocular structures (e.g., the lens). A diagrammatic representation of UVR attenuation in the eye is shown in **Figure 3**.

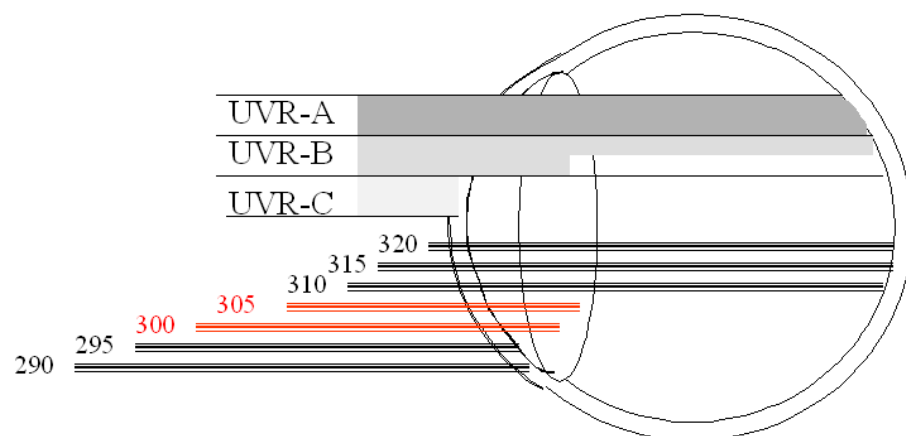


Figure 3. UVR transmission to the eye.

The corneal transmittance of UVR depends on wavelength. A 62-year-old human cornea transmits 0% UVR at 290 nm, 10% at 300 nm, and 63% at 380 nm.¹⁵ Increasing corneal transmittance is seen when comparing human (10%), rabbit (13%), rat (32%), and mouse (37%) samples at 300 nm,¹⁵ indicating that transmittance strongly depends on corneal thickness. There also is considerable variability among individuals,¹¹ and some reports indicate that transmittance of UVR decreases with age.^{16, 17} However, van den Berg,¹⁸ using a

mathematical model, argued that corneal transmittance of light (320–700 nm) is essentially age independent.

In the human lens, transmission of UVR is not only wavelength dependent but also is age dependent. Absorbance of UVR in the lens increases with age.^{15, 16, 19, 20} The young lens attenuates UVR primarily between 300 and 400 nm, but it exhibits a small window of transmission centered at 320 nm. The aged lens absorbs UVR throughout the entire spectrum and also in the visible region to at least 550 nm.¹⁵ The human lens attenuates almost all the UVR-B that passes the cornea and effectively attenuates UVR-A. Only about $\leq 1\%$ of UVR-A reaches the retina.¹⁶ UVR-A and UVR-B are therefore potentially harmful to the lens.

1.2 Lens and Cataract

1.2.1 Lens anatomy and physiology

The adult human lens is a biconvex, asymmetric disc with a larger radius of curvature on the anterior surface. Structurally the lens is divided into a lens capsule, a subcapsular epithelium and fiber cells (**Figure 4**).

The lens capsule is an acellular elastic structure. The principal component of the capsule is type IV collagen. The capsule is freely permeable to low-molecular-weight compounds, but it restricts the movement of larger colloidal material.

The epithelium is a single-layer cuboidal epithelium. It can be functionally divided into 3 zones: the central zone, the pre-equatorial/germinative zone, and the equatorial/transitional zone. The central zone of epithelium is usually nonproliferating. The cells located in the germinative zone proliferate and elongate. When the cells reach the transitional zone, they start to produce new cell membrane.

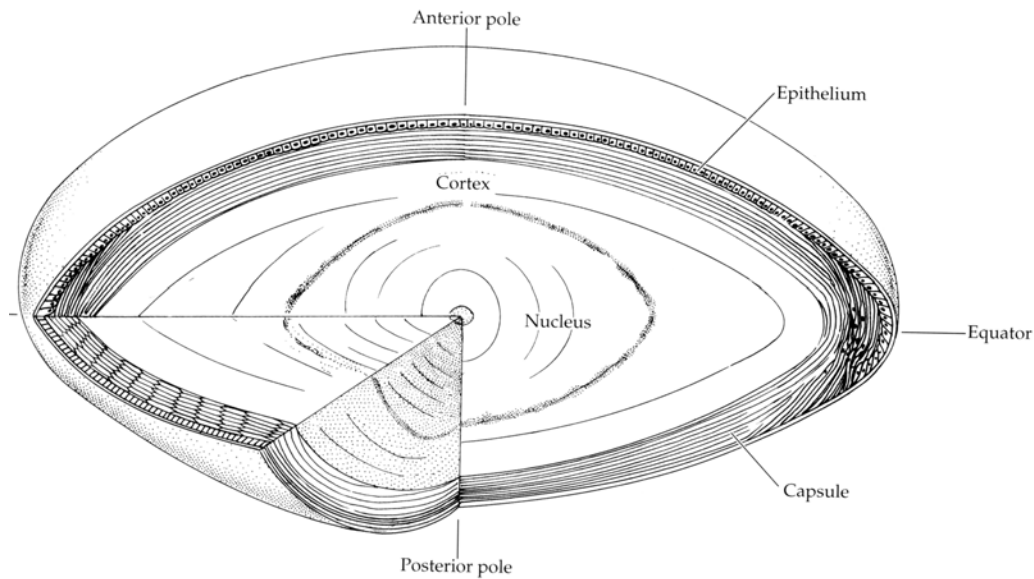


Figure 4. The human lens. (Reprinted with permission from Sinauer Associates, Inc.)

The lens fiber cells elongate from the equator, with one end moving towards the anterior pole and the other end moving towards the posterior pole. The ends of fiber cells in the anterior and posterior portion of the lens form the lens sutures.²¹

The lens, along with the cornea, is responsible for bringing light rays into precise focus on the retina. There are no blood vessels or nerves present within the lens, and the lens gets nutrition from the aqueous humour. The epithelial cells are responsible for the majority of active transport of ions, amino acids and facilitated diffusion of glucose.²²

The metabolism of the lens is essential for maintenance of transparency. The energy source in the lens is glucose. The lens generates 70% of its ATP through anaerobic glycolysis.^{23, 24} Anaerobic glycolysis generates 2 moles of ATP from 1 mole of glucose. The advantage of anaerobic glycolysis is independence of oxygen which would be highly toxic in an ambient continuously exposed to high energy optical radiation. ATP production via the citric acid cycle in the lens only takes place in the epithelium. Aerobic metabolism creates 38 mol of ATP per 1 mol of glucose; 20% of ATP needed in the lens is generated by aerobic metabolism.

To maintain lens transparency, control of lens hydration is critical. The human lens consists of about 65% water. The normal lens maintains a high content of potassium and a low content of sodium actively with $\text{Na}^+ - \text{K}^+$ ATPase while consuming ATP. These concentration gradients maintain iso-osmolarity between each lens cell and the extracellular space and between the lens and the aqueous humour. However, diffusion of sodium and chloride into the lens and potassium out of the lens continuously strive to decrease the concentration gradients with the net effect that particle concentration and water increases intracellularly²¹. Localized swelling causes a local disturbance of refractive index.

More than one third of the wet weight in the lens is protein. The majority of the water-insoluble proteins are membrane proteins or cytoskeletal proteins. It is the water-soluble lens proteins, the crystallins, that account for almost 90% of total lens protein content.²⁵ Crystallins can be divided into 3 groups (α , β , and γ) according to molecular weight. The α -crystallins are responsible for 85% of light scattering.²⁶ They perform a “chaperone” function, protecting the β - and γ -crystallins.²¹ β -Crystallin is a stabilizing lens protein, whereas the γ -crystallins are predominant in the nuclear region of the lens. In this region, a steep refractive index gradient and a relatively low water content are found.²⁵

1.2.2 The aging lens

There are at least 2 types of biological systems related to senescence. In an open system, aged cells are replaced by division or are refreshed by component renewal. In a closed system, the cell systems or tissues are retained throughout life and have limited capacity for repair; once a fault or damage occurs, there is little opportunity of ridding it from the system. Thus, aging in a closed system represent a lifelong accumulation of error. The aging in the lens is of this type.

The lens is unique among organelles in that it consists solely of a single cell type in varying stages of cytodifferentiation and retains within it all the cells formed during its lifetime.²⁷ The oldest cells are contained within the nucleus of the lens and new cells are added superficially throughout life. As the lens cell becomes older and more embedded within the lens, it loses its organelles and nuclei and becomes more metabolically inert. Lens proteins change as they age. The insoluble fraction rises throughout life in the normal lens (**Figure 5-A**). Proteins

tend to aggregate and become insoluble, and if aggregation is sufficiently extensive, it will be manifest as a decrease in transparency, i.e., cataract (**Figure 5-B**).

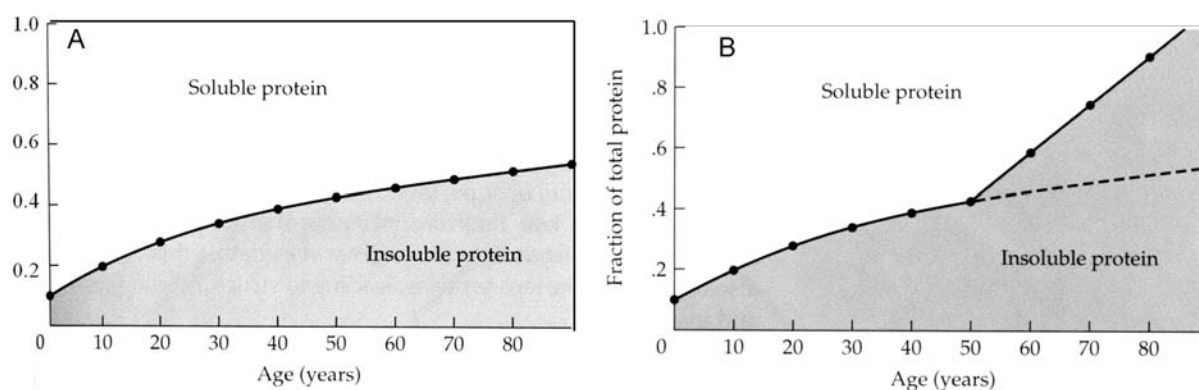


Figure 5. (A) In the normal lens, the insoluble fraction of lens protein increases gradually with age. **(B)** In the cataractous lens, the fraction of insoluble protein increases more rapidly than in normal lenses. (Reprinted with permission from Sinauer Associates, Inc.)

With age, the ability of the lens to transmit short-wavelength radiation decreases. The lens nucleus becomes increasingly yellow to brown with increasing age. The yellow or brown color in the lens is due to blue light absorption that reduces the intensity of blue light on the retina.²⁸ The increasing amount of blue light absorbing pigment in the lens nucleus probably serves a functional role of protecting the retina from the toxic effects of blue light.^{29, 30} Chemically, these blue light-absorbing pigments are thought to be photodegradation products of molecules that absorb UVR-A. For example, tryptophan absorbs UVR-A and becomes degraded through N-formyl kynurenine into higher oxidative states of kynurenine.^{31, 32}

Unfortunately, the lenses of common experimental animals (rabbit, rat, mice, pig, and cow) used for studies of UVR-induced cataract lack the blue light-absorbing pigment. However, the primary absorption of UVR in the lens and its conversion into photochemical modification is the same for all species. In the present thesis, the effect of aging on sensitivity, expressed as inverse threshold dose, for cataract development after exposure to a near-threshold dose of UVR was studied in the albino rat (studies I, II, and III). No other information is available on age sensitivity for UVR-induced cataract. The present results

should therefore be considered when developing safety standards. However, the potential modulation of blue light-absorbing pigment in the lens of other species, including humans, requires further investigation.

1.2.3 Definition of Cataract and classification

Water and protein are major constituents of the lens. In the normal lens, the protein is arranged to transmit light with minimal light scattering. Under certain circumstances, the protein may aggregate and cause light scattering. Light scattering in the lens interferes with vision by decreasing contrast on the retina. Cataract is defined as an opacity in the normally transparent crystalline lens of the eye that impairs normal light transmittance through the lens and may or may not produce an impairment of vision in humans.³³

Cataract is a major cause of visual impairment and blindness. Age related cataract is the leading cause of blindness in the world today. Etiologically, cataract can be divided into age-related, drug-induced, traumatic, metabolic, or congenital cataract as well as cataract associated with nutritional effects, with uveitis, or with other systemic diseases.³⁴

1.2.4 Age-related Cataract

Age-related cataract is the most common type. Age related cataract may be classified based on slit lamp microscope observation as *cortical*, *nuclear* or *subcapsular*. Many patients have mixed types.

There are several methods that were developed for clinical cataract classification. The most recent are the Lens Opacities Classification System I, II and III (LOCS I, II and III),³⁵⁻³⁷ the Cooperative Cataract Research Epidemiology Study Group method (CCESG),³⁸ the Oxford Clinical Cataract Classification,³⁹ and A simplified cataract grading system.⁴⁰

The etiology of age-related cataract is multifactorial. Increased relative risk for cataract is associated with increased exposure to UVR, increasing age, diabetes, renal failure, severe diarrhea, heavy smoking, hypertension, high alcohol consumption, excessive heat, and malnutrition.⁴¹⁻⁴⁵

It was estimated that 7 million cases of cataract are operated on each year and 16 million are left without operation.⁴⁶ The number of cataract surgery is likely to double in the next 20 years due to the aging of the population.⁴⁷

Unfortunately, despite the advances of modern medicine, the only way to treat cataract is through surgery. Cataract surgery has been performed for 2500 years, beginning with the fifth century B.C., with continuous development in available techniques. In couching, the earliest described cataract surgical technique, the cataractous lens is displaced away from the pupil to the vitreous cavity in the back of the eye. In the 18th and 19th centuries, surgical approaches evolved to include extracapsular (ECCE) and intracapsular (ICCE) cataract extraction. Just 50 years ago, Ridley initiated an important development in modern cataract lens replacement by implanting the first polymethylmetacrylate intraocular lenses (IOL).⁴⁸ In recent decades, a safe and easy-to-use technique to restore normal vision at the time of surgery has been developed—Ultrasonic phacoemulsification (Phaco) cataract extraction combined with IOL implantation.

In the United States, high-quality surgery has been readily available since 1991, but at an annual cost exceeding US\$3.4 billion.⁴⁹ McCarty⁵⁰ indicates that the prevalence of cataract operation will double over the next year in Australia. The demand for cataract surgery could potentially be decreased through implementation of effective primary prevention strategies, although no successful strategies are currently known. It has been estimated that a delay in onset of only 10 years could reduce the need for cataract surgery by as much as 50%.⁵¹

To delay or decrease the incidence of cataract, it is necessary to understand the mechanisms of cataract development and the risk factors associated with the development of cataract. A significant association between sunlight (especially UVR-B) and cortical cataract has been reported.^{10, 52-56} Because there is near-universal exposure to UVR-B, the proportion of cataract in the community that is potentially due to UVR-B is relatively high.⁵⁰

1.3 Photobiology and photochemistry

Photobiology is the study of how electromagnetic radiation in the 100-1000 nm wavelength region interacts with living matter. There are 3 types of photobiological damage mechanisms.⁵⁷ In photochemical damage, photon energy is transformed into chemical reactions in biomolecules. In thermal damage, the photon energy causes molecular vibration and loss of biochemical structure and function known as denaturation. In mechanical damage the photon energy is transformed into fast thermal expansion and ablative explosion, ablation, or ionization and secondarily causes disruptive cavitation, photodisruption. UVR at low intensity causes photochemical damage in lens.

Photochemically induced cell damage may occur by a direct phototoxic reaction, in which the absorbed photon energy produces a toxic molecule. Alternatively, the absorbed energy may excite a primary energy-transmitting molecule—a photosensitizer—that secondarily releases its energy to a final target molecule, which then becomes toxic. This is known as a photosensitized reaction. The sensitizer acts via 2 principle pathways. In the type I, or radical, pathway a radiation-excited sensitizer directly or indirectly interacts with the target molecule. In the type II, or singlet oxygen pathway, the sensitizer interacts with oxygen in the tissue through formation of reactive oxygen species (ROS). The resulting photochemical reaction may lead to conformational change resulting in inactivation of an enzyme, structural protein aggregation, or membrane damage. The epithelial cell damage induced by UVR-B initiates local variations in the refractive index, thereby causing increased light scattering in the lens.

There are several anti-oxidative molecules in the eye such as the tripeptide glutathione (GSH), water-soluble ascorbic acid (Vitamin C) in the aqueous humor,^{58, 59} and vitamin E in membranes.^{60, 61} Together, the antioxidant molecules and enzymes function as a protective system.

The UVR-induced photobiologic response in the lens is often modified by complex biologic mechanisms. At the same dose of UVR exposure, a photochemical reaction should follow the Bunsen-Roscoe law of reciprocity, where the photochemical effect is determined by the total dose of radiation. However, if repair occurs during damage induction, the law of reciprocity may not hold for the biologic response. To judge the significance of biologic

modulation of the primary photochemical event in UVR-induced cataract, the impact of exposure time on threshold dose (study IV) and the impact of interexposure interval on threshold dose for repeated exposures (study V) were investigated.

1.4 UVR-induced cataract

It has long been observed that the prevalence of cataract and blindness due to cataract varies widely by geographic location.^{55, 62, 63} A dose-response relation between UVR-B exposure and cataract has been reported in epidemiologic as well as experimental studies.^{52, 64-67} The greater the number of important explanatory variables controlled for in epidemiologic studies, the more efficient those studies will be. Information on relevance of explanatory variables is usually easier to gather in experimental studies.

1.4.1 Epidemiological studies

In a cross-sectional study of Chesapeake Bay watermen, the risk for cortical opacity was found to increase with increasing dose of solar UVR.⁵² The association of UVR exposure and increased risk for cortical opacities was confirmed for the male population in the Beaver Dam Eye Study.⁶⁴ Epidemiologic studies indicate that UVR-B is a significant factor in development of cortical cataract.⁶⁸⁻⁷⁰

Recent research indicates that both cumulative lifetime UVR exposure and exposure after the teenage years specifically are correlated with the presence of nuclear opacities.^{71, 72} Excessive exposure to UVR in childhood may contribute to age-related nuclear cataract, because the cortical lens fibers move to the center of the nucleus with the growth of the lens.

The advantage of epidemiologic studies is that data can be gathered from humans and directly applied to humans. There are, however, a number of factors that are difficult to quantify or that cannot be elucidated with epidemiology, such as the action spectrum, primary photobiology of the damage, and impact of genetic constitution. Therefore, experimental studies are needed.

1.4.2 Experimental studies

Experimental research on UVR-induced cataract had already begun by the end of the 19th century. In one of the first experiments, Widmark⁷³ exposed a pig lens placed on his arm to UVR from a carbon arc lamp. His skin was severely damaged except where the pig lens had been. He correctly hypothesized that UVR was absorbed by the lens.

Pitts et al.² investigated the UVR action spectrum for cataract development in the rabbit within 48 hours after a high-dose exposure. Using qualitative observations of cataract with better spectral resolution than had been employed previously,⁷⁴ these investigators confirmed that the wavelength region around 300 nm is the most cataractogenic zone.² This was later confirmed by Merriam et al.,³ who measured cataract quantitatively. However, it may be premature to exclude UVR-A or even short-wavelength visible light in the etiology of human cataract.⁷⁵ It has been reported that guinea pig exposed in vivo to chronic, low-level UVR-A (340–410 nm) can develop nuclear cataract.⁷⁶

Extensive research has been carried out to elucidate the mechanisms of UVR-induced cataract both in the lens epithelium and in the lens fibers. Multiple mechanisms have been proposed. The finding of unscheduled DNA synthesis after in vivo exposure to UVR in the 300 nm wavelength region⁷⁷⁻⁷⁹ was suggested to be indirect evidence for DNA-damage due to the exposure.

UVR was found to damage cell membrane pumps, thereby allowing influx of calcium into lens epithelial cells, and increasing the concentrations of sodium while decreasing the concentrations of potassium.⁸⁰⁻⁸³ UVR was also shown to inactivate a number of metabolic enzymes^{84, 85} and to disrupt the crystalline's tertiary structure.⁸⁶ In the fiber cells, it has been observed that UVR leads to degradation and modification of lens crystallins.^{87, 88} It was further shown that lens epithelial cells damaged by in vivo exposure to UVR may become eliminated through apoptosis.⁸⁹ A summary of the mechanisms of cataract induced by UVR is shown in **Figure 6**.

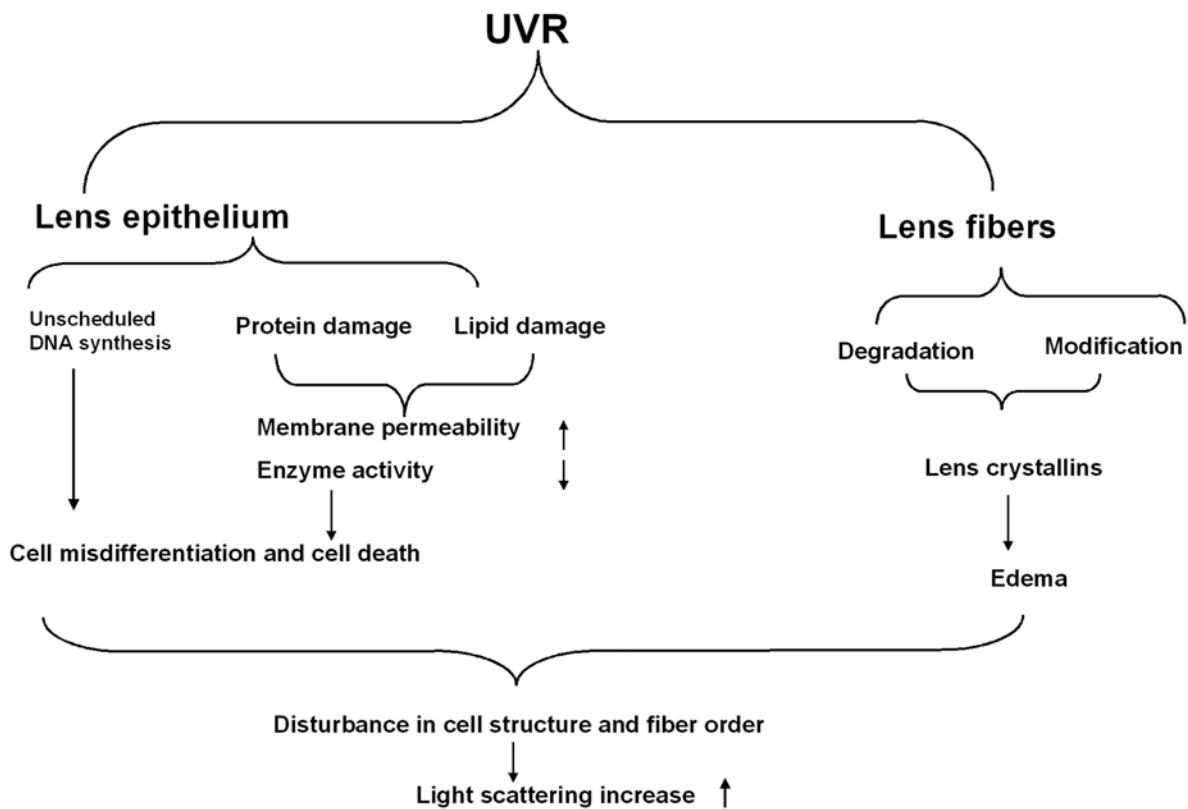


Figure 6. Mechanisms by which UVR induces cataract.

In order to quantify cataract, Söderberg developed an objective method for measurement of the overall intensity of forward light scattering in the lens.⁹⁰ This method ignores the location of the cataract. Applying this method, it was demonstrated that the intensity of light scattering in the lens after in vivo exposure to UVR in the 300-nm wavelength region increases with an exponential decline to asymptote at a time constant of about 20 hours.⁹¹ It was later shown that there is no further increase of light scattering by 1 week after exposure.⁹²

The quantitative method for measurement of overall intensity forward light scattering in the lens⁹⁰ further allowed investigators to demonstrate that in 6-week-old albino rats, cataract induced by UVR in the 300-nm wavelength region fits a continuous dose-response function.⁶⁶ Löfgren et al.⁹³ showed that young rats are more sensitive to UVR-B than old rats and that there is no difference in sensitivity to UVR with regard to sex. It was shown, by varying the exposure

time at an equivalent in vivo dose of UVR, that exposures around 15 minutes provoke more light scattering than shorter or longer exposures.⁹⁴ Ayala et al.⁹⁵ also fractionated the in vivo exposure into 2 equivalent exposures and studied the impact of the time interval between the 2 exposures on the intensity of forward light scattering. They found that there is a maximum intensity of forward light scattering expressed when the interval between the 2 exposures is on the order of 72 hours.

1.5 Dose-response functions and threshold dose

A dose response function expresses the functional causal relationship between a quantity of an agent and the quantity of response as a result of that agent. A response may be binary, multinomial or continuous in character. Due to biological and measurement variation, there is always a random variation of response to a given dose.

Often, there is a need to express a *significant response*. If the dose-response function is binary, the response is defined only as “event” or “no event” without any grading.⁹⁶ However, the probability for an event after a particular dose varies. Therefore, for binary dose-response functions, significant response has to be expressed as the probability of an event.⁹⁶ If the dose-response function is continuous, the significant response has to be defined on an objective and meaningful basis. It may be argued that it is illogical to attempt to define a threshold if there is a continuous dose-response function. However, for construction of safety standards, it is necessary to define thresholds for such functions.

The *threshold dose* refers to the quantity of an agent, below which the significant response does not occur and above which the significant response always occurs.⁹⁶ Radiation thresholds are generally derived for limited acute (short-term) effects. Applied to radiation, the threshold dose is the dose below which there is no significant effect of the radiation on the biological response of interest. For a binary significant response, the threshold dose is the dose below which there is an insignificant risk that the adverse effect will occur.⁹⁶ However a priority should also be placed on consideration of a continuous dose-response relation in radiation damage.

1.5.1 Threshold estimation for binary dose response functions

A classical example of binary toxic dose response is death or survival after exposure to a toxic agent. Due to biological variability in sensitivity, the dose required to evoke the binary effect varies slightly from one individual to another. For this reason, there is no universal threshold dose that holds for all individuals. If a large sample of individuals is exposed to a low dose of the toxic agent, a low percentage of the individuals will develop the toxic reaction. If the dose is increased a higher percentage of individuals will develop the toxic reaction.

Based on this observation, Finney⁹⁶ developed statistical methods to experimentally estimate the dose when an arbitrary % of all individuals express the toxic response. The dose at which 50% of the sample expresses the damage has become a standard for expression of toxicity for binary events and is known as the effective dose 50 (ED_{50}). The uncertainty of the ED_{50} estimation is expressed as a confidence interval for the population ED_{50} .

1.5.2 Threshold estimation for continuous dose-response functions

A typical example of a continuous toxic dose response function is loss of coordination due to alcohol consumption when loss of coordination is measured on a continuous scale.

One strategy is to choose a limit for normality that objectively sets a limit on the adverse response variable, considering normal variation among individuals who have not been exposed to the toxic agent. Thereafter, the dose corresponding to the limit can be evaluated based on the dose-response function.^{97, 98} The uncertainty of the dose estimation can be expressed as the confidence interval for the population threshold dose.

1.6 Safety limit estimation for cataract induced by UVR

The current threshold doses for UVR induced cataract, defined by the International Commission for Non-Ionizing Radiation Protection (ICNIRP), is based on the total literature on experimental and clinical experience of UVR-induced cataract. The critical explanatory variable is wavelength.

In the fundamental experiment for avoidance of cataract, Pitts et al.² derived the action spectrum. These authors exposed pigmented rabbits eyes to UVR in vivo, observed the eyes in a slit-lamp microscope, and graded the appearance observed. If there was no change within 48 hours, the eye received a higher dose. The highest dose associated with no damage or only limited lens damage was considered the threshold dose. Only one animal was used for each threshold estimation. In the pigmented rabbit, the estimated threshold dose was determined to be 1.5 kJ /m² for transient cataract at 300 nm and 5 kJ /m² for permanent cataract. This experiment thus applied a simplified design for estimation of a binary dose-response.

It was found that UVR-induced cataract follows a continuous dose response function by quantitative measurement of cataract.⁶⁶ Therefore, new strategies for estimation of threshold dose for continuous dose-response functions were developed.^{97, 98}

1.6.1 Maximum acceptable dose (MAD)

The concept of maximal acceptable dose (MAD) was developed by Söderberg et al.⁹⁸ based on the principle that there is a continuous dose-response function for UVR-induced cataract.

Clinically normal lenses scatter a baseline amount of light. The level of light scattering in clinically normal lenses varies from individual to individual according to a normal distribution.⁷⁸ The mean, μ , and standard deviation, σ , for intensity of forward light scattering in clinically normal lenses may be estimated with insignificant error if the sample size is large enough. Then, a tolerance limit for normality, I_{Limit} , may be calculated based on the standardized normal distribution, Z , (**Equation 3**).⁹⁹

$$\text{Equation 3} \quad I_{Limit} = \sigma Z(\alpha) + \mu$$

Here, σ and μ , respectively, are the standard deviation and the mean of the population light scattering in normal lenses. The parameter α is the probability that an observation of the stochastic variable of light scattering in normal lenses exceeds the limit. Thus the tolerance for normality is $1 - \alpha$. The magnitude of α for a nonexposed normal lens to be wrongly classified as pathologic is arbitrarily

chosen. If a high probability is chosen, there is a large risk of misclassification; if a low probability is chosen, there is small risk of incorrect classification.

In studies I and IV, the value for α was set to 2.5%. In other words, there is 2.5% risk of misclassifying a clinically normal lens as pathologic, the tolerance for normality being 97.5%. Thus, the MAD is described as $MAD_{0.975}$, with the subscript 0.975 determined by the result of $1 - \alpha$.

With quantitative measurement, the intensity of forward light scattering in a lens continuously increases with a higher dose.⁶⁶ As we intend to set the limit where the induced light scattering is significant compared with light scattering seen in nonexposed lenses, only the lower dose interval is relevant (**Figure 7**).

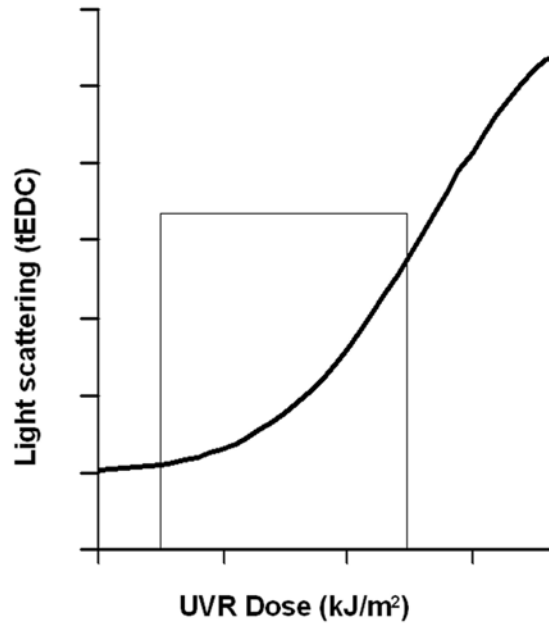


Figure 7. Relevant range of the dose-response function for UVR-induced light scattering in a lens, which is used for estimation of the toxicity threshold for UVR.

The low-dose region of the dose-response function for UVR induced light scattering can be approximated to a second order polynomial, omitting the first order term (**Equation 4**).

$$\text{Equation 4} \quad I = I_0 + kH_e^2 + \varepsilon$$

Here, I (tEDC) is UVR induced light scattering (y was used in study I), I_0 (tEDC) is the baseline light scattering at dose 0 (a was used in study I), k (tEDC) $[\text{kJ/m}^2]^{-2}$ is the increased rate of the dose-response function, H_e (kJ/m^2) is the UVR dose (x was used in study I), and ε is a random error due to biologic variation and measurement error.

There is a limit for the intensity of light scattering that corresponds to the selected tolerance for normality, $1 - \alpha$, (I_{limit} , Equation 3). If this limit is projected on the dose-response function, the dose of UVR that on an average induces an equivalent amount of light scattering ($I = I_{\text{limit}}$) can be estimated. This dose is the MAD, in this case $\text{MAD}_{1-\alpha}$ (**Figure 8**).⁹⁸

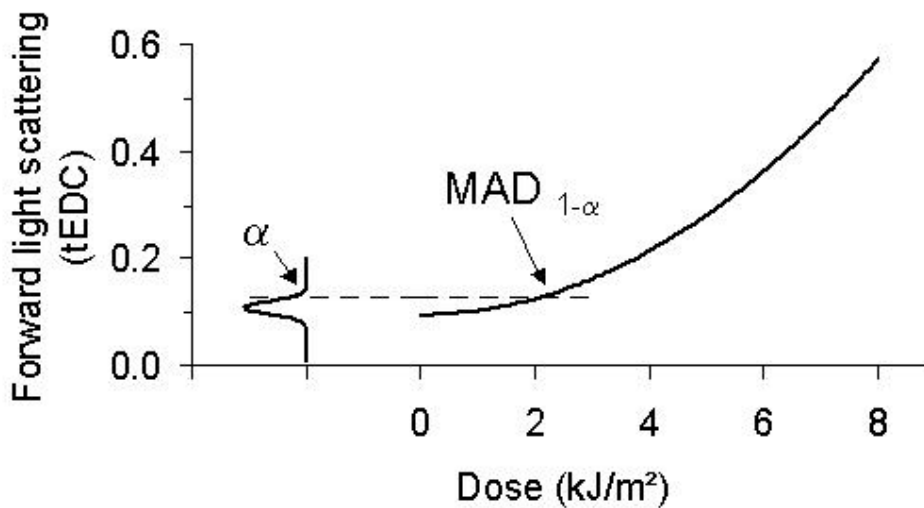


Figure 8. Maximum acceptable dose (MAD).⁹⁸ (Left) The frequency distribution for light scattering in nonexposed control lenses is shown. α = probability for the nonexposed control lens to wrongly be classified as pathologic although it is normal (short arrow). (Right) The population mean dose-response function. The projected arrow on the dose axis indicates $\text{MAD}_{1-\alpha}$. The dashed line indicates the limit between physiologic and pathologic light scattering. (Reprinted with permission from *Acta Ophthalmol Scand*.⁹⁸)

A disadvantage of MAD is that in order to derive the tolerance limit for normality, a large sample is needed to obtain sufficiently good estimates of the mean and standard deviation of light scattering in clinically normal lenses. In experimental research, normally only limited samples are available. Further, the dose that on average corresponds to the tolerance for normality does not have a direct public health meaning for the individual exposed.

1.6.2 Maximum Tolerable Dose (MTD)

The Maximum Tolerable Dose (MTD)⁹⁸ was developed to overcome the disadvantages of MAD.

In the MTD concept, the difference in light scattering between a pair of an exposed and a contralateral nonexposed lens is the primary observation. The population dose-response function (**Figure 9**) is estimated by running a small sample experiment including observations at dose 0.

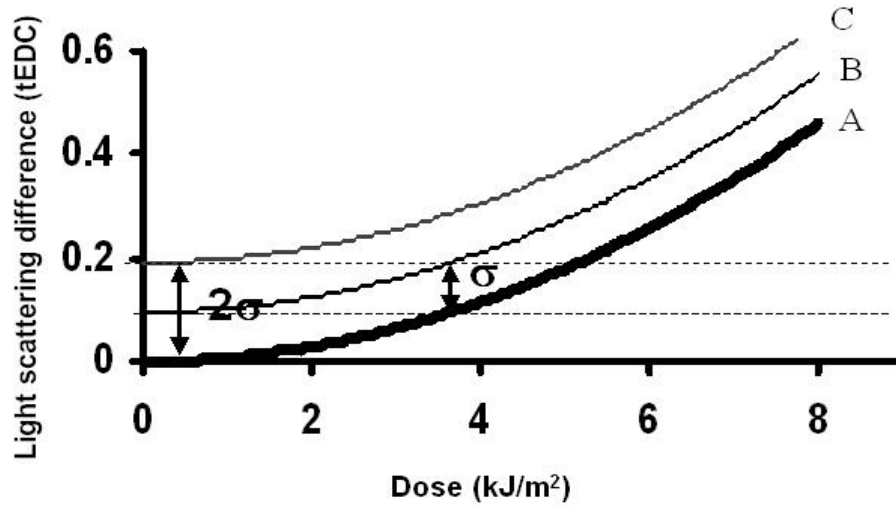


Figure 9. Difference of light scattering between exposed and contralateral nonexposed lens as a function of UVR dose. σ is the standard deviation for difference of light scattering between exposed and contralateral nonexposed lens as a function of dose in the population of pairs of lenses from unilaterally UVR exposed rats. *Line A* depicts the population mean difference of light scattering between a paired exposed and contralateral not-exposed lenses as a function of dose. *Line B* depicts 1 standard deviation (σ) above the population mean dose-response curve. *Line C* depicts 2 standard deviation (2σ) above the population mean dose-response curve.

The experimental data are fitted to a second-order polynomial omitting the zero and the first-order term (**Equation 5**),

$$\text{Equation 5} \quad I_d = kH_e^2 + \varepsilon.$$

Here, I_d (tEDC) is the difference of intensity in forward light scattering between the exposed and the contralateral nonexposed eye, k (tEDC $[\text{kJ/m}^2]^{-2}$) is the increased rate of the dose-response function, H_e (kJ/m^2) is the UVR dose, and ε is a random error due to biologic variation and measurement error. The random

error is assumed to belong to a normal distribution, with the expected mean equal to zero and the expected standard deviation equal to σ . A fit of the experimental data provides an estimate of σ as the residual standard deviation.

The tolerance limit for normality is set in the model to e.g. 2σ , meaning that there is a 2.3 % risk⁹⁹ for wrong classification of a difference between paired-normal lenses as pathologic instead of normal (**Figure 10**).

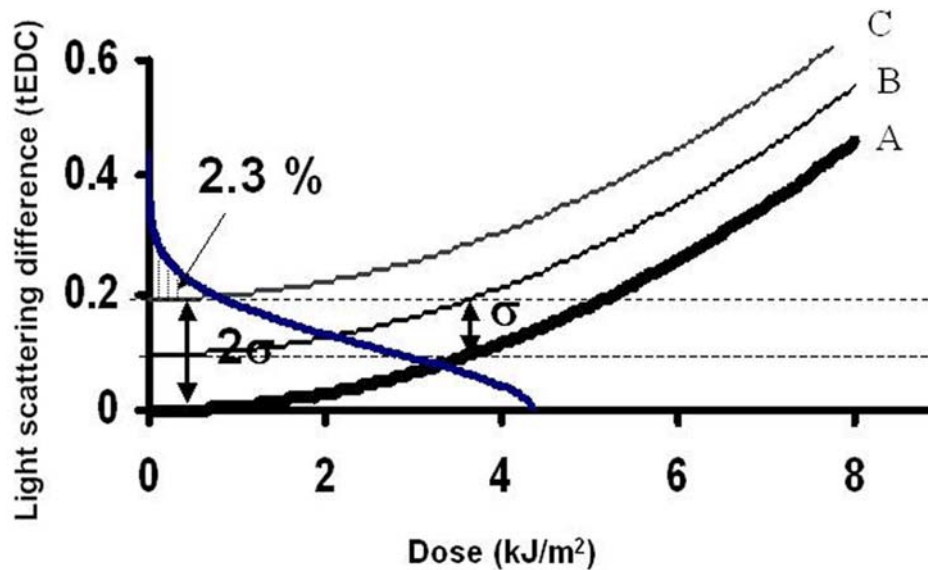


Figure 10. Tolerance limit for normality of difference of light scattering between an exposed and a contralateral nonexposed lens, projected on the dose-response function for UVR-induced difference of light scattering. *A* = dose-response function of population mean difference of light scattering between paired exposed and contralateral nonexposed lenses. *B* = 1 standard deviation (σ) above the dose-response function. *C* = 2 standard deviations (2σ) above the dose-response function.

Further, the criterion for significant toxicity is set, e.g., to 1σ above the dose-response function, meaning that there is a 16% probability⁹⁹ of finding a lens that expresses light scattering above the criterion for normality in order for the dose to induce a significant response (**Figure 11**).

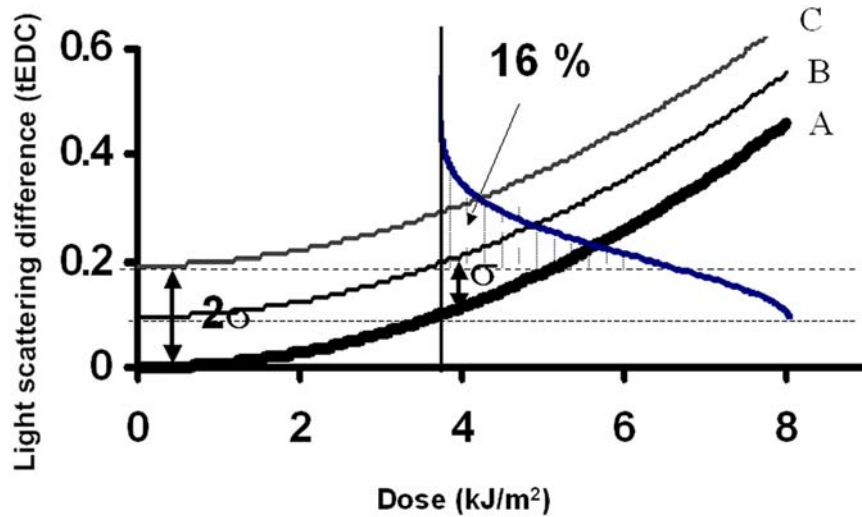


Figure 11. Significant response. A = dose-response function for population mean difference. B = 1 standard deviation (σ) above the dose-response function. C = 2 standard deviations (2σ) above the dose-response function.

The dose corresponding to the significant response is defined as the Maximum Tolerable Dose (MTD). The MTD has 2 arbitrarily chosen parameters; the tolerance for normality (in this instance 1-2.3%) and the criterion for significant response (in this instance 16%). MTD is therefore denoted here as $MTD_{2.3:16}$ (Figure 12).

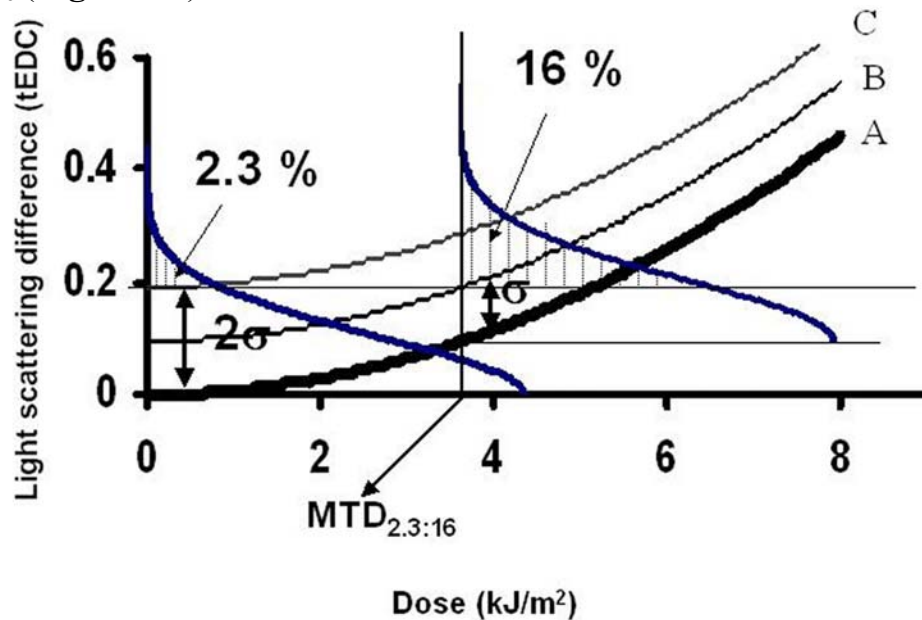


Figure 12. Maximum Tolerable Dose ($MTD_{2.3:16}$) with tolerance for normality set to 1-2.3% and limit for significant response set so that there is 16 % probability that exposed lenses express more light scattering difference than the tolerance limit for normality.

In Figure, it can be seen that $MTD_{2.3;16}$ also can be expressed as (**Equation 6**),

$$\textbf{Equation 6} \quad 2\sigma = k(MTD_{2.3;16})^2 + \sigma \text{ or } MTD_{2.3;16} = \sqrt{\frac{\sigma}{k}}$$

The strategy for MTD estimation can be generalized to all continuous dose response events.

2. Aims of the study

The aims of the present study were:

- To determine the influence of age on threshold dose for avoidance of UVR-B-induced cataract in albino rats.
- To derive a general expression for the influence of age on the threshold dose, for avoidance of UVR-B-induced cataract for the entire lifespan.
- To determine if the age dependent sensitivity of the lens to UVR is associated with the change of the geometrical dimensions of the eye during aging, or related to the lens per se.
- To investigate the influence of exposure time on threshold dose for avoidance of cataract after exposure to UVR-B.
- To investigate the influence of interexposure time between 2 repeated exposures, on threshold dose for avoidance of UVR-B-induced cataract.

3. Methods

3.1 Experimental Animals

The experimental animal was outbred female Sprague-Dawley (SD) rat. This rat was selected for all experiments because it is available at uniform size in large numbers at an affordable price. However, it should be pointed out that the rat eye has a much thinner cornea than the human eye and therefore is expected to have a higher sensitivity for cataract after exposure to UVR than humans. In addition, the rat lens lacks the yellow lenticular pigment found in the human lens. It has also been proven that the albino rat is more sensitive to UVR-B than the pigmented Brown Norway rat.¹⁰⁰

The mean life span for SD rats is about 106 weeks.¹⁰¹ The 2-year survival rate for SD rats is <50%, with cancer being the most common cause of death.^{102, 103}

The survival of the SD rat is directly related to the amount of food consumed. The 2-year survival rate for the female SD rat fed ad libitum is only 37%, but it can be much higher if the rat is on a restricted diet.¹⁰³ The rats were divided into feeding groups. One group was fed ad libitum group, while the diet-restricted groups were given measured fractions of adult SD rat ad libitum daily food consumption. The 25% DR group was given 17 g/day (75% of ad libitum), and the 55% DR group was given 10 g/day (45% of ad libitum). The mean survival for the female SD rat for different amounts of food consumed is shown in **Figure 13**.¹⁰³

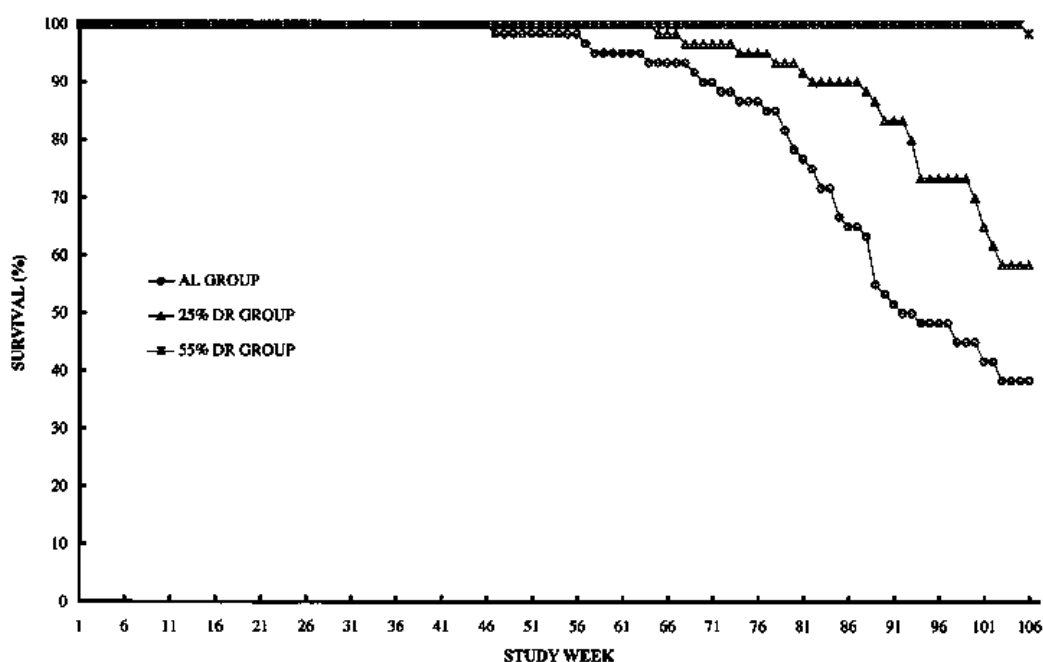


Figure 13.¹⁰³ Mean survival for the female Sprague-Dawley (SD) rat. AL = ad libitum; DR = diet restricted; DR 25% = 75% of adult SD rats fed AL; DR 55% = 45% of adult SD rats fed AL. (Reprinted with permission from the Society of Toxicology.)

The rats in the first age experiment (study I) were fed ad libitum, because it was not then known that food intake is important for life expectancy. Therefore, in order to maintain the same feeding conditions, the rats in the second age experiment (study II) were also fed ad libitum. The oldest age group was set at 60 week to include rats that were as old and as healthy as possible. A 3-week-old rat is weanling, a 6-week-old rat is past weanling stage but is still prepubertal, an 18-week-old rat is a fertile young adult, a 26-week-old rat is an adult, and a 60-week-old rat is elderly.

The weight of the rat increases with increasing age. The weight increase of rats fed with the *ad libitum* in our experiments shows that the rate of weight increase decreased after the age of 26 weeks (**Figure 14-A**).

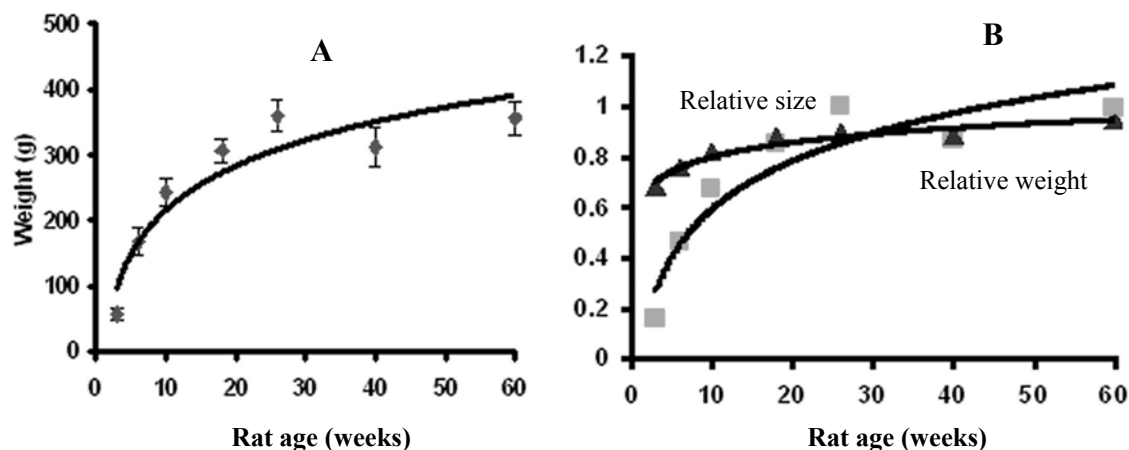


Figure 14. (A) Rat weight increases with increasing of age ($n = 40$ for the 18-week-old group; $n = 20$ for each of the other rat groups) (B) The relative rat weight and lens size as a function of age.

If the maximum relative weight was 1 (where $1=360$ g), and maximum size of rat lens was 1 (where $1=5$ mm), the relative age and size as a function of age can be obtained and is given in Figure 14-B. It is seen that while the diameter approaches the asymptote early, the weight of rat increases throughout lifetime.

All animals were treated in accordance with the Association for Research and Vision in Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research. Ethical approval was obtained from the Northern Stockholm Animal Experiments Ethics Committee.

3.2 Experimental instruments

3.2.1 UVR source

UVR from a 350-W high-pressure mercury lamp (Oriel 6286; LOT-Oriel, Darmstadt, Germany) was collimated and passed first through a water filter and then through a double monochromator (Oriel 2x 77250; LOT-Oriel) (**Figure 15**).

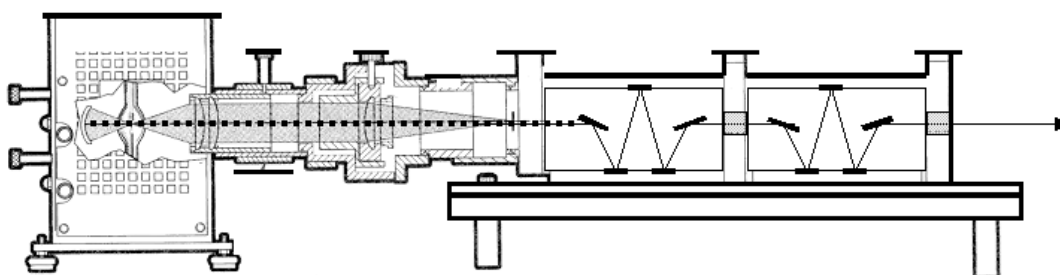


Figure 15. UVR exposure source.

The advantage of using a double monochromator is that stray light is blocked with improved contrast between wanted and not wanted wavebands as a result. The monochromator in all five experiments was set for a maximum throughput at 300 nm and the entrance and output slit were adjusted to achieve a full width at half maximum (FWHM) of 10 nm (295 nm–305 nm).

The spectral distribution of the output beam was measured with a fiberoptic spectrometer (Ocean Optics PC 2000; Ocean Optics, Dunedin, Florida, USA). The wavelength distribution is shown in **Figure 16**. The maximum intensity was 302 nm, and the true FWHM was 9 nm.

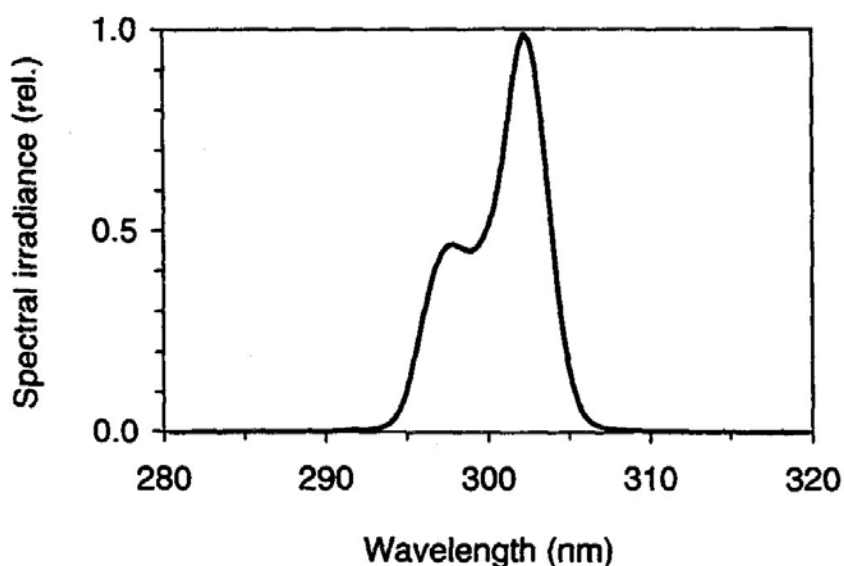


Figure 16. Relative spectral irradiance measured in the beam of the source used in the experiments.

The irradiance at the corneal plane was measured with a thermopile (model 7104; Oriel Instruments, Stratford, Connecticut, USA). In the thermopile, the incident radiation causes an increase in temperature. The rise in temperature is detected with metal junctions fused so that 1 piece of metal bridges 2 pieces of a different kind of metal. If the 2 junctions are exposed to different temperatures, a voltage is generated between the 2 pieces of metal (**Figure 17**).

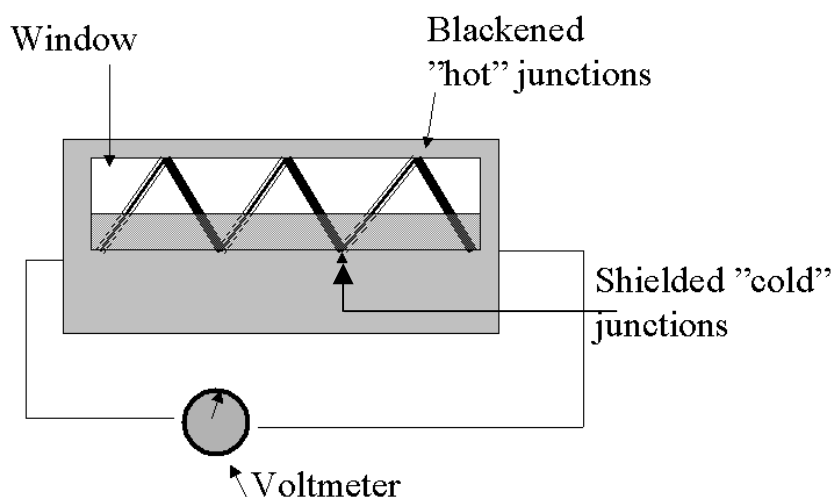


Figure 17. The principal of a thermopile for measurement of irradiance.

The instrument was calibrated regularly to a National Institutes of Standards (NIST) traceable source by the Swedish national bureau of standards (Statens Provningsanstalt, Sweden).

3.2.2 Light scattering measurement

Cataract development after UVR-B exposure was quantified as intensity of forward light scattering (tEDC). The intensity of forward light scattering is measured with the Light Dissemination Meter developed by Söderberg.⁹⁰ The principle of the instrument is shown in **Figure 18**.

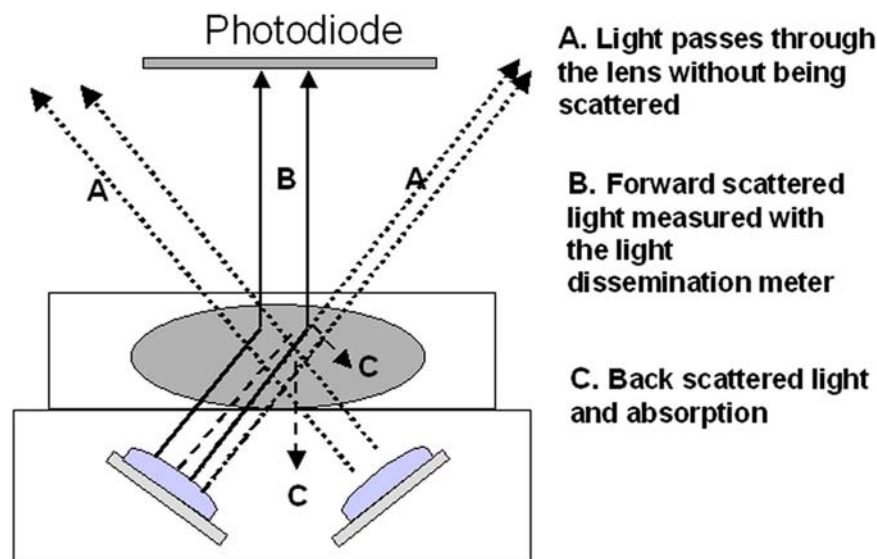


Figure 18. The principle of measurement of intensity of forward light scattering in the lens.

A probing white light from a cold light source is directed at an angle of 45° towards the posterior surface of the lens. A fraction of the light scattered forward from the lens measured (**Figure 18**-line B) is collected by the optics, and focussed on a photodiode. The objective is not depicted in **Figure 18**. If the lens measured is crystal clear, the illuminating light (**Figure 18**-line A) goes through lens without being collected onto the photo detector. If there is light scattering in the lens measured, a fraction of the light scattered is collected and projected onto the photodiode. The photodiode produces a current proportional to the intensity of the light on the surface of the photodiode. The current is voltage converted and read as a voltage measurement. The normal lens exhibits a baseline intensity of forward light scattering.⁹⁰ If the lens is totally opaque, the probing light is attenuated through back scattering and absorption (**Figure 18**-line C) after multiple scattering. Then, the intensity of forward light scattering decreases with increasing scattering, similarly to sunlight hitting dark rain clouds.

All measurements were calibrated to standard dilutions of a commercially available lipid emulsion of Diazepam (Stesolid Novum, Dumex-Alpha A/S, Danmark). The intensity of forward light scattering therefore is expressed as Equivalent Diazepam Concentration (EDC). Early measurements demonstrated

that primary readings of intensity of forward light scattering in lenses should be log transformed in order to become approximately normal distributed.⁹⁰ Therefore log transformation is done on a routine basis to facilitate statistical analysis of experimental data. The unit for the log transformed data has been nominated tEDC. To control for drift of the intensity of the probing light, the voltage driving the lamp was adjusted to obtain a standard signal with an opaque plastic standard light scatterer. A clinically normal lens typically expresses about 0.1 tEDC and an opaque lens a maximum of about 1 tEDC. The technique allows detection of less than 1% change in light scattering.

3.3 Experimental procedure

3.3.1 UVR exposure in vivo

Ten minutes preceding the UVR-B exposure, the animal was anaesthetized with a mixture of 40 mg/kg ketamine and 4 mg/kg xylazine, injected intraperitoneally. Five minutes after the injection, the mydriatic tropicamide was instilled in both eyes and the eyes were examined with a slit lamp microscope. Animals with lens opacities as observed in the slit lamp were excluded from experiment before exposure. After another 5 minutes, one eye of each animal was exposed to a narrow beam of UVR-B covering only the cornea and the eyelids, and the other eye was left as a not exposed control.

One week after the exposure, the animal was sacrificed by carbon dioxide asphyxiation. The eyes were enucleated, and both lenses were extracted and placed in balanced salt solution (BSS). Remnants of the ciliary body were removed from the lens equator. The light scattering of both the exposed and the contralateral not-exposed lens were measured. The macroscopic appearance of each lens was documented with photography.

The intensity of forward light scattering in not-exposed lenses varied between 0.1-0.2 tEDC. It was noted during pre-experiments that if the time used for dissection of the lens and removal of ciliary body remnants is long, high readings of forward light scattering were obtained. The time between dissection and light scattering measurement was therefore shorter than 5 minutes for all the experiments.

3.3.2 UVR exposure in vitro

The rats were killed by CO₂ asphyxiation. The eyes were enucleated and both lenses were extracted by a posterior approach. Both lenses were then immediately immersed in 6 ml balanced salt solution (BSS) in sterile plastic culture Petri dishes. One lens was exposed at room temperature to 1.5 kJ/m² UVR-B for 15 minutes immediately after immersion of both lenses. The lenses were positioned anterior side down and the radiation was directed from beneath using an aluminum mirror (**Figure 19**). The other lens was kept in the same room but was not exposed to UVR-B.

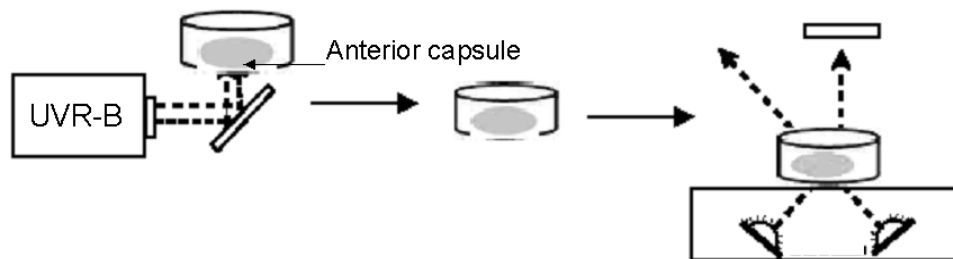


Figure 19. Lens in vitro UVR-B exposure, incubation and light scattering measurement.

Immediately after the UVR-B exposure, both lenses from each rat were transferred to culture medium in new dishes with vented lids. The light scattering of each lens was measured daily in the first week and every other day during the second and third weeks of culture.

3.4 Macro-photography

The morphological changes in the lens after UVR-B exposure were documented by macrophotography, with incident bright-field illumination against a dark background with white grids and/or dark-field illumination against a dark background (**Figure 20**).

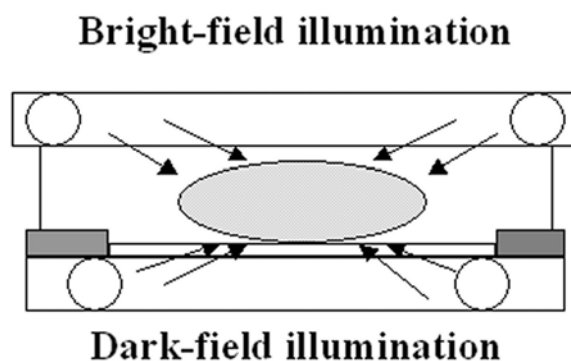


Figure 20. Two types of illumination in macrophotography.

With bright-field illumination, the light from a paraxial cold light ring illuminates the lens from above. The white grid under lens reflects the lens changes as follows; clear lens— regular grid; anterior subcapsular haze— irregular grid; total lens opacity— invisible grid. The grid also documents the size of the lens, with the distance between the white wire being 0.79 mm.

With dark-field illumination, the light comes from an angle under the lens. Scattering in the lens is then detected as white spots. Local refractive index deviations are more visible in dark-field illumination than in bright-field illumination. Subcapsular punctiform opacities observed under dark-field illumination are the first signs of UVR-B-induced cataract. These are invisible under bright-field illumination. Therefore, only macro-photography with dark-field illumination was used in study III and V.

3.5 Experimental design

3.5.1 Design for estimation of MAD

The experiments for studies I and IV were designed for estimation of MAD as a function of age and exposure time, respectively. For each MAD estimation, a regression experiment was run and the parameters of the dose-response functions were estimated with regression analysis.

To optimize the regression, MAD has to be estimated, preferably based on previous experiments. The dose range of interests should distribute around the

assumed MAD (\tilde{MAD}) and should be split into at least 4 levels of equally spaced doses. Subdoses in studies I and IV were selected according to the strategy $\tilde{MAD} \times 0$, $\tilde{MAD} \times 2^{-1}$, $\tilde{MAD} \times 2^0$, $\tilde{MAD} \times 2^1$, $\tilde{MAD} \times 2^2$. In other words, when \tilde{MAD} was 2 kJ/m^2 , the respective subdoses were 0, 1, 2, 4, and 8 kJ/m^2 . This design was used to both give a strong weight to doses around the assumed MAD and at the same time include doses considerably higher than assumed MAD where light scattering can be measured with high precision (**Figure 21**).

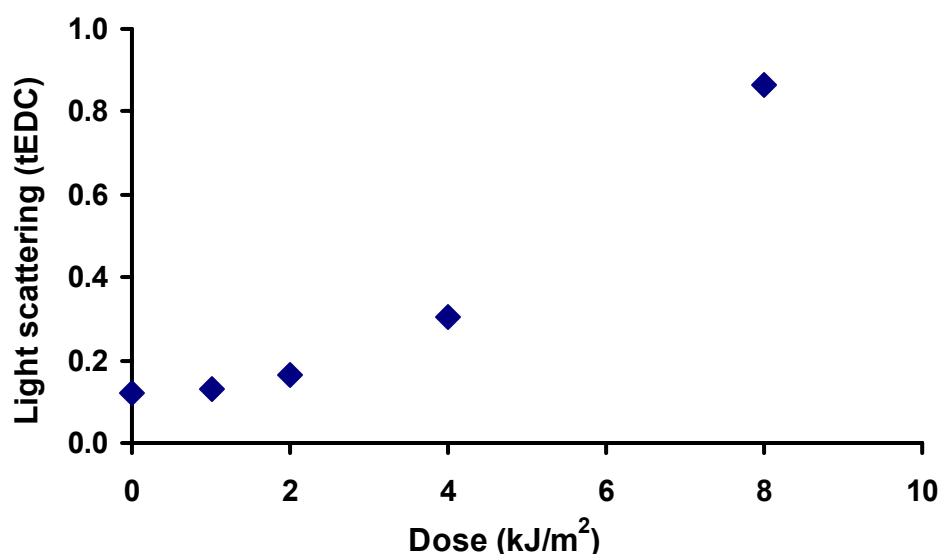


Figure 21. The dose-response function between light scattering and exposure doses.

In study I, the assumed MAD was based on a previous experiment showing that young rats are more sensitive to UVR than their older counterparts.⁹³ The assumed MAD was set to 0.75, 1, 1.5, and 2 kJ/m^2 for the 3-, 6-, 10-, and 18-week-old rat groups, respectively. For each of the age groups, the doses assigned to the subgroups were then selected according to the strategy described in the paragraph above (Figure 2, study I). In study IV, the same assumed MAD of 2 kJ/m^2 was used for all exposure-time groups, and the doses appointed to subgroups were then selected according to the strategy described above. The experimental design is given in **Figure 22**.

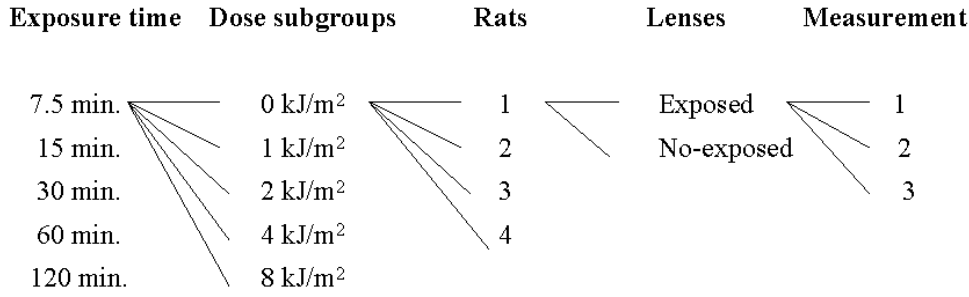


Figure 22. Experimental design for investigation of the influence of exposure time on MAD.

The square of the dose used in the experiment was set as the explanatory variable data in the regression. This allowed use of standard straight-line regression.

3.5.2 Design for MTD estimation

The MTD was based on the light scattering differences between paired exposed and contralateral not-exposed lenses. The dose-response of the light scattering difference was modelled as a second order polynomial omitting the zero and the first order term (**Equation 5**). The regression parameters were estimated by a small sample experiment, and MTD was calculated from the regression parameters (**Equation 6**).

As in MAD design, the parameters of the dose-response function were determined with regression analysis. In the regression, the linear relation between light scattering and H_e^2 is analyzed. To optimize the regression, the dose interval squared should be split into ≥ 4 ranges. The square root for the limits of the ranges should then be used for the experiment.

For experimental MTD determination, the zero dose should be included. To achieve the highest precision of the MTD estimate, the mean dose used should be close to the MTD. For MTD estimation, we aimed at squared doses distributed as evenly possible within the interval of interest (**Figure 23**).

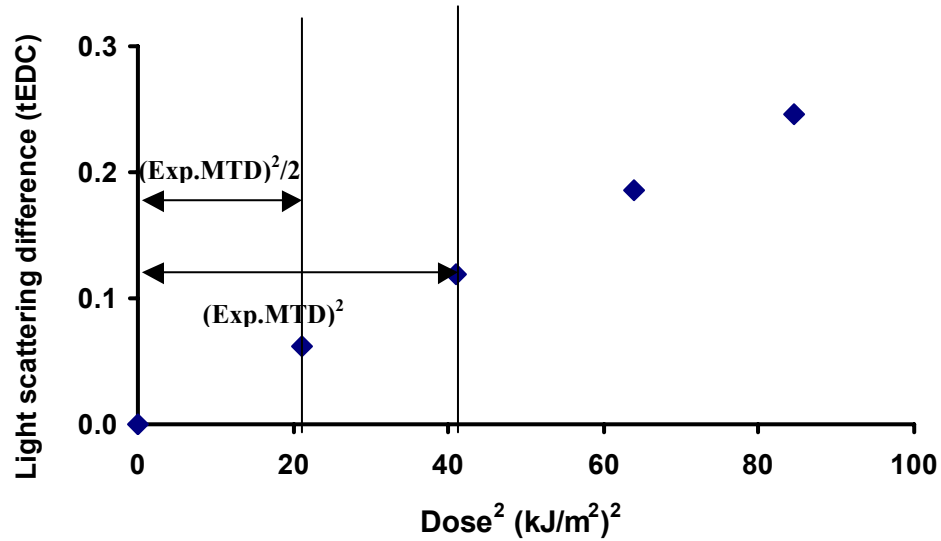


Figure 23. Experimental design of dose selection for experimental MTD estimation.

The doses for sub-dose groups were calculated according to **Equation 7**.

$$\text{Equation 7} \quad \sqrt{(g-1) \frac{(MTD)^2}{2}}$$

Here, g (1..5) is the order of subdose group, and MTD is the assumed MTD based on the previous and/or preliminary experiments. For example, in study II the assumed MTD was 6.5 kJ/m^2 based on the previous experiment.¹⁰⁴ The respective doses selected for the first, second, third, fourth, and fifth dose groups in the experiment were 0, 4.6, 6.4, 8, and 9.2 kJ/m^2 (Figure 2, study II). We used three independent observations at each dose applied.

3.5.3 In vitro UVR exposure experiment

The dose was set at the lens anterior surface, and the same exposure dose; 1.5 kJ/m^2 was used for all age groups. The 1.5 kJ/m^2 UVR-B dose was chosen to mimic about 4 kJ/m^2 in vivo dose at the corneal plane, considering a transmittance of 40% through cornea and 90% through the aqueous.^{75, 105} The exposure time of 15 minutes was the same as in our earlier in vivo UVR-B exposure experiments. The design of the experiment to investigate the influence of age under in vitro UVR exposure is given in **Figure 24**.

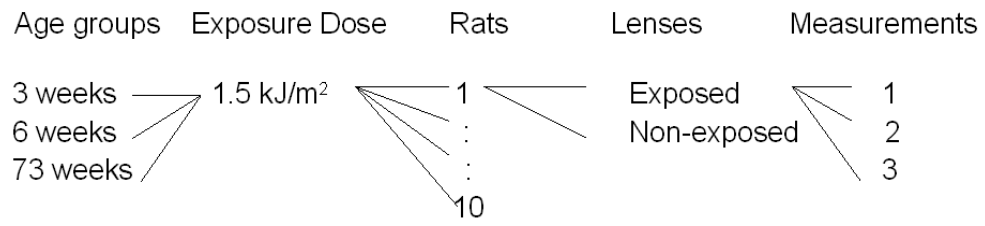


Figure 24. Experimental design of in vitro UVR exposure.

4. Results and Discussion

When measuring intensity of forward light scattering, UVR induced cataract follows a continuous dose-response function.⁶⁶ The MAD⁹⁷ and further developed into MTD⁹⁸ have been developed as concepts for threshold dose for continuous dose-response relationships. In the present thesis, different factors that may affect threshold dose for UVR cataract have been investigated using MAD or MTD.

4.1 Threshold dose for UVR-B cataract as a function of age

4.1.1 Threshold dose for UVR-B cataract in 3 to 18 weeks old rats

The MAD was estimated based on the principle that light scattering in normal lenses has a normal frequency distribution (**Figure 8**). The light scattering in nonexposed lenses varies from individual to individual in each age group in accordance with the normal distribution.⁷⁸

The homogeneity of variances of light scattering among the different age-groups was tested for by Bartlett's test.¹⁰⁶ There was a significant differences among the different age-groups ($p < 0.05$). The frequency distribution for light scattering for nonexposed contralateral lenses in each age-group was therefore estimated separately.

Because the mean and the standard deviation for normal nonexposed control lenses were estimated from limited samples of contralateral nonexposed eyes ($n = 20$), the probability for misclassification of a normal lens as pathologic is not exactly known in the strict statistical sense. To be precise, $n = 20$ should be used instead of $n \cong \infty$ to estimate MAD, but when we designed the experiment for study I $n \cong \infty$ was the best approximation available. However, because the strategy for MAD estimation was used consistently for all the age groups, the relative difference of MAD among age groups is still correct.

The lens morphologic changes 1 week after in vivo exposure to 2 kJ/m^2 UVR-B show that significant subcapsular and equatorial cataract developed in 3-week-old rats. Only light equatorial cataract developed in lenses from 6-week-old rats. Detectable local equatorial opacity developed in lenses from 10-week-old rats.

There was no cataract development in the lenses from the 18-week-old rats (Figure 3, study I). The lens sizes in the 3- to 18-week-old animals demonstrated fast lens growth. The mean diameter of the lenses was 3.6 mm for 3-week-old rats and 4.6 mm for 18-week-old rats (Figure 3, study I).

The $MAD_{0.975}$ for avoidance of cataract after UVR exposure was estimated at 1.4, 2.7, 4.3, and 5.2 kJ/m^2 , respectively for the 3-, 6-, 10-, and 18-week-old rats. It should be noted that $MAD_{0.975}$ was almost 4 times lower for the 3-week-old rats than for the 18-week-old rats (Figure 6, study I). The current result strongly suggests that UVR-B sensitivity is age dependent.

There are several factors that might contribute to higher sensitivity to UVR-B in younger compared with older rats. A young rat has a higher rate of lens cell division. The fast growth of a lens requires intense protein synthesis, and UVR-B inhibits protein synthesis.⁸⁴⁻⁸⁶ It has also been shown when the same corneal dose of UVR-B is applied, that the lenticular doses are different for young rats (3 weeks old) and old rats (52 weeks old) because the younger rats have thinner corneas, shallower anterior chambers, and smaller lenses.⁹³ The age dependence of MAD may therefore be due to the relatively higher lenticular UVR-B dose in the young rats compared with the older ones. In order to exclude the impact of anatomic effects, in vitro UVR-B exposure with the same lenticular dose for all age groups was used in study III.

The thresholds estimated for various ages in the experiments cannot be directly extrapolated to humans. There are anatomic differences between the rat¹⁰⁷ and human¹⁰⁸ eye. The human eye also has a higher concentration of antioxidant molecules such as GSH, vitamin E, and vitamin C, probably making humans lenses more resistant to UVR-B. However, it is plausible that the same trend of age-dependent UVR-B sensitivity exists in humans. It has been reported that high sun exposure early in life is strongly related to a high incidence of nuclear cataract later in life.¹⁰⁹ A near- or above-threshold dose of UVR in childhood may damage the lens epithelium and superficial fibers. A relative overefficiency for cell biologic function in young lenses may maintain transparency despite accumulation of UVR-induced damage. With older age and decreasing efficiency, the accumulated damage may become expressed as age-related cataract. In light of the present results, age should be considered when setting safety limits for avoidance, prevention, or postponement of cataract.

4.1.2 Threshold dose for UVR-B cataract in 18 to 60 weeks old rats

When planning this experiment, MAD⁹⁷ had been developed into MTD.⁹⁸ MTD is preferable because it provides a statistically meaningful estimate of a threshold for continuous dose response functions. The interpretation of the real meaning of MAD is less intuitive and the estimation requires an approximation of mean and standard deviation for light scattering in normal not-exposed lenses, from a small sample.

In study II, it was found that there is essentially no change of sensitivity to UVR in the 18- to 60-week age interval (Figure 5, study II). This was also reflected by the fact that the MTD did not change significantly within this interval (Figure 4, study II). When analyzing cataract development after in vivo exposure to a high dose of UVR-B (8 kJ/m^2), we found intense equatorial and subcapsular cataract in 6-week-old rats. However, only low-degree subcapsular cataract developed in 60-week-old rats (**Figure 25**).

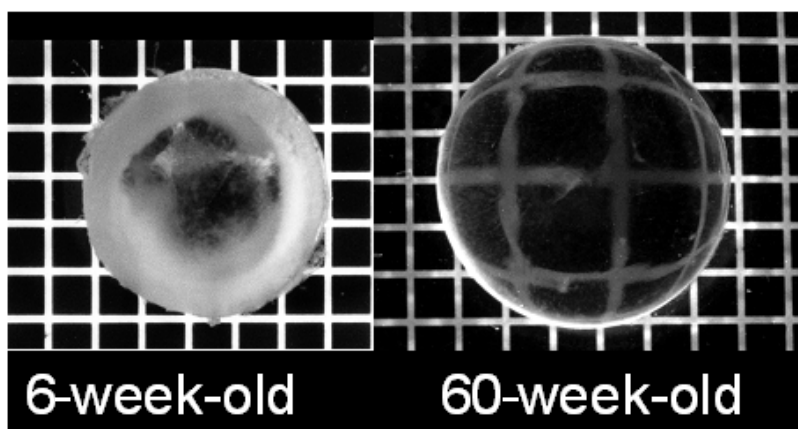


Figure 25. Cataract development in 6-week-old and 60-week-old rats 1 week after in vivo exposure to UVR-B 8 kJ/m^2 .

The decreased sensitivity to UVR-B in the old lens may be caused by declining mitotic activity in the lens epithelium associated with less DNA replication and decreasing overall lens growth associated with less overall synthetic activity. For comparison, we retrospectively applied the MTD concept (**Equation 6**) to the data from study I, and plotted the estimated MTD for all age groups in study I and study II in the same graph (Figure 6, study II).

It was found that MTD increases with an exponential decline in the age interval 3 to 60 weeks. The MTDs in Figure 6, study II, were transformed into relative MTD using MTD_{max} as the reference. Age was transformed into relative age using a maximum age of 106 weeks.¹⁰² MTD_{max} was obtained from the nonlinear regression according to Equation 4 in study II.

The estimated relative MTD (MTD_{Rel} [unitless]), as a function of relative age (Age_{Rel} [unitless]), was fitted to a nonlinear model (**Equation 8**).

$$\text{Equation 8} \quad MTD_{Rel} = (1 - e^{-\beta \cdot Age_{Rel}}) + \varepsilon$$

Here, β ($[Age_{Rel}]^{-1}$) is the increase rate and ε is a random error expressing biological variability and measurement error.

The exponentially declining increase of relative MTD with increasing relative age is shown in **Figure 26**.

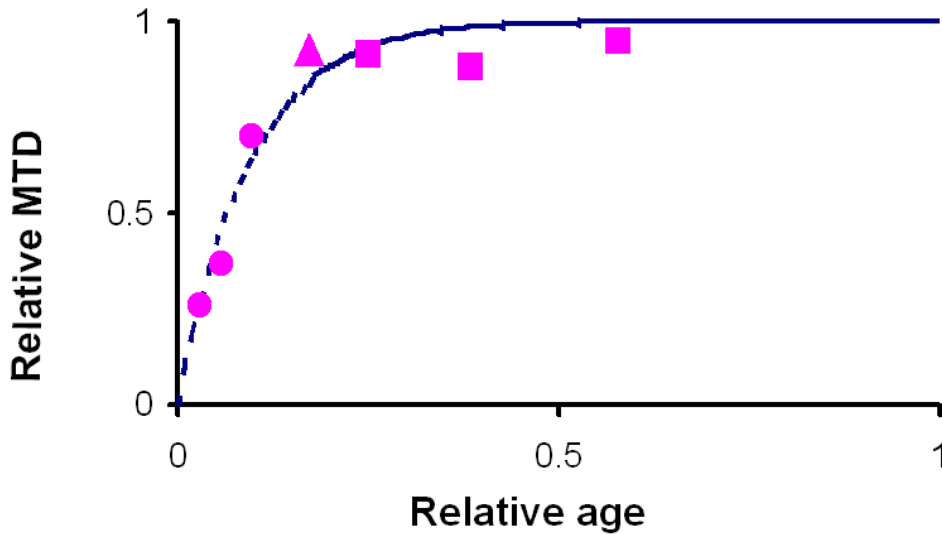


Figure 26. Effect of relative age on relative threshold dose for cataract induced by *in vivo* exposure to UVR in the 300 nm wavelength region. . (Both relative age and relative threshold are unitless.) ● = data from study I. ▲ = pooled data from both study I and study II. ■ = data from study II.

It is shown in **Figure 26** that sensitivity to UVR-B decreases with increasing age during the first third of the rat life span and remains stable during the later two thirds of the life span. Thus, most of the change of the threshold occurs during the early phase of the life span.

Until specific data are available for different species, the present data on relative MTD can be adapted to any species, including humans. One possible way to improve the knowledge about human lenses is to study them in vitro. If similar in vitro investigations are done on different experimental animals and compared with the results for in vivo exposures, it may be possible to extrapolate the human in vitro data to the in vivo situation with better confidence.

The dose dependence for UVR induced cataract has been well established both for chronic low dose exposure in epidemiological investigations^{8, 43, 64, 70} and for high dose experimental studies.^{92, 110, 111} The UV Index¹¹² has been introduced for guidance of the public on UVR exposure. A commonly adopted safety limit for avoidance of acute UVR cataract is based on the ICNIRP safety standard.¹¹³

Excessive exposure to UVR in childhood has been recognized as a risk factor for development of skin cancer later in life.¹¹⁴⁻¹¹⁷ However, the possibility that overexposure to UVR in childhood may be a risk factor for age related cataract has not previously been considered. UVR exposure elicits photochemical damage through production of reactive oxygen species (ROS), leading to enzyme inactivation, protein aggregation, and or DNA alteration and secondarily cell damage. These molecular events hold both for UVR-induced skin damage and for UVR induced cataract. Like for skin cancer, it is probable that excessive exposure to UVR in childhood, added to degenerative changes in the lens during aging, may contribute to age-related cataract later in life.

The Safety Exposure Duration (SED) in second can be calculated by **Equation 9**.¹¹⁸

$$\text{Equation 9} \quad SED = H_L / E_e$$

Here, H_L (J/cm^2) is the threshold dose for lens at 300 nm, and E_e (W/cm^2) is the biologically efficient source irradiance. If H_L for young individuals is set to $1.4 \text{ kJ}/\text{m}^2$, which is the biologically efficient threshold dose for permanent lens damage for a 3-week old rat at 300 nm, the SED is $1.4 \times 10^3 / E_e$. However, if the threshold dose is set to $5 \text{ kJ}/\text{m}^2$, which is the biologically efficient threshold dose for permanent lens damage for an 18-week old rat, the SED is $5 \times 10^3 / E_e$. This demonstrates that younger individuals have an SED of only $1.4/5 = 28 \%$ of the

SED for old individuals. Thus younger individuals will develop acute cataract at approximately 1/3 of the time that is required for older for a certain irradiance.

When the latest ICNIRP standard¹¹³ was updated, the present data on age dependence of UVR sensitivity in the lens was not available. Hopefully the current data on age-dependent UVR sensitivity will be considered in future safety standards for avoidance of UVR induced cataract.

4.1.3 Age-dependent UVR sensitivity to in vitro exposure

In study III, lenses of varying age were exposed in vitro to the same UVR-B dose in order to exclude the possibility that anatomic differences are responsible for the age-dependent sensitivity of lenses exposed in vivo.

The 1.5 kJ/m² UVR-B dose was chosen to mimic an in vivo dose of about 4 kJ/m² at the corneal plane, which is above the threshold for 3- and 6-week-old rats but below the threshold for 73-week-old rats.

Temperature-sensitive changes were observed in the in vitro cultured lenses. Two hours after moving the cultured lenses from room temperature (20°C) into the incubator (37°C), both exposed and nonexposed lenses developed temporary osmotic cataract. Haze and edema were present in the anterior lens cortex. The haze disappeared at day 1 after culture. The possibility that the haze development partly was caused by changing from BSS to culture medium cannot be ruled out. It was concluded that in in vitro cultured lenses, a valid measurement of light scattering can be taken only after a period of adaptation to the culture.

At day 1 in culture, nonexposed lenses for all 3 age groups were transparent. Exposed lenses from all age groups developed limited anterior subcapsular punctiform opacities (Figure 5, study III). The anterior subcapsular punctiform opacities were the first signs of UVR cataract in all age groups, after both in vitro and in vivo exposure. The punctiform opacities became large, merging together at the equator and forming the equatorial cataract. The equatorial cataract developed from the equator to the center of lens, following the lens cortical fibers, and formed a cortical cataract at day 3 for 3-week-old and day 7 for 6-week-old groups (Figure 5, study III). However, cortical cataract did not develop in lenses from the 73-week-old animals until day 17 in culture.

Nuclear cataract also developed both in lenses from 3-week-old rats at day 15 and in 6-week-old rats at day 17 in culture. Very-high-density nuclear cataract developed in lenses from the 3-week-old group of rats at day 17 after exposure. These lenses showed a dark nucleus in dark-field illumination macrophotographs. No nuclear cataract developed in lenses from the 73-week-old rats (Figure 5, study III).

Development of cataract was observed in cultured lenses after in vitro exposure to UVR. The subcapsular punctiform opacities probably resulted from damage to the lens epithelial damage. UVR can induce DNA, protein, and lipid damage in the epithelial cells. If the damage cannot be repaired, it is expected that cell metabolism decreases. Damage to lipids and proteins in membranes may cause an increase in membrane permeability. Lack of ATP and/or damage to the Na^+ - K^+ -ATPase leads to an increase in intracellular sodium and a concurrent decrease in intracellular potassium. This causes a net increase in lens solute and leads to osmotic swelling.⁸³ Oxidation of functional and structural proteins leads to protein aggregation. Osmotic swelling and aggregation of proteins cause variations in the refractive index, which is expressed as increased light scattering. After UVR exposure, the scattering is seen primarily in the cortical zone from the center of the lens, through the germinative zone—the most active metabolic zone—and all the way out to the equator.

Nuclear cataract was not expected in lenses from 3- and 6-week-old rats after exposure in vitro to UVR-B 1.5 kJ/m^2 (corresponding to approximately 4 kJ/m^2 in vivo). There is higher oxygen concentration in the incubator (20%–80%) than in the aqueous humor. Oxygen enters the lens via diffusion from the surrounding air. Oxygen crosses cell membranes and, if not consumed, would diffuse into the center of the lens.

Oxidation of protein in the lens nucleus is supposed to be the mechanism for nuclear cataract.¹¹⁹⁻¹²¹ A high above-threshold dose in the young rat may also contribute to nuclear cataract. Löfgren et al.⁹³ showed that nuclear cataract developed in 3-week-old rats after in vivo exposure to UVR-B 8 kJ/m^2 , but not in 6-, 17-, or 52-week-old rats.

However, nuclear cataract was not found in nonexposed lenses even at the end of the culture period. There is an active antioxidation system in the lens that

probably protected the nonexposed lenses against nuclear cataract formation. The UVR exposure probably caused a dysfunction in the antioxidation system that resulted in cataract development. It is interesting to note that the young lens was also more sensitive than the old lens to the in vitro culture (Figure 4, study III). Whereas most nonexposed lenses from 3-week-old rats can only survive until day 9 in culture, lenses from the 6-week-old rats and the 73-week-old rats can survive until day 19 and day 30, respectively. The higher metabolism in the young lens may be the reason for the higher sensitivity to the in vitro culture.

The light scattering differences between exposed lenses and their not-exposed contralaterals shown that the highest light scattering developed after in vitro exposure to 1.5 kJ/m^2 UVR-B in lenses from 3-week-old rats and the lowest light scattering developed in lenses from 73 week-old rats (Figure 27).

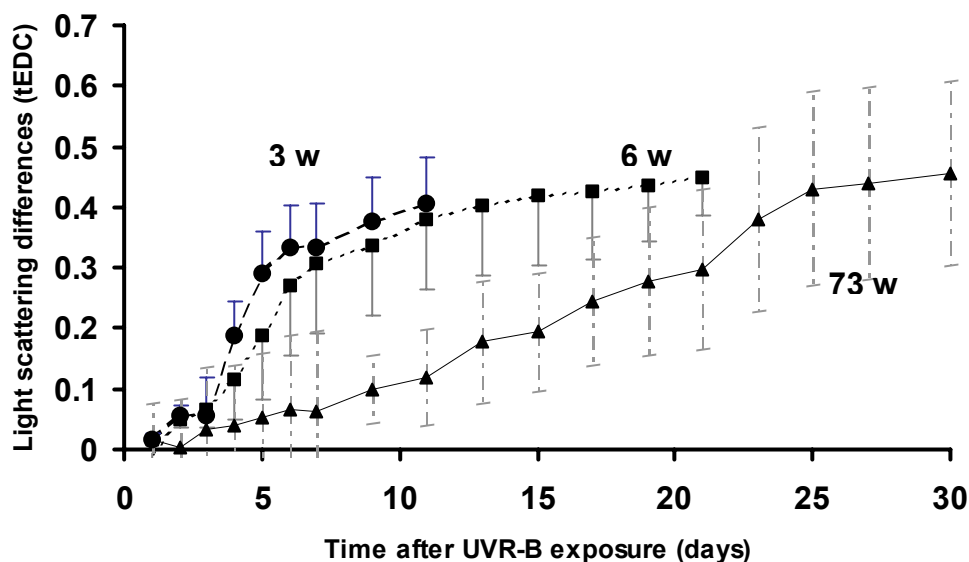


Figure 27. Light scattering development for lenses from 3-, 6-, and 73-week old rats after in vitro exposure to UVR-B 1.5 kJ/m^2 . ($n=10$ paired lenses), Bar is 95% confidence interval.

The results from the present experiment demonstrate that the age-dependent UVR-B sensitivity noticed in the in vivo exposures originates from lens biology rather than age-dependent geometry of the eye.

In vitro UVR exposure of a lens provides limited information about the impact of UVR on the total biology of the lens in vivo. However, when considering the lenses of human eyes, experimental exposure can only be done in vitro.

Therefore, comparison of effects after animal experiments in vivo and in vitro provides important insight into how in vitro information can be used to arrive at in vivo conclusions.

The current finding that UVR-B sensitivity in the lens is age dependent is important and should be considered in safety estimation until more data is available. Further studies in vivo experiments on diurnal animals and in vitro experiments on human lenses are needed for a more complete understanding of the significance of age dependence of sensitivity for UVR induced cataract in the human. Epidemiological studies intending to prove the benefit of protection of the eye against high dose exposure to UVR in childhood are needed.

4.2 Influence of exposure time on UVR cataract

The MAD for cataract after exposure to UVR was compared for exposure times in the interval 7.5 to 120 minutes. The 15-minute exposure time was associated with the lowest MAD (Table 2 and Figure 5, study IV). Cataract development after exposure to UVR-B 4 kJ/m^2 at varying exposure times is showed in **Figure 28**.

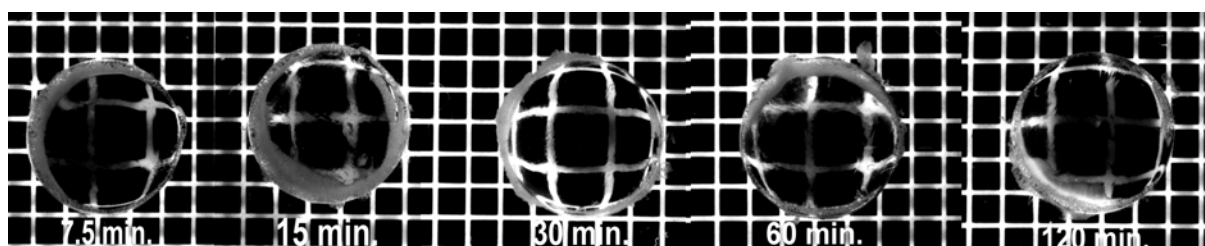


Figure 28. Cataract development 1 week after exposure to UVR-B 4 kJ/m^2 for varying periods of exposure.

It can be seen in Figure 28 that the most severe equatorial cataract developed in the 15-minute exposure time group. The implication of this finding is that the Bunsen-Roscoe¹²² reciprocity law for photochemical reactions does not hold for the biological damage expressed in the lens after in vivo exposure to UVR. Therefore, it appears that there must be some biologic modification of the primary photochemical event. A possibility is that photosensitizers are formed during the early phase of an exposure. This would potentiate the effect after an

initial exposure. If, however, defense mechanisms are triggered by the exposure, the defense mechanisms may limit the damage at extended exposures.

4.3 Influence of interexposure interval on UVR cataract

The impact on sensitivity of the interexposure interval between two exposures of UVR was investigated. Sensitivity was measured as the inverse of the threshold dose, which itself was estimated as MTD. The interexposure intervals 6 hours, and 1, 3, 9, and 30 days were examined. The lowest $MTD_{2.3:16}$ was found in the 1-day interexposure interval group, and the highest $MTD_{2.3:16}$ was found in the 30-day interexposure interval group (Figure 9, study V).

This result indicates that molecular defence and repair in the lens plays an important role modulating lens damage induced by UVR. Antioxidative molecules^{123, 124} and enzymes may repair proteins damaged by UVR induced oxidation. The total damage recorded in the current experiment after both exposures to UVR, is the damage evoked by the first exposure minus the repair elaborated by the lens between the two exposures, plus the damage evoked by the second exposure. It was also found that the lenses that were exposed to the most extended interexposure interval expressed the least light scattering.

Reactive oxygen species (ROS) induced by UVR may enhance the lens response to the second exposure.⁹⁵ DNA damage and inactivation of a number of metabolic enzymes^{84, 125} may explain the slightly lower threshold in the 1-day interval group compared with the other groups.

The cumulative effect of exposures of the cornea to UVR at 295 nm has been studied. Severe damage was induced if two repeated doses of 0.5 threshold dose were given with an interexposure interval of less than 4 hours. However, decreased corneal damage resulted if two repeated doses of 0.5 threshold dose were given with an interexposure interval of more than 8 hours.¹²⁶ These findings indicate that corneal repair starts 8 hours after an exposure to a 0.5 threshold dose.

Lens forward light scattering after two exposures of close to threshold exposure (4 kJ/m²) at 300 nm was the same when there was no interexposure interval as when the interexposure interval was 6 hours. Forward light scattering after an interexposure interval of 24 or 48 hours was nearly 2-fold greater than that

following two exposures with no or 6 hours interexposure intervals.¹²⁷ Lenses need also longer time (> 7 days) to repair the damage.¹²⁸

The finding that the highest sensitivity (lowest MTD) was found in the 1 day interexposure interval group is probably associated with the molecular biology of repair. Michael et al.⁸⁹ found that programmed cell death induced by threshold-dose UVR-B peaks around 24 hours after exposure. There is data indicating that the time delay of apoptosis depend on the wavelength of the UVR. It was reported that UVR-A (340-400 nm) induces immediate apoptosis (0-4 hours after insult) while UVR-B (290-320) and UVR-C (200-290 nm) induce apoptosis delayed at least 20 hours after the UVR insult.¹²⁹ A second exposure at a critical time of repair may result in more severe cell damage in the lens.

4.4 UVR-B-induced corneal changes and uveitis

A high-dose in vivo exposure to UVR-B-induces severe corneal damage. We observed corneal edema, opacities, and neovascularization. In addition, corneal damage is both dose dependent and age dependent. We observed more severe corneal damage in young rats than in old rats after the same UVR-B exposure (Figure 7, study I). Corneal opacities and neovascularization were also found in nonexposed corneas. This has been reported earlier and was related to proptosis induced by ketamine-xylazine anesthesia.¹³⁰ However, in the present study, more severe corneal changes were found at high-dose exposures. In addition, the corneal surface shape changed increasingly with increasing dose (**Figure 29**).

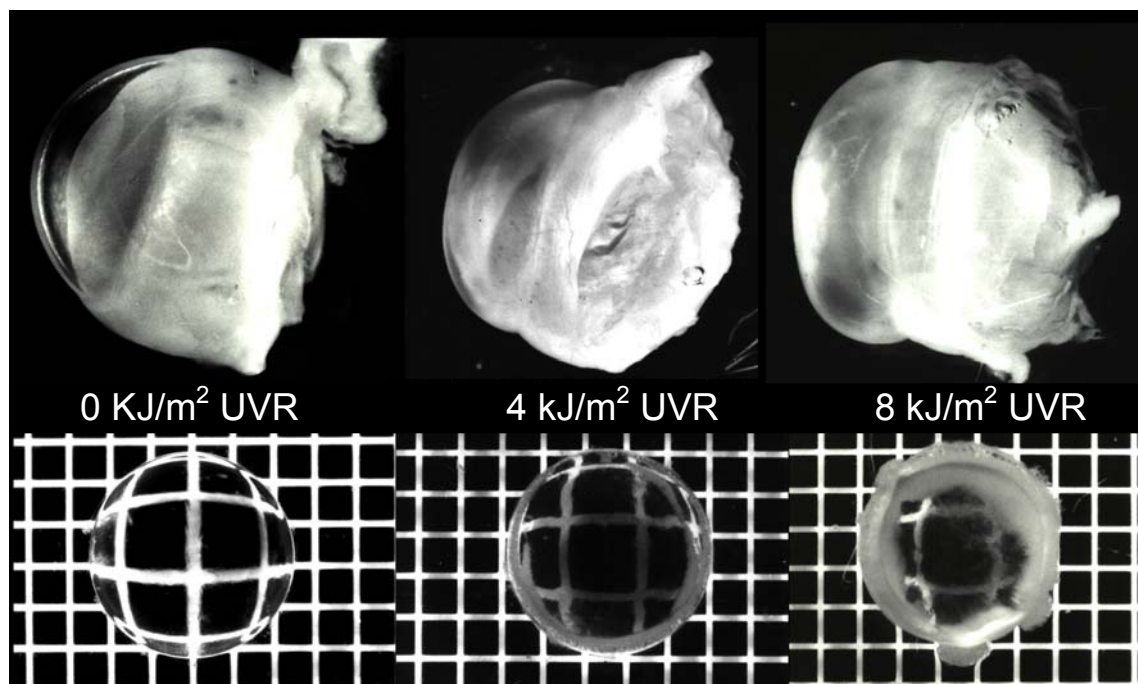


Figure 29. The parallel damages of cornea and lens after UVR-B exposure.

Dose-dependent uveitis induced by UVR was also observed. After a high dose exposure, hemorrhage in the anterior chamber, late anterior or posterior iris synechia and ciliary body edema were observed. It is possible that the uveitis observed induced secondary glaucoma and that an increased intraocular pressure caused the corneal shape changes (**Figure 29**). The severity of the corneal changes paralleled the severity of the lens damage.

Uveitis classically is not considered to cause UVR-induced cataract.⁴ The fact that cataract also occurs after exposure in vitro (study III) proves that UVR is directly toxic to the lens. The possibility cannot, however, be excluded that in vivo exposure, uveitis adds to the direct toxic effect of UVR, at least at high-dose exposures over a relatively short period. Thus, in vitro exposure of lenses may be a useful experimental design to specifically study a certain aspect of this problem. To fully comprehend the complete biology, however, in vivo exposures are necessary

5. Conclusions

We conclude, from the results of the investigation reported herein, that young rats are more sensitive to UVR-B than old rats.

The sensitivity to UVR-B increases with increasing age during the first third of the life and then remains stable during the later two thirds of the life span. Age should therefore be considered in safety limit estimation for avoidance of cataract induced by UVR-B.

The lens per se has an age-dependent sensitivity to UVR-B.

The sensitivity of the in vivo exposed lens to UVR-B varies with the time of the exposure, with a minimum threshold dose at around 15 minutes. Therefore, the reciprocity law for the photochemistry of UVR cataract does not hold for the biological damage expressed.

The threshold dose for cataract after two equivalent near-threshold exposures increases with an increasing interval between the exposures. This indicates that repair takes place in the lens after exposure to UVR.

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