Brian C. Gilger · Cynthia S. Cook
Michael H. Brown *Editors*

Standards for Ocular Toxicology and Inflammation

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Preface

This book was developed and is endorsed by the Pharmaceutical/Toxicology Committee of the American College of Veterinary Ophthalmologists (ACVO), whose membership consists of board-certified veterinary ophthalmologists with interest and experience in the pharmaceutical and toxicology industry.

The mission of the ACVO pharmaceutical/toxicology committee is to increase awareness of the pharmaceutical industry and contract research organizations (CROs) about the specialty of veterinary ophthalmology, to protect the public by encouraging participation of trained veterinary ophthalmologists in studies of investigational drugs and devices, and to establish standards for ophthalmic examinations in pharmaceutical and toxicological studies. This book is being developed to assist in this mission, especially to develop a standard for conducting ophthalmic studies.

This book is a consensus document to provide standards and harmonization for procedures, terminology, and scoring schemes for ocular toxicology studies. This information will be used by industry, pharmaceutical companies, and government agencies to help improve the drug development process and to reduce and refine the use of animals in research.

The purpose of Chap. 1 is to review laboratory animal ophthalmic examination procedures and techniques as it pertains to the pharmaceutical industry and preclinical research studies and to develop standards for the conduct of these examinations.

In Chap. 2, the authors provide harmonized protocols for commonly performed ophthalmic procedures in laboratory animals, including techniques such as intracameral, intravitreal, subconjunctival, retrobulbar, and subretinal injections, to assist in development of institutional standard operating procedures (SOP) documentation. Having similar protocols and SOPs among researchers and institutions will allow better comparison between studies, more efficient use of animals, and enhance the quality of ocular research.

The goal of Chap. 3 is to provide a consensus for the harmonization of preclinical terminology for ultimate adoption for studies submitted to the United States Food and Drug Administration (FDA) and other global regulatory agencies. Provided is a list of terminology for each anatomical section of the eye with definitions, synonyms, and justification for the descriptive-based terminology recommended for the harmonization process, including a number of representative lesion images.

Chapter 4 provides incidence data on spontaneous ophthalmic abnormalities in the most commonly used species compiled from CROs in North America. This data will allow differentiation between test article related ocular findings and background incidental lesions and thus enhance interpretation of ophthalmic findings, improve speed of drug development, reduce the number of studies that need to be repeated, and reduce the overall number of animals used in toxicology research and drug development.

Chapter 5 provides a comprehensive review of the approaches and methods used to perform clinical ocular scoring of the ocular anterior and posterior segment in laboratory animals in toxicologic and preclinical drug development studies. Following this review, there is an introduction of an enhanced scoring scheme modified from previous systems to improve the applicability and predictive value of clinical observations made in support of modern preclinical ocular drug and device development programs.

ACVO diplomates act as consultants to sponsoring pharmaceutical companies or CROs in performing ophthalmic examination of animals. These studies are designed to evaluate the potential for ocular toxicity or other adverse effects arising from the systemic, topical, or other administration of drugs or compounds, the application of medical devices, or certain surgical procedures. The ACVO recognizes that insuring public safety is the goal of such studies and that diplomates of the ACVO have unique training and experience in ophthalmology in a variety of animal species that make them exceptionally qualified for conducting ophthalmic examinations in such studies. A diplomate of the American College of Veterinary Ophthalmologists (Diplomate, ACVO), in addition to being a licensed veterinarian, has completed a minimum of 3–5 years of postgraduate specialty training in veterinary ophthalmology. This training encompasses the diagnosis and treatment of eye conditions in a variety of animal species, including those commonly used in toxicological testing. The ACVO is the only credentialing body in North America that supervises the training and certification of those qualified to perform ophthalmic diagnosis in animals. Sponsors engaging the services of a CRO must be advised of the participation, or lack thereof, by veterinary ophthalmologists and the potential limitations that may arise if such studies do not involve veterinary ophthalmologists.

The ACVO recommends that, to ensure the highest quality of toxicological testing, ACVO diplomates be used exclusively for study design, ophthalmic examinations of treated animals, and evaluation of study results in pharmacologic and toxicologic testing.

The authors of this book thank the ACVO for their support and Springer Scientific for developing this book.

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Chapter 1
Standards for Conducting Ophthalmic Examinations in Laboratory Animals

David A. Wilkie, Brian C. Gilger, and Joshua T. Bartoe

Abstract Ocular toxicology pertains to toxicological effects on the eye of drugs administered topically, periocularly, intraocularly, or systemically. The ophthalmic examination is able to provide detailed in-life information and is used in combination with clinical observations, clinical pathology, and histopathology to assess potential toxicologic effects. The ophthalmologist must be familiar with the wide range of species used in the field of toxicology, be familiar with the anatomic variations associated with these species, be able to differentiate an inherited or a breed-related finding from a study-related effect, be competent with the required ophthalmic equipment, and be capable of examining this wide range of animals.

Keywords Laboratory animal · Examination · Ophthalmology · Ocular toxicology · Standards

Introduction

The purpose of this chapter is to discuss laboratory animal ophthalmic examination procedures as it pertains to the pharmaceutical industry and preclinical research studies. The industries of interest include contract toxicology laboratories and researchers in an academic environment that may require the expertise of a board-certified veterinary ophthalmologist.

Contract research organizations (CROs) evaluate products for pharmaceutical and agricultural use and are governed by Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) guidelines. In addition, they may test cosmetics, contact lenses and associated materials, intraocular devices, and a host of other products that might have an ocular use, contact the eye, or be applied topically

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or be inhaled, ingested, or injected. The evaluation of potential drug effects and toxicity must integrate the disciplines of pharmacology, toxicology, pathology, and ophthalmology [1]. Toxicology studies conducted for regulatory purposes need to be conducted in compliance with good laboratory practice (GLP). All personnel, including the consulting ophthalmologist, involved in animal studies will be expected to be familiar with GLP and will usually be required to take annual GLP-refresher courses.

Systemic and ocular toxicity studies require evaluation of both systemic toxicity using clinical observations, body weight, and clinical and histologic pathology and ocular toxicity using detailed ophthalmic examinations [1]. The eye, as it pertains to toxicology, can be considered in one of three ways. With respect to undesirable ophthalmic toxicologic effects, the ophthalmologist is concerned with (1) undesirable ocular effects when the eye is the target organ of interest with the drug of interest applied to the eye, (2) undesirable systemic effects associated with an ocularly applied agent, and (3) undesirable ocular effects from an agent applied in a systemic manner (oral, dermal, injection, inhalation) with resulting ocular effects [2–5]. In addition to drug effects, animals may be used to evaluate the effects and side effects of a procedure or device. With regard to the eye, this may include evaluation of a new intraocular device such as an intraocular lens or viscoelastic agent or evaluation of a new surgical procedure.

The eye, because of its large blood flow by organ weight, makes it a prime target for various systemic toxicities. In addition to the adnexal structures, vascularized intraocular structures include the retina and uveal tissues (iris, ciliary body, and choroid) [6]. The transparent nature of the eye and the ability to visualize arteries, veins, and neural tissue make the eye an organ where toxicities may be readily detectable. This makes the eye unique in that it is possible to conduct a detailed assessment during the in-life portion of a study [1].

This chapter will emphasize the routine ophthalmic examination of laboratory animals. It will also provide information on more advanced ophthalmic diagnostic tools that are becoming more commonplace in the area of ocular toxicology.

Routine Examination

Prior to study initiation, the ophthalmologist should review all ophthalmic procedures, discuss with the study director and/or sponsor any issues or concerns with the study design or the examination procedures, and then follow standard operating procedures (SOPs) when conducting their examinations. It is the position of the American College of Veterinary Ophthalmologists (ACVO), that in order to ensure public safety that the status of Diplomate of the ACVO is the minimum qualification for performing these ocular examinations and assessment of findings in a laboratory animal study that is intended to support applications to the FDA (or other similar regulating agencies) for entry into human clinical trials. Evaluation of toxicological effects of pharmaceutical agents involves assessment by a number of personnel, many of which are board-certified specialists, including pathologists, cardiologists,

and others in addition to ophthalmologists. Sponsors engaging the services of a CRO must be advised of the participation of veterinary ophthalmologists and the potential limitations and liability that may arise if such studies do not involve veterinary ophthalmologists.

A board-certified veterinary ophthalmologist is uniquely qualified to consult in the development of the experimental design (including the species selected, appropriate diagnostic tests, and frequency of exams) and the assessment of ocular effects of test materials being evaluated. Coordination between the testing agency and the veterinary ophthalmologist is essential throughout the process, including protocol development, establishing SOPs, and the identification and assessment of ocular findings. If ocular abnormalities are identified, communication between the ophthalmologist, study director, and the pathologist will allow correlation of clinical and histopathologic findings.

The components of an ophthalmic examination may vary depending on the species involved and the specific objective of the study. However, if the purpose of such a study is to screen for adverse effects on any ocular tissue including, at a minimum, the adnexal structures (eyelids and conjunctiva), anterior segment (cornea, anterior chamber, iris, and lens), and posterior segment (vitreous and fundus), the following must be included:

1. Pharmacologic pupillary dilation
2. Darkened ambient light conditions
3. Indirect and/or direct ophthalmoscopy
4. Slit-lamp biomicroscopy

Additional procedures may be included depending on the objective of the examination. These may include, but are not limited to, corneal staining, corneal aesthesiometry, pachymetry, tonometry, fundus photography, fluorescein angiography, optical coherence tomography (OCT), and electrophysiological assessment of the visual system (e.g., electroretinography, multifocal electroretinography, visual evoked potentials). Topical anesthesia, sedation, or general anesthesia may or may not be required depending on the species, the procedure being performed, and individual animal.

The routine ophthalmic examination for all animals used in toxicologic studies should begin with the minimum database of the results of examinations using both slit-lamp biomicroscopy and indirect ophthalmoscopy. Regardless of the species of interest, these two examination techniques are essential to ensure an accurate and complete examination of both the anterior and posterior segments of the eye. Together these two examinations must, at a minimum, include evaluation of the adnexal structures (eyelids and conjunctiva), anterior segment (cornea, anterior chamber, iris, and lens), and the posterior segment (vitreous and fundus).

Ophthalmic examinations should be conducted on eyes that have been pharmacologically dilated and should be performed in a darkened examination room. While some have advocated the use of 10% phenylephrine HCl to aid in dilation of rodents [7, 8], this is generally not required. Pharmacologic dilation is most commonly performed using tropicamide at a concentration of 0.5% for rodents and 1% for larger mammals. The ophthalmologist should be familiar with the length of time

required to achieve mydriasis and the duration of the mydriasis for the species being examined. In general, 10–15 min is the minimum time required to achieve acceptable mydriasis, and this may be slightly longer in heavily pigmented eyes. The duration of mydriasis is directly related to the amount of intraocular melanin. In albinotic rodents, mydriasis will last no more than 1 h, while in a pigmented eye of a dog or primate, the effect will persist for 3–5 h. This information is important, so the ophthalmologist knows when to begin dilation and how many animals should be dilated at one time. The latter will depend on how many animals the ophthalmologist can examine in a given time period. For the basic examination, slit-lamp biomicroscopy and indirect ophthalmoscopy, animals are either manually restrained (rodent, dog, rabbit, pig, guinea pig, cat) or sedated or anesthetized (primates). Rats and mice may be held in a dose-hold and presented to the ophthalmologist with their heads restrained. Some ophthalmologists prefer the eyes to be slightly proptosed in rodents. Dogs and rabbits are most often examined on a table. Rabbits seem to do best if there is a towel on the table as this decreases their movements and may provide some comfort. Alternatively, some ophthalmologists prefer to use restraint bags for rabbits and cats to minimize movement during the examination. For rabbits, the ophthalmologist should be seated slightly below the level of the restraint table to allow easy visualization of the rabbit's optic nerve and retinal vasculature which are located in the superior fundus. For dogs, the examiner may be seated or standing. Since primates will be usually anesthetized or heavily sedated, they may be manually restrained or placed on an examination table. If more extensive examinations such as electrodiagnostic testing or OCT are necessary, sedation or anesthesia may be required regardless of the species.

Additional examination procedures such as direct ophthalmoscopy, corneal staining, tonometry, pachymetry, fluorescein angiography, photographic documentation (anterior or posterior segment), electrodiagnostic testing, ultrasonography, OCT, and other tests may be indicated depending on the study and toxicologic effects of interest. If any of these additional tests are required, the order in which tests are performed and when to perform pupil dilation must be considered. For example, determination of intraocular pressure (IOP) should be performed prior to pupil dilation. In addition, if repeated IOP measurements are required during a study, they should be performed at the same time of day to avoid diurnal pressure fluctuation and performed preferably by the same individual to limit interobserver variation. When possible, examination of the cornea, including fluorescein staining, should be performed prior to procedures that may result in corneal changes as a result of corneal contact (pachymetry, tonometry) or the use of topical anesthesia. If sedation is required for the ophthalmic examination, then consideration must be given to dosing and feeding schedules, clinical observations, and clinical pathology sampling. Finally, from an animal well-being standpoint, a balance must be struck between multiple procedures performed on the same day as compared with multiple repeated days requiring sedation [1].

Animals will be most commonly identified by tattoo, ear tag, or microchip. If a tattoo or ear tag is used, the animal handler will need to have enough light during the examination to read the identification to ensure accurate data collection. This

may be provided by a separate light source elsewhere in the examination room or if possible by performing the ophthalmic examinations in a darkened anteroom allowing the handlers to keep the main animal room lights on. When identified by microchip, a computer scanner will allow animal identification to be linked to the computer program for data entry. To ensure accuracy, each animal must be identified to both the ophthalmologist and the data entry person at the time of the examination. The data entry person must verify that the animal being examined and the animal for which the data are being entered correspond.

Depending on the compound being evaluated and the SOP, the ophthalmologist will be expected to wear shoe covers, a lab coat or surgical scrubs, and gloves at a minimum and may be required to wear a Tyvek® suit, surgical cap, mask, and occasionally a respirator. When working with nonhuman primates (NHP), annual testing for tuberculosis (TB) using a TB intradermal PPD skin test or the new QuantiFERON®-TB blood test will generally be required of all personnel including the ophthalmologist.

The ophthalmologist must be familiar with what is normal for the species being examined and what are the common spontaneous abnormalities for that species, age of animal, and breed/strain (see Chaps. 3 and 4). Normal differences in albino vs. pigmented eyes and characteristics of species-specific retinal vasculature patterns (holangiotic vs. merangiotic vs. paurangiotic vs. anangiotic) should be well known to the ophthalmologist. The presence or absence of a tapetum lucidum and whether the animal has a fovea should be considered. In addition, the examination techniques to be used, type of biomicroscope and indirect ophthalmoscope, diameter and diopter strength of the indirect lens, and number of animals that can be examined in an hour must be understood. The role of the veterinary ophthalmologist is to perform a pretest examination designed to identify those animals not ideally suited to the study due to presence of pre-existing ocular changes and to establish a baseline database to which comparisons of interim and terminal findings can be made. Animals are then subsequently examined one or more times during the study, at termination of the study and possibly in a recovery phase depending on the study duration and design. Typically, studies are divided into acute, subacute, subchronic, and chronic depending on the study duration. The ophthalmologist must then interpret findings in light of the species examined, pretest data, compound evaluated, additional study procedures performed (anesthesia, orbital blood collection), and dose group outcome.

Since most laboratory studies involve a significant number of animals, organization and efficiency are essential. In general, most canine, primate, swine, feline, and rabbit studies involve 40–60 animals, while rats and mice may involve 250–1500 animals in a single study. An individual ophthalmologist generally requires 2–3 animal handlers, a data entry person, and in studies over 250 animals 1–2 individuals to go ahead of the animal handlers to dilate the pupils. For efficiency, an animal should be in front of the ophthalmologist at all times. The ophthalmologists' findings are reported verbally to the data entry individual who then either enters it into a computer program or records on a paper for later entry into a computer database. These data are then verified at the end of the examination, and both the ophthalmologist and data entry individual date and initial the accuracy of the report.

When an ophthalmic abnormality is observed, it must be characterized with respect to diagnosis, location, and severity. Depending on the laboratory, some will use a standardized scheme for recording of clinical observations such as Provantis® that has a set of preloaded ophthalmic terms for organ location (cornea, lens, iris, etc.), clinical signs/diagnosis (opacity, coloboma, degeneration, hemorrhage, etc.), specific location (cortex, nucleus, tapetal, anterior, posterior, etc.), and severity (slight, moderate, severe). Other CROs may have their own in-house computer or paper-based recording system. The ophthalmologist should be familiar with each laboratory's recording system and terminology to be consistent both within and between studies. See Chap. 3 for terminology harmonization designed to streamline and allow easy comparisons across the industry. When an abnormality is observed, correlation between dose groups is important when evaluating the incidence and severity of lesions so that any association with the test article can be assessed [1]. While it would be best if animals were examined out of dosing order so as to mask the ophthalmologist with respect to dose group being examined, this is often not possible given the way animals are housed and entered into the data collection system.

The ophthalmologist should also have a standardized scoring or grading scheme to assign a severity to any abnormalities seen. In general, a grading scheme of slight, moderate, and severe/marked is most common. When using this grading scheme for the transparent media (cornea, aqueous humor, lens, and vitreous humor), a grading of slight would imply a lesion that does not obstruct visualization of the deeper tissues past the lesion, a moderate grade implies a lesion that interferes with but does not fully obstruct the view of the tissues deep to the lesion, and a severe/marked lesion fully obstructs the view of structures deep to the lesion. The reader is directed to Chap. 3 (Harmonization of Lesion Nomenclature) for updated information on lesion descriptions.

For studies involving topical ophthalmic application of a drug or an ocular/intraocular device, a more specific detailed biomicroscopic examination protocol with standardized scoring or grading criteria is frequently used. The reader is directed to Chap. 5 (Standard Ocular Irritation/Inflammatory Scoring: Anterior and Posterior Segments) for more information on ocular inflammatory scoring criteria.

Slit-Lamp Biomicroscopy

Slit-lamp biomicroscopy is used to examine the ocular anterior segment including the eyelids, conjunctiva, third eyelid, tear film, cornea, anterior chamber, iris, lens, and anterior vitreous humor. Biomicroscopy provides a magnified view of the living eye using a light that can be varied in intensity, width, height, and color. In general, for laboratory animals, this is performed using a handheld slit lamp of the ophthalmologist's preference. The slit lamp used for routine examination should be portable, lightweight, and easy to use on a variety of species. The two most common portable slit lamps used for laboratory animals are the Zeiss HSO-10 and the Kowa SL-15 or SL-17 (Fig. 1.1). The Zeiss HSO-10 provides a 12× magnification with a

Fig. 1.1 A Kowa SL-15 handheld slit lamp is battery-powered and portable. As with the Zeiss HSO-10, they are suited for use in all laboratory animal species

125 mm working distance and is both lightweight and easy to use on all species. The Kowa SL-15 has either a 10× or 16× magnification with a 100 mm working distance. Unlike the Zeiss, the Kowa works from a battery pack. Both have fixed slit widths (0.15 and 0.75 mm Zeiss; 0.1, 0.2, and 0.8 mm Kowa), and both have a cobalt blue filter for visualization of fluorescein staining. If higher magnification or photographic documentation is required, a table-mounted slit lamp may be used (Fig. 1.2). Table slit lamps provide higher-quality optics, increased magnification, and variable width and height of the slit beam and with additional attachments can allow for photographic documentation, gonioscopy, or specular microscopy. Table slit lamps are however significantly more expensive, less portable, and more difficult to use on a large number of animals or un-sedated animals.

The slit lamp performs two major functions. First, it provides magnification for a more detailed examination of the eye. Second, it makes use of an aperture, decreasing the beam of light to a slit allowing an optical cross section of the eye to be obtained (Figs. 1.3 and 1.4). This allows precise localization of the depth of a lesion and allows visualization of subtle changes that cannot be seen with full illumination (Figs. 1.5, 1.6, 1.7, 1.8, and 1.9). The term for this type of illumination and examination is an optical section, and it is the most common method of biomicroscopic examination. Using a narrow slit beam, a highly magnified optical section of the eye is obtained. The direction of the slit beam may be varied so that the structures may be viewed using either direct illumination or by retroillumination [1]. This allows the examiner to detect and localize, with respect to depth, abnormalities in the ante-

Fig. 1.2 Haag-Streit BM 900 table-mounted slit lamp. This provides excellent optics, higher magnification, and variable slit beam width and height and additionally can be used for other procedures such as photographic documentation and specular microscopy

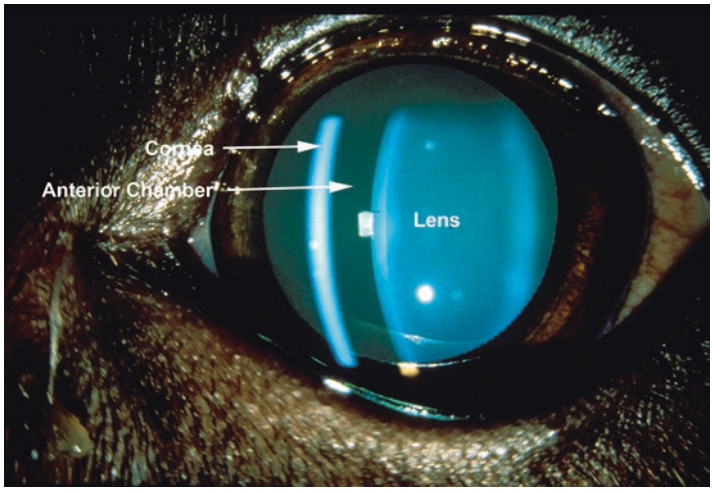

Fig. 1.3 A normal slit-lamp examination using the technique of optical section to examine the anterior segment of a normal Beagle

rior segment of the eye. For example, a corneal lesion can be localized to superficial, stromal, or endothelial; aqueous humor opacities such as cells, flare, or hemorrhage are detectable and quantifiable, and lesions of the lens may be localized to anterior, posterior, and equatorial and further to capsular, cortical, or nuclear. Interpretation

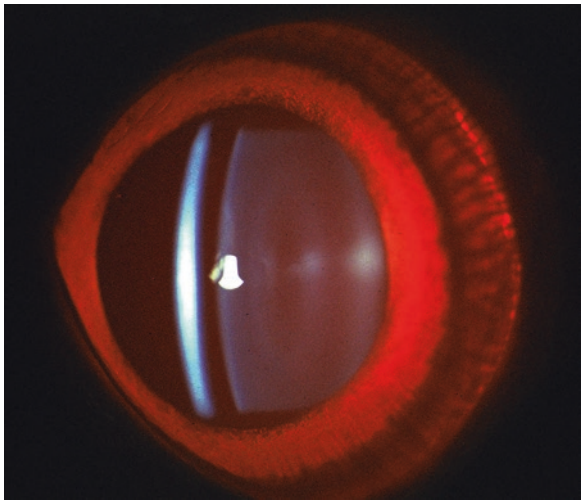

Fig. 1.4 A normal slit-lamp examination using the technique of optical section to examine the anterior segment of a normal New Zealand white rabbit

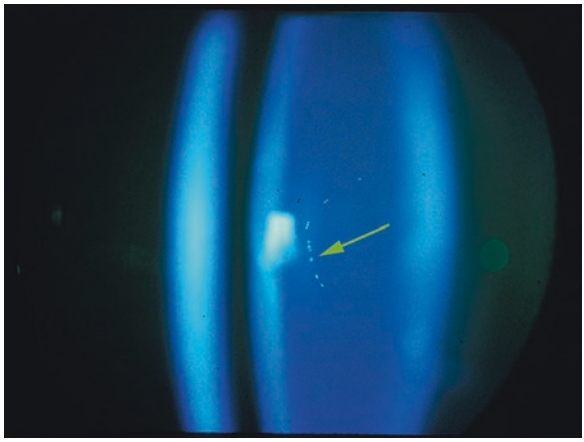

Fig. 1.5 Multiple anterior cortical suture opacities/cataracts are noted (arrow) in a Beagle on pretest examination

of the findings on slit-lamp biomicroscopy requires extensive knowledge of normal biomicroscopic anatomy as well as background lesions that occur as incidental findings in the species and strain being examined [1].

Indirect/Direct Ophthalmoscopy

Indirect ophthalmoscopy is the preferred technique of choice for routine screening of the posterior segment in all laboratory animal species. Indirect ophthalmoscopy provides a binocular, inverted, and reversed aerial image with a wide field of view. It requires an indirect headset and a condensing lens. Once perfected, the technique of indirect examination also allows for a more rapid examination of the entire

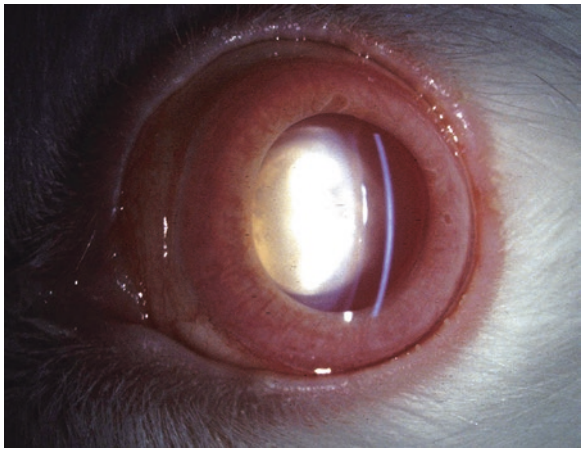

Fig. 1.6 An immature, nuclear cataract noted in a New Zealand white rabbit on pretest examination

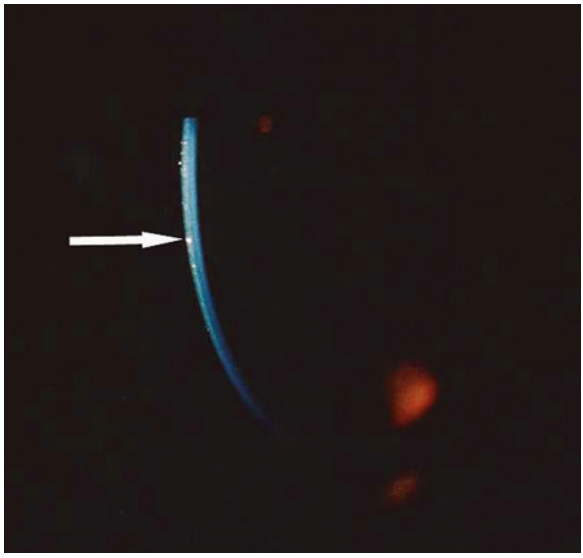

Fig. 1.7 Multifocal, corneal anterior stromal opacities (arrow) observed as a treatment-related effect in high-dose Beagles during a chronic study

posterior segment as it provides a wider panoramic field of view, allowing the examiner to evaluate more animals, more accurately in a shorter period of time. The indirect headset of choice should be lightweight, comfortable, and easy to manipulate out of the way with one hand and have a small pupil setting. The ease of manipulation will allow the examiner to move efficiently between indirect and biomicroscopic examination techniques. While several excellent choices are available, the Keeler All Pupil® is very portable (Fig. 1.10). Alternatively, the Heine indirect ophthalmoscope also offers excellent optics and can be fitted with a portable power supply. The indirect condensing lens of choice varies by species examined and by the examiners' choice. In general, a 2.2 Volk Pan Retinal or 28–30 diopter lens works well for routine screening examination of most larger species,

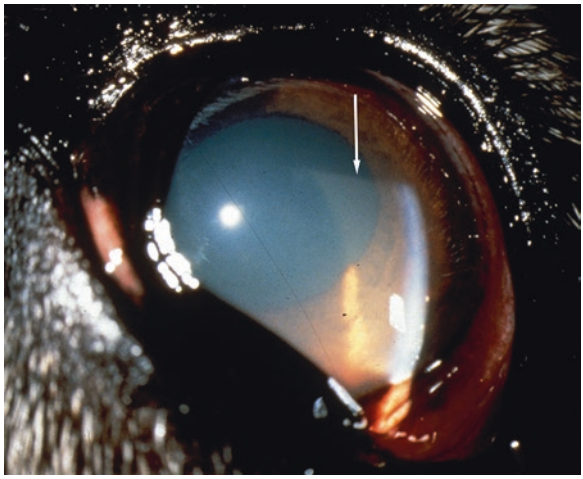

Fig. 1.8 Aqueous flare is noted in the anterior chamber as a cloudiness (arrow) in the normally transparent aqueous humor

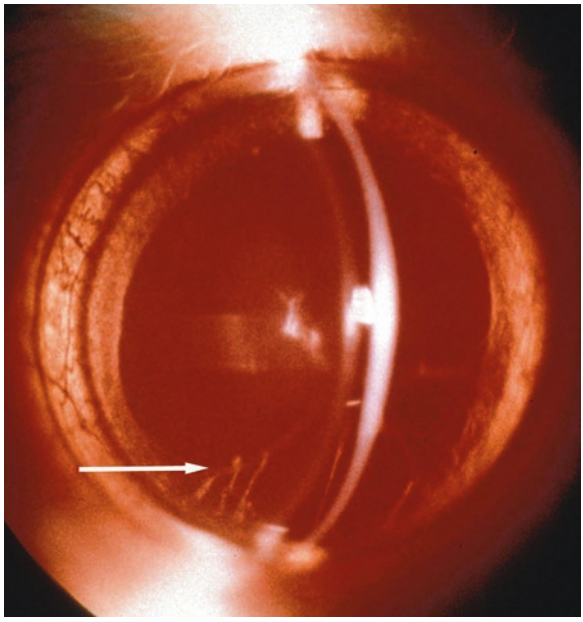

Fig. 1.9 A Sprague-Dawley rat with several persistent pupillary membranes (arrow) noted on pretest examination

and a 28, 40, or 60 diopter lens works best for rats and mice. The addition of a 20 diopter and/or a 15 diopter lens may be advised for higher magnification of the fundus in the canine and to examine the fovea and optic nerve in greater detail in NHP. Alternately, a direct ophthalmoscope (Fig. 1.11) can be used to examine the optic nerve head and fovea in NHP, but given its small field and monocular view, it may be less than optimal. In addition, when performing direct ophthalmoscopy, significant opacity of the cornea, aqueous, lens, or vitreous will severely impair or prevent visualization of the posterior segment. Lastly, the use of a direct

Fig. 1.10 (**a**) The Heine Omega 500 indirect ophthalmoscope and 28D Volk lens. (**b**) The Keeler All Pupil® Vantage indirect ophthalmoscope and 28D Volk condensing lens for examination of the ocular fundus in a New Zealand white rabbit

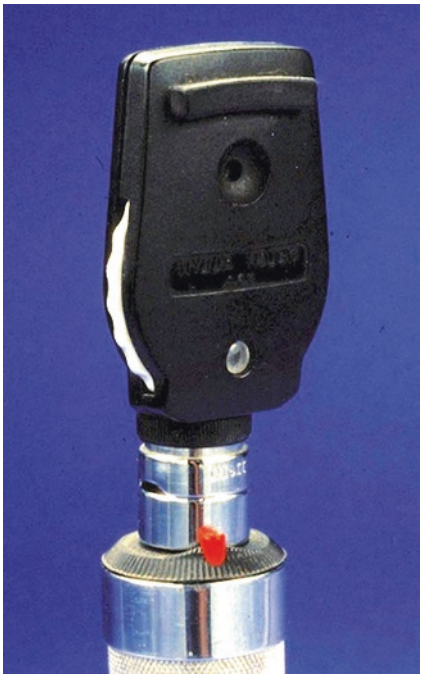

Fig. 1.11 A Welch-Allyn direct ophthalmoscope

ophthalmoscope requires the examiner to be in very close proximity to the animal's face which may present a safety concern when working with NHP due to infectious disease transmission and possible arousal from sedation.

Prior to indirect ophthalmoscopy, a short-acting mydriatic agent is required to dilate the pupil. Tropicamide 0.5–1.0% is the mydriatic of choice. When performing indirect ophthalmoscopy, the examiner remains at arm's length and places the condensing lens just anterior to the cornea. This technique is used to examine the posterior vitreous, optic nerve, retinal vasculature, retina, and choroid. In addition, opacities of the clear media (cornea, aqueous, lens, and vitreous humor) are readily detectable using retroillumination. Given the wide variety of laboratory animals, the examiner must be familiar with the variation of normal anatomy and species differences. The retinal vasculature will vary with the species: anangiotic (guinea pig), merangiotic (rabbit), and holangiotic (rodent, dog, pig, NHP). The pigmentation of the retinal pigment epithelium (RPE) and the choroid vary between albinotic, subalbinotic, and pigmented animals of the same species (mouse, rat, rabbit, dog, etc.). Some species such as the dog will have a tapetum lucidum located in the superior choroid, but this can be absent in color dilute or lemon beagles or young dogs in which the tapetum has not yet developed. Finally, NHP are foveated, and this region must be examined carefully for abnormalities.

Additional techniques to evaluate the retina may include fluorescein angiography, OCT, confocal scanning laser ophthalmoscopy, fundus photography, and electrodiagnostic testing. When these tests are used correctly and in combination, they

can provide additional en face, cross-sectional, and functional information of the retina that may then be correlated with histopathology.

Pretest Examination

Prior to study initiation, a pretest ophthalmic examination should be performed on all study animals. This is done for two reasons. The first is to eliminate from study any animals with current significant or potentially progressive ophthalmic abnormalities. The second is to establish a baseline of ocular findings to compare to as the study progresses and subsequent ophthalmic examinations are performed. Examples of pretest abnormalities that should automatically result in an animal's elimination from the study would include all ocular findings with a severity score of moderate or higher and all abnormalities that currently prevent, or may prevent if progressive, complete examination of intraocular structures. Examples of ocular findings that may be progressive during the course of the study and should result in elimination include cataract, intraocular hemorrhage, uveitis, and any other findings that may result in progressive opacification and interfere with a complete ophthalmic examination on subsequent examinations.

Common background abnormalities will vary by species and are described in detail in Chaps. 3 and 4. Whenever possible, animals with ocular abnormalities are eliminated from inclusion in the study. However, some abnormalities are so common as to preclude elimination. The most common example of this would be corneal dystrophy in the rat and mouse. The prevalence of corneal dystrophy varies by stain, age, and sex of the rat. Please see Chap. 4 (Spontaneous Incidence of Ocular Abnormalities in Laboratory Animals) for more information on incidence of spontaneous common ocular abnormalities in laboratory animals.

Additional Ophthalmic Diagnostic Procedures

There are numerous noninvasive ophthalmic diagnostic techniques that, depending on the tissue of interest, can provide both structural and functional information of both the anterior and posterior segments of the eye. Some of the more common techniques are discussed below, and a more detailed discussion of these and additional techniques as they apply to toxicologic, ophthalmic research and clinical application have recently been published [7, 9].