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LASER HAZARDS:
BIOMEDICAL THRESHOLD LEVEL INVESTIGATIONS

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"In conducting the research described in this report, the investigators adhered to the 'Guide for Laboratory Animal Facilities and Care' as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care, of the Institute of Laboratory Animal Resources, National Academy of Sciences - National Research Council."
The review of current research data and areas requiring future investigation delineates project areas of prime interest. Through research, laser safety standards have been developed. Current studies are fulfilling the immediate requirements associated with the test, development, and deployment of new laser devices. The establishment of various biological and functional criteria, which accurately reflect the changes associated with laser irradiation of the eye and skin, continues to provide valuable information on current and proposed safety standards.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TABLE OF CONTENTS</td>
<td>i</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>BIOMEDICAL DATA: OCULAR EXPOSURE</td>
<td></td>
</tr>
<tr>
<td>Exposure Conditions</td>
<td>2</td>
</tr>
<tr>
<td>Response Criteria</td>
<td>3</td>
</tr>
<tr>
<td>Visible Changes</td>
<td>4</td>
</tr>
<tr>
<td>Histological Changes</td>
<td>4</td>
</tr>
<tr>
<td>Functional Changes</td>
<td>4</td>
</tr>
<tr>
<td>DAMAGE MECHANISMS</td>
<td>5</td>
</tr>
<tr>
<td>BIOLOGICAL DATA AND MAXIMUM PERMISSIBLE EXPOSURE</td>
<td>6</td>
</tr>
<tr>
<td>DATA FROM LASER RESEARCH: SKIN EXPOSURES</td>
<td>8</td>
</tr>
<tr>
<td>STANDARDS AND FUTURE RESEARCH</td>
<td>9</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>10</td>
</tr>
<tr>
<td>FIGURE 1</td>
<td>12</td>
</tr>
<tr>
<td>TABLE I</td>
<td>13</td>
</tr>
<tr>
<td>DISTRIBUTION LIST</td>
<td>14</td>
</tr>
</tbody>
</table>
INTRODUCTION

A laser is a source of highly collimated monochromatic optical radiation in pulse durations ranging from $10^{-11}$ second (mode-locked laser) and $10^{-8}$ second (Q-switched laser) to continuous wave (cw) emission laser. Some wavelengths are readily identified by laser names, e.g. 488.0 and 514.5 nm (argon laser), 632.8 nm (HeNe laser), 694.3 nm (ruby laser), 905 nm (GaAs laser), 1060 nm (neodymium laser), and 10600 nm (CO$_2$ laser). The low beam divergence of the laser provides the capability of accurate irradiation of specified targets at long range. Through appropriate time modulation of the laser output, the signal reflected from the target can be used for rangefinding, tracking, or guiding missiles. When the target is equipped with a detection system, and the laser is restricted to low output levels, it becomes a communications device or a direct fire simulation training device.

The present and projected military uses of lasers and laser devices are increasing as new requirements are generated. Hazards accompany the use of these devices. In field situations, sudden loss of vision, sudden sensation of glare, or sudden sensation of heat may be hazards caused by laser beams. Long-term vision problems could be experienced by persons subjected to the continuous viewing of low-level laser radiation, such as in holography, ranging, pattern making, and other jobs in military and industrial settings. A thorough knowledge of immediate and long-term biological effects of laser and laser devices is essential to establish permissible exposure levels.

Through research, laser safety standards have been developed.$^1$-$^3$ Current studies are fulfilling the immediate requirements associated with the test, development, and deployment of new laser devices. The establishment of various biological and functional criteria, which accurately reflect the changes associated with laser irradiation of the eye and skin, continues to provide valuable information on current and proposed safety standards.

The subjects used in the majority of the research have either been rhesus monkey (ocular exposures) or pigs (skin exposures). Occasionally, other animal species have been used when the use of the nonhuman primate (rhesus monkey) would be prohibitively expensive and when other vertebrate ocular systems would suffice. The eye and the skin are the two biological systems most frequently affected by lasers. More intensive research has been done on the effects to the ocular mechanism than to the skin. Therefore, the greater portion of this

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$^1$Department of the Army Regulation No. 40-46, 1974.


paper will be devoted to the research studies in the eye -- the biomedical data, damage mechanisms, and correlation of biological data and safe operating levels. A lesser portion of the paper will provide a description of the research studies on laser hazards to the skin and it will conclude with proposals for future research as we now see it.

This is an overview of laser safety research. The report is written for military physicians who have only superficial knowledge and understanding of lasers and military uses of lasers.

BIOMEDICAL DATA: OCULAR EXPOSURE

Exposure Conditions

Most experiments have been designed to irradiate anesthetized animals in a setting in which laser exposures are precisely placed under the visual (funduscopic or biomicroscopic) control of the investigator. The animal's pupils are maximally dilated and their ocular systems rendered incapable of changing focus. A typical laser eye exposure system is shown (Fig. 1) in which a Q-switched ruby laser source is used. The retina is viewed with a fundus camera. A gonioplatform aids in the precise alignment of the rhesus eye. A calibrated ultrafast detector coupled to an oscilloscope is used to determine the absolute energy incident on the cornea. The intensity of the exposure is controlled by bulk neutral density filters. A low powered HeNe laser is aligned collinearly with the Q-switched ruby laser in order to locate the placement of the exposure at the desired retinal site. A laser blocking filter located in the fundus camera optics protects the investigator from accidental exposure to the laser source.

Various laser wavelengths through the visible, near infrared, and far infrared spectral regions have been studied with essentially the same exposure arrangement. These experiments have resulted in the establishment of dose response relationships for direct observation criteria.4-8


In some recent experiments, several approaches for exposing awake subjects have been devised. Unlike the previous laser exposure system, these approaches do not involve alteration of normal optical characteristics of the eye by pharmacological agents. In most of these experiments, trained (operant-conditioned) animals were employed.

Response Criteria

Several criteria are used to evaluate the results of laser exposures. Direct observation of the irradiation site has been the primary method of evaluating changes in the cornea or retina. In these experiments, the criterion for damage has been visible change one hour or 24 hours after exposure.

At a more subtle level, a second criterion has been used. Light and electron microscopic studies of retinal and corneal tissue have been used not only to investigate specific cellular damage mechanisms but, in many cases, to confirm the presence of cellular changes in cases where no gross morphological changes were previously noted following irradiation.

A third criterion of change resulting from laser irradiation is functional. The presence of either gross or microscopic changes in the eye indicates that morphological changes have occurred. Such changes, however, do not reveal the effect on the ability to "see." Different techniques are required to answer questions concerned with change in function. Visual acuity (Landolt ring), spectral sensitivity, and dark adaptation are currently being studied. We have used functional criteria also in measuring the ability of rhesus monkeys to detect CO\textsubscript{2} laser irradiation. All of these measurements involve behavioral and electrophysiological methods.

For all but behavioral and some electron microscopic criteria, a probit analysis has been performed. The presence or absence of changes in appearance of the target area is noted for each irradiance studied. The ratios of observed responses to the total number of exposures at given doses are then plotted on logarithmic probability paper where the ordinate is probability (percentage observed) and the abscissa reflects the dose.

References:


From the plot, the ED$_{50}$ (effective dose required to produce an observable response 50 percent of the time) is obtained. Confidence intervals about the dose response curve are calculated. Because of the experimental design the ED$_{50}$ has greatest statistical significance and is often referred to as the damage threshold.

Visible Changes

Dose response data have been obtained in this and other laboratories for a large range of wavelengths, exposure duration, and irradiance diameters. The ED$_{50}$ levels obtained for visible criterion show general dependencies on each of these variables. The ED$_{50}$ energy levels for damage by shorter wavelength lasers is lower than that for longer wavelength lasers. For Q-switched exposure the ED$_{50}$ at a wavelength of 530 nm is approximately five times lower than for 694.3 nm and 25 times lower than for 1060 nm. The total intraocular energy increases with exposure duration for the range from 10 usec to 10 seconds. The threshold retinal radiant exposure is inversely related to retinal image diameter over a range of 40 to 1000 microns. This dependency holds over a wide range of laser exposure durations and wavelengths.

Histological Changes

The ED$_{50}$ required to produce a minimal disturbance of retinal pigment epithelium is 20 to 30 percent lower when light microscopic examination is used than when a fundusscopic observational criterion is used. This finding has been confirmed for both Q-switched ruby (694.3 nm) and cw argon (514.5 nm) laser sources. Transmission electron microscopy has further shown that marked alterations of the photoreceptors are produced at irradiation levels approximately ten times lower than the funduscopically derived ED$_{50}$ for Q-switched ruby laser exposures. Current long-term follow-up studies of more than a year post exposure indicate that such changes are relatively permanent. The threshold for these ultrastructural changes has not been determined but it is known to be many times below the ED$_{50}$ for the visibly observed opacity.

Functional Changes

Foveal irradiation at suprathreshold levels involves an immediate decrease of Landolt visual acuity in the rhesus monkey. Long-term follow-up of irradiated animals from six months to several years after exposure suggests that while significant visual acuity "recovery" may occur over time, tests of rhesus spectral sensitivity indicate a significant loss of photopic color vision. In addition, several ongoing investigations indicate that low-level chronic laser exposures at 514.5 nm can produce long-term alteration of photopic spectral sensitivity. Concurrent electrophysiological investigations of retinal function indicate that neural inhibitory connections are apparently affected at irradiance levels at which permanent functional changes are
first seen to occur. Preliminary evidence suggests that permanent alterations in photopic spectral sensitivity can occur at levels at least ten times lower than those promulgated as being safe for extended source viewing. The low limits of these data are still under investigation.

Results of studies in which rhesus monkeys have been trained to detect exposure to CO₂ (10600 nm) laser radiation indicate that the cornea is much less sensitive to CO₂ laser radiation than the skin when behavioral criteria are used.¹⁰

DAMAGE MECHANISMS

Essentially, two types of damage mechanisms have been postulated to explain laser tissue interactions. These are generally classified as "thermal" and "non-thermal." Thermal mechanisms involve either denaturation of protein or deactivation of critical enzyme systems.¹⁵ The most vulnerable tissue to a specific laser wavelength is determined primarily by the absorption properties of the tissue. The retina is particularly vulnerable to collimated laser light in the visible and near infrared region of the spectrum because the light is transmitted by the ocular medium and focused to a small spot on the retina. For far infrared wavelengths, the cornea is susceptible since most of the incident energy is absorbed in the first hundred microns of tissue. Within the exposed tissue, the light energy is absorbed and for the most part converted into heat. This thermal episode creates a time-temperature history at each point within the tissue where denaturation of protein or enzymatic changes can occur at specific rates. For visible and infrared laser exposure durations from 10 msec to 5000 msec, a maximum temperature rise of 20 to 35°C is required to produce an observable "burn."¹⁵ Such mechanisms are adequate to explain much of the existing direct observational data and some gross histological and functional findings. However, such mechanisms are not adequate to explain permanent tissue or functional changes which occur at irradiation levels where temperature changes of less than 1°C are involved.⁶,⁹ While there is no currently acceptable non-thermal explanation for low irradiance effects, some evidence exists to suggest that photochemical and/or photoreceptor processes are intimately involved. Selective spectral changes at such levels suggest that these effects may be specific to certain photoreceptor types and their synaptic linkages.

The retinal radiant exposure (joules/cm²) required to produce an observable lesion for large retinal irradiance diameters (1000 microns) is lower than for smaller retinal irradiance diameters (50 microns). This spot size dependence is partially explained by heat flow. Heat

flow away from the center of a small irradiated spot may significantly relax the thermal stress on the area. Thus a higher radiant exposure would be required for a minute spot than for large irradiance diameters where heat flow to the center area from the nearby irradiated surround is sufficient to negate the heat flow away from the area. This spot size dependence has been reported for exposure durations from $10^{-8}$ seconds to several seconds.\textsuperscript{5}

Q-switched ruby and neodymium lasers produce single pulses of 20 $\times$ $10^{-9}$ second duration. In these exposures, the retinal heat flow occurs after the laser exposure is complete. The photon energy absorbed by the melanin in the pigment epithelium is transferred to the surrounding tissues. Observed tissue effects are the result of this thermal insult as well as to the mechanical rupture of cell structure from the acoustic or shock components of the brief pulse. Localized ionization effects may also contribute to the degradation of biological processes.

Non-thermal effects can be thought of as any biochemical or biophysical change within the photoreceptor which may change the cell without appreciable change in temperature above the ambient range. For example, direct absorption of laser radiation may produce shock waves which disrupt lamellar membranes. Laser light may saturate the photochemical process of the rhodopsin cycle producing intermediate chemical blocks.

In many laser systems the output is not a simple cw or "one-shot" burst of energy, but is composed of many short pulses in a train of pulses. Each component spike in this train may have cumulative effects upon the tissue. With laser systems such as gallium arsenide (905.0 nm), and in recent experiments in which neodymium, ruby, and argon lasers were used, circular retinal lesions have been produced with a vertically elongated retinal image.\textsuperscript{13} The configuration of the beam, thus, does not match the observed lesion. The explanation of this and other inconsistencies must be determined in order to define the mechanisms of damage.

**BIOLOGICAL DATA AND MAXIMUM PERMISSIBLE EXPOSURE**

Dose response data have been obtained for many different laser exposure conditions by several different laboratories. From these data, from known environmental light levels, and from literature delineating broadbanded non-ionizing radiation effects, the maximum permissible exposures (MPE) for humans have been derived.\textsuperscript{15} In general, the MPE was obtained by dividing the ED$_{50}$ obtained for the "worst" case condition using direct observational criteria by a factor of 10 to 100. The "worst" case condition might be defined as that experimental condition for a given wavelength and exposure duration where the least amount of energy per pulse is required to produce the effect. For example, for
intrabeam viewing of visible laser radiation, the "worst" is produced when the maximally dilated emmetropic eye focused the light to a minimal spot (≈ 50 microns) in the macular region of the retina. The rationale for the factor of 10 to 100 was based on many considerations such as the slope of the dose-response curves, the relationship between the direct observational ED50 and the dose required to produce morphological or functional changes, and the applicability of the animal model to human exposure. For exposure conditions where no bioeffects data are available, a continuous curve was extrapolated that was consistent with trends exhibited by the available data and the absorption characteristic of the tissue involved. MPEs were derived for the intrabeam viewing and viewing of an extended source for the wavelength region from 200 nm to 10^6 nm and exposure durations from 1 x 10^{-9} to 3 x 10^4 seconds.\textsuperscript{1,2}

The military documents which propagate maximum permissible exposures (MPE) are Army Regulation 40-46\textsuperscript{1} with its supportive document TB Med 279\textsuperscript{2} and Air Force Manual 161.\textsuperscript{3} In addition, the ANSI Standard 7.136 and the Bureau of Radiological Health's Laser Performance Standard provide MPEs and maximum accessible emission limits. From these documents, the "safe" viewing envelope can be calculated and the classification of a given laser system can be determined so that the required safety procedures can be followed. With some exceptions the permissible exposure values promulgated by these documents agree because essentially the same data has been used for their derivation.

Documents which provide MPE data for intrabeam viewing of a laser beam report the corneal radiant exposure (joules/cm^2) as a function of wavelength and exposure duration. When extended source criteria are met (i.e., the angle subtended by the source exceeds the designated value), the maximum permissible integrated radiance (watts/cm^2 steradian) or the irradiance (watts/cm^2) incident on the diffuse target is given. These directly measurable quantities are useful when evaluating a given laser exposure situation; however, the relationship between these values and the existing bioeffects data are not immediately obvious. As an example, if one considers the relationship between the MPE and the bioeffects data obtained for a Q-switched ruby laser (wavelength, 694.3 nm) with pulse duration of 30 nsec, the MPE incident upon the cornea is 0.5 \mu joulles/cm^2.\textsuperscript{1,2}

A total intraocular energy (TIE) of 20 \mu joulles (i.e., ED50) is required to inflict a paramacular retinal lesion which can be observed one hour after exposure. The TIE is defined as the total energy incident upon the cornea in a beam smaller than the pupillary aperture. In this situation where a lesion of minimal retinal irradiance diameter is produced, the beam diameter at the cornea is 3 mm. In the same situation, the corneal radiant exposure is 283 \mu joulles/cm\textsuperscript{2} (over 500 times the MPE). If the beam diameter at the cornea were 8 mm and the ED50 (TIE) were 24.7 \mu joulles, the corneal radiant exposure would be 49 \mu joulles/cm\textsuperscript{2} (100 times the MPE). For these two sets of conditions,
the retinal radiant exposure was comparable, although the corneal radiant exposure and apparent safety factor differed by a factor of 6. The ED$_{50}$ TIE for macular exposure is approximately one half the para-macular ED$_{50}$.

This reduces the safety margin to a factor of 50 when comparing the safe corneal radiant exposure to that required to produce a macular lesion 50 percent of the time.

Further dose-response data were obtained by using a large retinal diameter (1000 microns). The ED$_{50}$ for the direct observation of a retinal lesion is 200 μjoules.

When this dose was decreased by a factor of 10, no ophthalmoscopically visible lesions were observed; however, definite changes in the outer segments of the photoreceptors were demonstrated by electron microscopy.

A summary of these data along with permissible exposure levels is given in Table I. The corneal radiant exposure for the production of a visible lesion in a 50 micron retinal spot is approximately the same as that required to produce an electron microscopically visible change in a 1000 micron retinal irradiance. If this is compared to the intrabeam MPE, it yields the same safety margin even though the effect and the calculated retinal radiant exposure are drastically different.

Since the retinal image diameter of 1000 micron subtends an angle greater than the minimum required for using extended source criterion, one can compare the TIE from a diffuser that is irradiated at the MPE for an extended source and imaged to 1000 microns on the retina. For a 7 mm pupillary aperture, the TIE is 41.7 μjoules. The results of this calculation are also shown in Table I. The TIE and the retinal radiant exposure are at levels two times above those where electron microscopic changes were reported. Using these calculations, we see that the ultrastructural alterations are at levels below those currently accepted as safe when we use extended source criterion. The interpretation of the "safe level" must therefore be used with caution.

**DATA FROM LASER RESEARCH: SKIN EXPOSURES**

The military is presently developing high energy infrared laser systems for the engagement of military hardware. These systems pose definite hazards to the skin at great distances. Dose-response relationships have been determined for some exposure conditions. From these data the MPEs for the irradiation of the skin have been derived.

Laser skin effects have been studied on porcine and human skin. The response criterion most often used to describe the minimal effect is the observation of a minimal erythema observed from 4 to 24 hours after the exposure. Threshold doses for skin changes vary with exposure duration, wavelength, and the degree of pigmentation of the skin. The skin is particularly vulnerable to exposure in the ultra-
violet region of the spectrum. Doses required to induce the erythemic response have been determined for exposure durations from 1 msec to 40 sec for CO₂ laser radiation at 10600 nm. The visual response for the doses used ranged from no effect to the production of immediate blisters or spotty white burns. Using a 100 nsec CO₂ laser exposure, we observed complete ablation of the stratum corneum and upper epidermis at doses less than three times that required to produce a minimal erythema. Light microscopy revealed changes from minimal epidermal to deep dermal effects. In general the appearance of infrared laser burns are similar to typical clinical observed burns.

STANDARDS AND FUTURE RESEARCH

Currently laser safety standards have been criticized by military systems developers and the laser industry. They cite the standards as being "too conservative" and complex. We made a comparison of the standard for a specific wavelength (694.3 nm) and pulse duration (30 nsec) where bioeffects data are available. If one is concerned about ultrastructural changes to the retina from a single exposure, the present standard is certainly not too conservative for this particular case. Extrapolation to other exposure conditions is difficult, consequently, more bioeffects data are needed and an understanding of the underlying mechanisms is required.

Future areas of research will involve a concerted effort to establish the relationship between light and retinal photoreceptors. Laser effects at ultra-low levels may be a function of direct absorption of the energy in more superficial retinal layers. Repeated laser exposures may effect, in a cumulative fashion, the ability of the retinal or visual system to recover. If current ultrastructural data indicate persistence of retinal change years after initial exposure, the significance of the changes on visual function must be demonstrated. The appearance of retinal swelling or clouding may be as injurious to function as frank retinal opacity and this must be investigated. The chronic effects of repeated laser exposure of the skin have not yet been clearly defined. The research must provide the military surgeon with the clinical knowledge which may be necessary to him in the evaluation of possible laser injury.
REFERENCES


Comparison of dose response data for o-switched ruby exposures to the MPE

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<thead>
<tr>
<th>Diameter</th>
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**Abbreviations:**
- EM = electron microscopy
- MPE = maximum permissible exposure
- ED50 = effective dose for the observation of a response

*Experimental corneal beam diameter (pupillary diameter, mm).*
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