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Review

An overview of current techniques for ocular toxicity testing

Samantha L. Wilson [a,*], Mark Ahearne [b,c], Andrew Hopkinson [a]

[a] Academic Ophthalmology, Division of Clinical Neuroscience, University of Nottingham, Queen's Medical Centre Campus NG7 2UH, UK
[b] Trinity Centre for Bioengineering, Trinity Biomedical Sciences Institute, Trinity College Dublin, Ireland, UK
[c] Department of Mechanical and Manufacturing Engineering, School of Engineering, Trinity College Dublin, Ireland, UK

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ABSTRACT

Given the hazardous nature of many materials and substances, ocular toxicity testing is required to evaluate the dangers associated with these substances after their exposure to the eye. Historically, animal tests such as the Draize test were exclusively used to determine the level of ocular toxicity by applying a test substance to a live rabbit's eye and evaluating the biological response. In recent years, legislation in many developed countries has been introduced to try to reduce animal testing and promote alternative techniques. These techniques include *ex vivo* tests on deceased animal tissue, computational models that use algorithms to apply existing data to new chemicals and *in vitro* assays based on two dimensional (2D) and three dimensional (3D) cell culture models. Here we provide a comprehensive overview of the latest advances in ocular toxicity testing techniques, and discuss the regulatory framework used to evaluate their suitability.

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Abbreviations: BCOP, Bovine Cornea Opacity Permeability (test/assay); CAMVA, Chorioallantoic membrane vascular assay; CEET, Chicken enucleated eye test; CK, Cytokeratin; CM, Cytosensor Microphysiometer (test); CPSC, Consumer Product Safety Commission; CTPA, Cosmetic, Toiletry and Perfumery Association; DMSO, Dimethyl sulfoxide; DNA, Deoxyribonucleic acid; EC, European Commission; ECVAM, European Centre for the Validation of Alternative Methods; EET, Enucleated eye test; EIT, Eye irritation test; EURL-ECVAM, European Union Reference Laboratory for Alternatives to Animal Tests (formally ECVAM); FDA, Food and Drug Administration; FL, Fluorescein leakage; GHS, Globally Harmonized System (of classification); HCE, Human corneal epithelium; HET, Hen's egg test; HET-CAM, Hühner-embryonen test on chorioallantoic membrane; HO, Home Office (British); ICCVAM, Interagency Coordinating Committee on the Validation of Alternative Methods; ICE, Isolated chicken eye (test); IRAG, Interagency Regulatory Alternatives Group; IRE, Isolated rabbit eye (test); IVIS, *In vitro* irritancy score; JaCVAM, Japanese Centre for the Validation of Alternative Methods; LDH, Lactate dehydrogenase (leakage); LVET, Low volume eye irritation test; MAS, Maximum average score; MDCK, Madin–Dardy canine kidney (cells); MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NICEATM, NTP Interagency Center for the Evaluation of Alternative Toxicological Methods; NIEHS, National Committee of Environmental Health Sciences; NIOSHA, National Institute for Occupational Safety and Health Administartion; NRC, National Research Council; NTP, National Toxicology Programme; NZW, New Zealand White (rabbits); OECD, Organization for the Economic Co-operation and Countries Development; PLLBOA, Prototype laser light based opacitometer; QSAR, Quantitative structure–activity relationship; RCE, Rabbit corneal epithelium; REET, Rabbit enucleated eye test; SIRC, Statens Seruminstitut rabbit corneal (cells); SMI, Slug mucosal irritation (assay/test); STE, Short time exposure (test); TG, Test guidance; UN, United Nations; VMP, Validation Management Group.

* Corresponding author. Tel.: +44 7976 063 762.
E-mail address: Samantha.wilson@nottigham.ac.uk (S.L. Wilson).

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1. Introduction

The location, physiological structure and sensitivity of the ocular surface predispose it to exposure from a variety of potentially hazardous environmental conditions and substances on a daily basis. Many different materials and chemicals can result in damage to the cornea that may vary from irritation and inflammation causing mild discomfort to tissue corrosion resulting in irreversible blindness. These include household, industrial, agricultural and military products, cosmetics, toiletries and may even include certain ocular drugs and pharmaceuticals if incorrectly administered (Wilhelmus, 2001). While exposure to such substances may be incidental, accidental or intentional (Vinardell and Mitjans, 2008), most ocular incidents involve accidental exposure either in the workplace or at home *via* splashing with concentrated solutions, such as bleach or detergents, followed by rapid washing with water or removal *via* lacrimation (Shaw et al., 1991). To reduce the risk of exposure to dangerous substances all manufactured consumer products and their ingredients must be tested and their eye irritation potential assessed so that the public can be assured of their safety, or warned of the associated dangers. Eye toxicity tests are therefore required to ensure that the risks associated with products meet suitable safety criteria and are clearly labeled.

Historically, as toxicology testing has become more common, its reliance upon animal use has increased. This has primarily been due to the absence of more sophisticated assessment techniques and the low status of animals in society (Stephens and Mak, 2013). Ethical reconsideration of animal use for toxicology studies was driven by the emergence of the animal rights movement in the 1950s (Stephens and Mak, 2013) and its criticism of animal experimentation, in particular the use of Draize testing for cosmetics testing. In 1959, Russell and Burch performed a study based upon the philosophical concept of humanity, in which they observed that some biological experiments could be classed as "inhumane" based upon the levels of pain, distress and lasting harm experienced by the test animals (Russell et al., 1959). Their research provided the systematic basis of the 3R's: Replace, Reduce and Refine the use of sentient beings in experimental biology. This led to a general expansion of funding sources for *ex vivo* and *in vitro* alternative methods, to reduce the dependency on live animal testing, whilst also creating a political climate whereby alternative procedures were incorporated into federal and government legislation (Stephens and Mak, 2013). In this review, we will provide an overview of established and newly developed ocular toxicity tests and discuss their advantages and potential limitations.

2. Draize testing

Live animals have been used to assess and evaluate potentially harmful products to the eyes since the 18th century (Wilhelmus, 2001). The international standard assay for acute ocular toxicity is the rabbit *in vivo* Draize eye test (Draize et al., 1944) which was developed in the 1940s by the Food and Drugs Administration (FDA) in response to new laws implemented following permanent eye injuries occurring due to cosmetics use in the 1930s (Calabrese, 1987). Draize testing is a government endorsed protocol accepted by the Organization for Economic Co-operation and Countries Development (OECD, test guidance [TG] 405) (Huhtala et al., 2008; OECD, 2012b). New Zealand white (NZW) rabbits are most commonly used as they have large eyes with a well described anatomy and physiology, are easy to handle, readily available and are relatively inexpensive (Wilhelmus, 2001). The procedure involves the application of 0.1 ml (or 0.1 g solid) test substance onto the cornea and conjunctival sac of one eye of a conscious rabbit for up to 72 h while the other eye serves as an untreated control (Draize et al., 1944). The original Draize protocol used at least six rabbits per test, but this was reduced to three animals or a single animal when serious ocular damage is expected, with those with severe lesions being humanely euthanized. The latest Draize test guidelines include the application and delivery of analgesics and anesthetics (OECD, 2012b) to reduce animal pain and suffering. Rabbits are observed at selected intervals for up to 21 days for signs of irritation including redness, swelling, cloudiness, edema, hemorrhage, discharge and blindness (Huhtala et al., 2008). In cases where severe eye irritation or pain is observed, it is recommended that the animals are euthanized or removed from the study prior to the 21 day time point (OECD, 2012b). The observed degree of irritancy allows for chemicals to be classified, based on subjective scoring of the effect on the cornea, conjunctiva and iris, ranging from non-irritating to severely irritating. In fact, Draize testing is the only test formally accepted and validated to assess the full range of irritation severity. Both irreversible and reversible ocular effects can be identified using this test (Barile, 2010). Eye irritation was traditionally summarized as a "maximum average score" (MAS) which is an average value primarily focused on corneal injury, for individual animals at the time of scoring (Huhtala et al., 2008). However, many countries had their own scoring systems, which although similar in their approach, led to multiple classifications, labels, and data sheets for the same chemical, dependent upon which country the chemical was been marketed in. In response to this, and as a means of replacing the numerous different classification systems, with a single controlled and unified classification system, the United Nations (UN) developed the current internationally agreed, standard scoring system, known as the Globally Harmonized System (GHS), also known as the "purple book" (UN, 2013). The GHS utilizes pictograms, signal words, hazard and precautionary statements, and safety data sheets according to standardized levels of physical, health and environmental hazards. The GHS is based upon averaged single tissue observations which can account for the reversibility of the observed chemical effects (Eskes et al., 2005). With regards to eye irritation, there are two primary categories. Substances which cause serious irreversible (up to 21 days) damage/destruction to the cornea, iris and/or conjunctiva are Category 1; substances which cause reversible (within 21 days) irritation including corneal opacity, iritis, redness or chemosis are Category 2. Category 2 chemicals can be split into two subcategories: 2A, irritating to eyes, chemicals which cause reversible irritation to eyes within 21 days; and 2B, mildly irritating to eyes, chemicals