Maximum acceptable dose of ultraviolet radiation: a safety limit for cataract

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ABSTRACT.

Purpose: **To develop a method for experimental estimation of toxicity for continuous dose–response relationships. To apply this method to cataract induced by ultraviolet radiation (UVR) in young rats.**

Methods: **After establishing experimentally the frequency distribution of light scattering of normal physiologically clear lenses, the lower limit of pathological light scattering is defined such that a certain fraction, for example 97.5%, of normal lenses scatter less light.**

Results: **The dose–response function for UVR and cataract is determined experimentally. With this function, the dose corresponding to the lower limit of pathological light scattering may be determined as the maximum acceptable dose (MAD). The $MAD_{0.975}$ for UVR 300 nm was determined to be 2.2 kJ/m^2.**

Conclusions: **The method can serve as a basis for establishing safety standards for UVR-induced cataract and probably other continuous dose–response functions.**

Key words: quantitative – dose–response – threshold – ultraviolet radiation – cataract

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Introduction

This paper presents a method for experimental estimation of the maximum acceptable dose (MAD) for the quantitative dose–response function for toxicity of ultraviolet radiation (UVR) to the crystalline lens.

Most toxicology data are based on experiments that assume a binary dose–response function. The toxic effects are expressed as percent incidence occurring in an exposed population (Brown 1980). The dose causing a certain incidence of effect is called the 'effective dose' (ED). The percentage of effect is given as subscript. For example, if an effect is produced in 10% of the exposed population, it is written as ED_{10}.

The most common method for estimating the ED is described as probit analysis, developed by Finney (1971). This is a statistical theory for threshold estimation for quantal responses. The method describes 'effect' or 'no effect' after a certain dose of a toxic agent. The response is illustrated by plotting the proportion of the sample responding (ordinate) as a function of dose (abscissa). Such dose–response curves have a narrow sigmoid shape. In probit analysis, the ordinate is substituted by a probability unit ('probit') and the abscissa is converted to log-scale (Finney 1971; Brown 1980). By doing so the sigmoid curve is transformed into a straight line, and the ED for different percentages of incidence can be easily estimated.

Current safety limits to avoid cataract after exposure of the eye to UVR are based on an experiment, designed assuming that there is a discrete dose limit below which there is no cataract and above which there is (Pitts et al. 1977). Clinical experience and a safety factor of 10 are usually incorporated into the safety limits. For example, the American Conference for Governmental and Industrial Hygienists (ACGIH 1996) has published safety recommendations as relative spectral effectiveness data, together with guidelines for calculating an effective irradiance or dose as a threshold limit or a maximum exposure time.

Cataract is the clinical term for reduced visual function due to optical disturbances in the crystalline lens which scatter light. Forward scattered light reduces contrast and blurs the retinal image; backward scattered light may be apparent to an observer as a varying degree of discolouration in the pupil of the eye. The crystalline lens always expresses some baseline scattering, even when visual function is normal, but the degree of baseline scattering

varies from one individual to another. In an experiment with 60 untreated animals, it was found that the baseline scattering in rat lenses varies from individual to individual in accordance with the normal distribution (Söderberg et al. 1990). In the present paper, non-pathological lenses with normal baseline scattering are described as 'physiologically clear lenses'.

Epidemiological studies (Taylor 1990; Cruickshanks et al. 1992) and experimental studies in rats, mice, rabbits and squirrels (Pitts et al. 1977; Jose & Pitts 1985; Söderberg 1988, 1990; Zigman et al. 1991; Hightower & McCready 1993; Breadsell et al. 1994) show a relationship between UVR exposure and subsequent lens opacities. Morphologically, these events correspond to swelling and disruption of lens epithelial cells and cortical lens fibres (Söderberg 1988; Breadsell et al. 1994). Swollen mitochondria and subcapsular vacuoles as well as chromatin condensation and nuclear fragmentation are found in the epithelium (Söderberg 1988). Epithelial hyperplasia is observed following longterm or above-threshold exposure (Söderberg 1988; Wegener 1994).

Epidemiological studies have the advantage of investigating the influence of UVR exposure over the longterm, with results that can be directly applied to the human in a general sense. It is still difficult, however, to calculate the dose of UVR exposure for each individual subject over decades of exposure. Although dosimetry in experimental exposure on animals can be well controlled and cofactors for cataract can be excluded, the results must then be translated to the human.

Our group has focused on *in vivo* exposure in rats and analyses of forward light scattering in dissected lenses. The intensity of forward light scattering is measured with the light dissemination meter developed by Söderberg et al. (1990). This instrument uses the principle of dark-field illumination, in which the illuminating light transilluminates a transparent object at such a flat angle that it cannot enter the objective aperture. If the object contains scattering particles, the transillumination light is scattered. Light that is scattered in the forward direction at an angle of 45° reaches the objective. Light within an angle of 5° is collected by the objective and is measured by a photodiode (Söderberg et al. 1990; Michael 2000). The scattering standard was a lipid emulsion of diazepam (Diazemuls, KabiVitrum, Stockholm, Sweden), with the unit of measure therefore expressed as transformed Equivalent Diazemuls Concentration (tEDC) (Söderberg et al. 1990). Typical values for normal and very opaque rat lenses are about 0.1 tEDC and about 1 tEDC, respectively.

The maximum response of the crystalline lens to one-dose UVR peaks 1 week after exposure (Söderberg 1990; Michael et al. 1996), and the relationship between UVR dose and cataract is continuous and may be expressed as a sigmoid function (Fig. 1) (Michael et al. 1998; Michael 2000).

The aim of the present paper is to derive an experimental method to objectively determine an MAD for continuous dose–response relationships, using UVR-induced cataract as an example. The general concept consists of three steps as follows:

(1) setting a lower limit of pathological light scattering for normal lenses from non-exposed eyes;

(2) getting the experimental dose–response function from the contralateral exposed eyes, and

(3) estimating the maximum acceptable dose with this data.

Material and Methods

Female Sprague-Dawley rats (6 weeks old, 150 g) were used as the experimental animals. Altogether, 20 rats were divided into five groups of four receiving 0, 1, 2, 4 or 8 kJ/m^2 unilaterally. The 0 kJ/m^2 group of rats were anaesthetized and put on the exposure bench, but given no UVR.

The animals were anaesthetized with xylazine (14 mg/kg) and ketamine (94 mg/kg) in saline solution by intraperitoneal injection 10 mins prior to exposure. Both pupils were dilated with tropicamide (5 mg/ml) 5 mins prior to exposure. One eye was exposed to UVR 300 nm from a high pressure mercury lamp filtered with a water filter and spectrally limited by a monochromator (full width at half maximum: 9 nm) (Michael 2000).

One week after exposure, the rats were killed and all eyes were enucleated. Each lens was removed by a posterior scleral incision and placed in balanced salt solution (BSS) in a Petri dish. With a dissecting microscope, remnants of the ciliary body were removed, taking care to avoid damage to the lens. Immediately thereafter the lens was put into a receptacle to record the intensity of forward light scattering quantitatively under dark-field illumination (Söderberg et al. 1990). The measurements of forward light scattering were calibrated to concentration of a commercially available standard scattering solution (Stesolid Novum, diazepam, Kabi Pharmacia, Stockholm, Sweden) and are therefore given in transformed equivalent Diazemuls concentration (tEDC) (Söderberg et al. 1990).

The crystalline lens always expresses some baseline scattering. This level of light scattering of physiologically clear

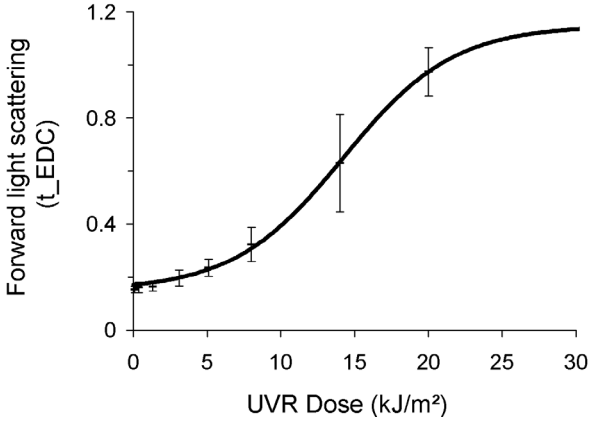

Fig. 1. Dose–response function for UVR-induced cataract from earlier experiments. Bars are the 95% confidence interval for the mean of forward light scattering in the crystalline lens 1 week after *in vivo* exposure to UVR. Sample size was $n = 20$ for values at 5 and 20 kJ/m^2 and $n = 10$ for all other doses. Data are fitted to a logistic model (solid line) and are sourced from Michael et al. (1996), (1998).

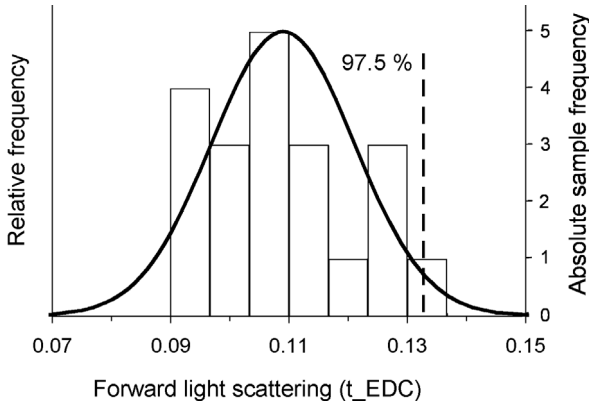

Fig. 2. Non-exposed contralateral lenses from the current experiment (histogram, $n = 20$) and estimated frequency distribution for forward light scattering in crystalline lenses (bold line). The end-point for pathological light scattering has been set such that 97.5% of normal physiologically clear lenses scatter less.

lenses varies from individual to individual in accordance with the normal distribution (Söderberg et al. 1990). For this reason, the lower limit or beginning of pathological light scattering must be defined statistically.

The distribution of the baseline scattering was estimated from the lenses of the non-exposed eyes of all 20 rats (histogram in Fig. 2). From these data, the mean and standard deviation were calculated to get the equivalent normal distribution function (solid curve in Fig. 2). The lower limit of pathological light scattering is then defined as the maximal level of light scattering that a certain fraction of normal physiologically clear lenses express. The selection of this fraction p is arbitrary; for this purposes of this paper we have used 97.5% (Fig. 2).

The mean and standard deviation for intensity of forward light scattering in normal physiologically clear lenses can be estimated with sufficient precision if the sample is large enough. From the estimated mean, μ (unit here tEDC), and standard deviation, σ (unit here tEDC), the beginning of pathological light scattering, y_{limit} (unit here tEDC) can be calculated based on the normal distribution function Z (Zar 1999) (equation 1):

$$y_{limit} = \sigma Z(p) + \mu$$

In search of an index for toxicity of UVR on the crystalline lens, only the low dose region of the dose–response function (Fig. 1) is of interest. In a small sample experiment, the low dose region of the dose–response function for UVR-induced cataract may be modelled with a second order polynomial, omitting the first order term (equation 2):

$$y = a + kx^2$$

Here, y is the dependent variable expressing intensity of forward light scattering (unit here tEDC) and x is the independent variable expressing dose (kJ/m^2). The parameters a and k can easily be estimated in a small sample experiment designed for regression.

If the limit for pathological light scattering y_{limit} (equation 1) is projected on the dose–response function y (equation 2) obtained with regression analysis of experimental data, the dose corresponding to that point can be estimated. We suggest that this dose represents the maximum acceptable dose (MAD).

Results

The mean and standard deviation for the frequency distribution for physiologically clear lenses were estimated from the contralateral non-exposed lenses. The estimated mean was $\mu = 0.109$ tEDC, and the standard deviation, $\sigma = 0.0121$ tEDC. The limit for pathological forward light scattering such that 97.5% of normal lenses express less light scattering was estimated (equation 1) to be 0.133 tEDC (Fig. 2).

The fit for the polynomial regression (equation 2) and the experimental data for the exposed lenses were good (Fig. 3).

The parameters for the regression were estimated as follows: $a = 9.61 \cdot 10^{-2}$ with $SD = 2.53 \cdot 10^{-2}$ and $k = 7.46 \cdot 10^{-3}$ with $SD = 8.76 \cdot 10^{-4}$ from a sample where $n = 20$. It should be noted that the variance increases with increasing levels of intensity of forward light scattering.

When the beginning of pathological forward light scattering was projected on the dose–response curve, $MAD_{0.975}$ was found to be 2.2 kJ/m^2 (Fig. 4).

Discussion

This paper presents a method to estimate quantitatively and objectively the toxicity of UVR to the lens. The estimation is illustrated for *in vivo* irradiation of rats to UVR and subsequent measurement of light scattering in dissected lenses. Light scattering measurements in dissected lenses allow the measurement of light scattering in

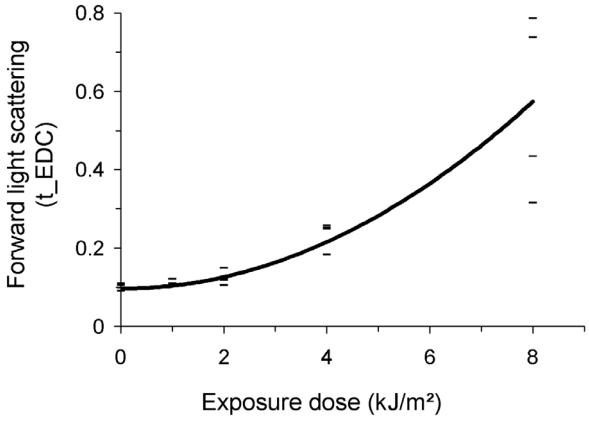

Fig. 3. Dose–response function of UVR cataract from the current experiment. Data of 20 exposed lenses (dashes) and fit of the data (solid line). There were four lenses for each UVR dose group, including one group with 0 kJ/m^2. ($y = 9.61 \cdot 10^{-2} + 7.46 \cdot 10^{-3}\ x^2$)

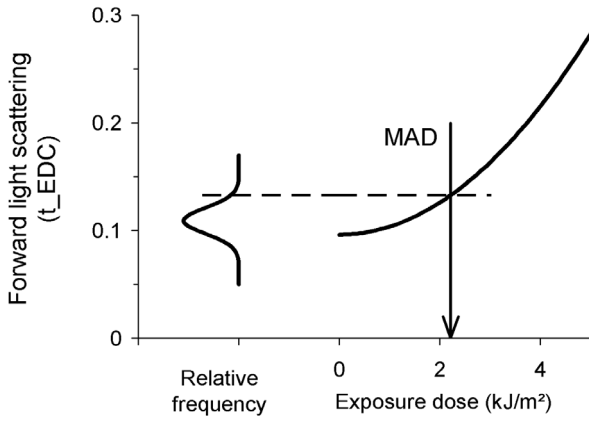

Fig. 4. Estimation of $MAD_{0.975}$. The limit for pathological forward light scattering derived from the 20 non-exposed lenses (left: relative frequency) is projected (dashed line) onto the dose–response function from the 20 exposed contralateral lenses of the same animals (right). The intersection gives the MAD, here 2.2 kJ/m^2 (arrow).

the forward direction under constant conditions. Compared to rabbit lenses, rat lenses are much less sensitive to dissection trauma. However, to minimize variability, dissection was randomized between exposed and contralateral eyes and light scattering was always measured immediately after dissection.

Baseline values for measured forward light scattering may vary between experimental studies. Figure 1 shows data from previous studies (Michael et al. 1996, 1998), where non-exposed lenses had 0.15 tEDC, and Fig. 3 shows data from the current study, where non-exposed lenses had 0.11 tEDC. Such differences are coursed by systematic variations between experiments such as batch of rats, different calibration of dosimetry and light scattering measurements. Our suggested method takes these systematic variations into account by using the non-exposed contralaterals as controls. It is a relative method that compares the non-exposed lenses with the regression line of the exposed lenses, in this way excluding systematic variations of baseline values. Previously, it was shown that there is no contralateral effect for UVR rat exposure (Michael et al. 1996).

In the current experiment, the sample size was 20 rats. Therefore, the estimates of the mean and variance of forward light scattering of normal physiologically clear lenses have limited precision. With increasing sample size, the estimates would converge against the population values. However, it has been shown previously (Söderberg et al. 1990) that when considering variability among rats, 20 animals is sufficient to estimate the dose–response function. In the experimentally derived dose–response relationship (Fig. 3), the variation increases with increasing intensity of forward light scattering, which is a common phenomenon because the measurement error is usually proportional to the signal. In this respect, the number of four animals in the high dose group seems low. However, for the purposes of estimating the regression line, the data of all 20 exposed lenses are considered. For a correct statistical estimation of the residual variance this has to be modelled as dependent on the intensity of forward light scattering.

There may be three types of idealized dose–response function (Sliney & Wolbarsht 1980). First, there may be a function with a threshold. Here, low doses induce no effect. At a certain dose there is a sharp increase of the dose–response function to response. Higher doses do not induce more response. Because of variations between individuals, this dose–response function can become stochastically modulated. The probability curve for response as a function of dose is then a sigmoid. Finally, there may be a continuous dose–response relationship. Here, increasing doses induce continuously more severe responses. Any single exposure would have a finite effect and a cumulative exposure would increase the response; the curve for response as a function of dose is then a sigmoid.

The development of an action spectrum for UVR-induced cataract based on slit-lamp observations and assuming a binary dose–response model (Pitts et al. 1977) represented an important step in terms of the design of safety standards. However, as the dose–response relationship for UVR-induced cataract was later found to be continuous (Michael et al. 1998), it is preferable to model the dose–response function of UVR-induced cataract as continuous. Moreover, in an epidemiological study, Sasaki (1997) found an exponential increase in lens opacities with increasing age. This continuous increase was found for the entire lens, but was most pronounced for the lens cortex.

The $MAD_{0.975}$ for rat lenses was found to be of the same order of magnitude as the threshold for permanent cataract stated for rabbits by Pitts et al. (1977).

It should, however, be kept in mind that the rabbit (and human) cornea is thicker than the rat cornea and therefore should filter out more UVR. The present MAD for rats – 2.2 kJ/m^2 incident on the cornea – corresponds to a dose incident on the lens of 0.97 kJ/m^2 in the rat and 0.44 kJ/m^2 in the human (Michael 2000). Another important aspect to be considered when translating UVR safety data between species is pigmentation. We have estimated the MAD in non-pigmented rats and it will probably be different in pigmented animals.

In the present paper, the estimation of the maximum acceptable dose was illustrated by an experiment of acute *in vivo* irradiation of rats and subsequent measurement of light scattering in dissected lenses. Therefore, the results are not directly transferable to humans. As it is impossible to expose humans to UVR to induce cataract, safety limits for UVR-induced cataract have to be based on animal experiments. A continuous record of clinical data of UVR damage has to be considered additionally in order to state public safety thresholds.

The maximum acceptable dose depends on the definition of the lower limit of pathological light scattering – in other words, the maximal level of light scattering that a certain fraction of normal physiologically clear lenses express. We suggest using 97.5% for this fraction (Fig. 2). However, the proposed method allows the freedom to choose this value in accordance with the objectives of the safety estimation under consideration. The MAD strategy allows for the objective, quantitative estimation of the threshold dose for

the first time. It represents, therefore, an important step towards well defined safety standards for continuous dose–response functions, such as UVR-induced cataract.

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