Ocular Safety: A Silent (*In Vitro*) Success Story

Rodger D. Curren and John W. Harbell

Institute for In Vitro Sciences, Inc., Suite 220, 21 Firstfield Road, Gaithersburg, MD 20878, USA

Summary — Ocular irritation testing has been one of the animal test methods most criticised by animal welfare advocates. Additional criticism has arisen from within the scientific community, based on the variability of the animal test results and the questionable relevance of the extremely high dose levels employed. As a result, the Draize eye irritation test has been one of the main targets for *in vitro* replacement. Despite extensive efforts, however, there is still no *in vitro* method that is fully validated as a regulatory replacement. In spite of this, many individual companies are using diverse *in vitro* ocular irritation tests to gain important safety and efficacy information about their products and raw materials, eliminating the need for animal testing in the process. This is done in a safe fashion by applying intelligent testing paradigms. ECVAM has played a major role in this success, through its many programmes that have emphasised the importance of understanding the true toxicological need, and then using *in vitro* tests to provide that information. Thus, even in the absence of a successfully validated regulatory assay, the desired result of reducing animal testing is being met.

Key words: *alternative methods, ECVAM, eye irritation,* in vitro*, validation.*

Introduction

Ocular irritation testing, and the associated pain and suffering of the test animals, has long been the target of animal welfare advocates. Their activities in informing the public about the details of this test (generally referred to as the Draize test; 1) have arguably given the field of alternative methods its strongest push. Recently, many scientists have also criticised the Draize test because of its high variability (2, 3), and the questionable relevance of its unrealistically high dose levels (4, 5). Many organisations have been involved in the search for *in vitro* alternatives to the Draize test, but as yet no *in vitro* method has been validated as a complete regulatory replacement. Although ECVAM has played a leading role in addressing this problem, it has kept the search for a Draize replacement in perspective, by emphasising the much greater animal use that exists in other areas of safety and efficacy testing. Today, numerous companies are productively employing *in vitro* methods for eye irritation testing, in significant areas where regulations do not specifically require the rabbit test. ECVAM has played a major role in this silent success. In fact, ECVAM not only assisted in this — it was itself strongly (and positively) affected by the search for ocular irritation alternatives.

Influence of the Search for Ocular Safety Alternatives on ECVAM

ECVAM was established at a time when many *in vitro* researchers were still secretly hoping for a miracle to solve the ocular irritation problem. We expected that the problem would be resolved with one of the many existing tests that were being promoted as having been fully evaluated with test materials covering a range of chemistries and physicochemical properties. We believed that one of these tests — although several lacked a clear mechanistic relationship to the response of the intact animal eye — would somehow respond similarly to the animal across an extremely diverse collection of pesticide ingredients, acids, bases, surfactants, inorganic salts, etc. Some also harboured the misconception that the Draize test was a reproducible measure of eye irritation.

As evidence of our beliefs, many of us joined in a major ocular irritation validation study that later became known as the British Home Office/European Commission (EC/HO) study (6). Thirty-seven laboratories tested 60 compounds using the validation study standards that were then the state of the art (6). Because nine different methods were being tested, it was widely assumed that one or more of these methods would prove to be capable of correctly assessing the irritancy potential of the materials, i.e. that an *in vitro* test would give results which correlated highly with the Draize rabbit test. However, at the study's conclusion, it was found that none of the tests was acceptable for predicting the Draize result for this diverse set of materials.

Instead of being created at a time when there was cause for celebration of a resounding validation success, ECVAM was fated to begin its life in the shadow of this costly and (in the opinion of many, but not all) unsuccessful EC/HO study. ECVAM was challenged with what appeared to be an uphill battle right from the start!

Many of us were concerned that ECVAM (and Michael Balls, its newly appointed Head) would

focus the majority of its early efforts in trying immediately to find an eye irritation test that would unerringly predict the Draize test results. However, ECVAM took a different — and far more effective — tack. The approach that ECVAM took was to learn from the "failure" by extracting as much useful toxicological and procedural information as possible from the study. Others might have decided to ignore the less-than-attractive results and let the study die quietly, or, potentially more fatal, embark on a grand ocular irritation replacement research programme. ECVAM calmly confronted the problem head on and supplied sufficient, but not extravagant, resources to understand the "failure", so that validation exercises for other toxicological endpoints would benefit. The lessons learned were not just applicable to ocular irritation — they actually helped define much of ECVAM's subsequent validation philosophy, a philosophy which has resulted in the number of positive accomplishments that are being discussed over the next three days at this ECVAM Status Seminar 2002.

As Table 1 shows, ECVAM and its collaborators recognised at least five major findings from a careful analysis of the EC/HO validation study. These findings not only helped set the standards for all future ECVAM validation studies, but they were also well publicised, so that others could apply them as well. Through a set of workshops (7–9), task force reports (10, 11) and individual publications (3, 12, 13), ECVAM and its collaborators refined and advanced this new science of validation. Their standards have significantly influenced all subsequent validation studies — conducted by ECVAM or by others — and have directly led to the significant successes we are celebrating in this meeting.

ECVAM's Influence on the Search for Ocular Safety Testing Alternatives

As previously stated, one mistake that could have happened after the EC/HO study was to focus ECVAM's entire efforts on the eye irritation problem, while ignoring many more important and imminently attackable areas. In fact, warnings against this were published (14) and Michael Balls took heed as he developed a long-term programme for this new entity called ECVAM. However, the evolving strategy did not completely ignore the minefield of ocular toxicology. Appropriate resources, both monetary and intellectual, were channelled in that direction by ECVAM, and they resulted in extremely helpful contributions (15–17). In retrospect, we can now see that the major and perhaps unmatchable resource that ECVAM provided was a sturdy intellectual platform to support discussions, arguments, experiments and logical analysis of the field of ocular safety testing. ECVAM became a place where people could come and exchange not only their exquisite factual knowledge of ocular toxicity, but also their unique reasoning patterns which were shaped by their various industrial, academic or regulatory perspectives. The ECVAM philosophy evolved to emphasise the need to determine in each situation: a) the problem that existed; and b) what biological information was really needed to solve that problem. Thus, relative to the present topic, we began to focus on what ocular toxicity information is really helpful for the toxicologist, rather than just blindly seeking to replace the Draize test. This approach has led to many positive changes throughout the field of alternatives. Changes have occurred by intelligently applying our

Table1: Five major lessons from the EC/HO (6) study that ECVAM has published and applied to subsequent validation efforts

Lesson Learned	Publication
Understand the mechanics of operating a validation study	Practical aspects of the validation of toxicity test procedures: the report and recommendations of ECVAM workshop 5 (7)
Question and carefully evaluate the animal test results	Practical aspects of the validation of toxicity test procedures: the report and recommendations of ECVAM workshop 5 (7)
Maintain scientific rigour in developing *in vitro* tests	The role of prevalidation in the development, validation and acceptance of alternative methods: ECVAM Prevalidation Task Force Report 1 (10)
Develop and use a prediction model	No prediction model, no validation study (36); the validation of toxicological prediction models (37)
Use meaningful statistical procedures	Recommendations for the application of biostatistical methods during the development and validation of alternative toxicological methods: ECVAM Biostatistics Task Force Report 1 (11)

growing understanding of the advantages of the *in vitro* systems, not by using brute force to copy every existing *in vivo* endpoint.

Ocular Irritation Testing Today

At this point, we should pause to remind ourselves of problems with the Draize test that cause concern for both scientists and animal welfare advocates. First is the obvious ethical consideration: severe pain and distress that can last for several days is often imposed on the animal. Second is the scientific consideration: the rabbit may not be an appropriate model, because of differences between it and humans in eye structure (for example, the presence of a nictitating membrane, thinner cornea, etc.), exposure parameters (for example, the exceptional high single dose applied), and response (for example, lack of significant tearing). In addition, the rabbit test results are extremely variable, because of animal–animal differences and the subjective nature of the scoring (2). In fact, if one asks whether the Draize test can actually predict its own result (i.e. is the Draize score a scientifically valid result or just an artefact?), a computer simulation reveals a far lower correlation between multiple tests of the same material than one would expect from the application of a regulatory test method (18). Such findings have caused a new generation of toxicologists to approach the eye irritation problem from a new and reductionist direction. This approach models different levels of organisation of the ocular tissues with different *in vitro* systems. Figure 1 illustrates several examples of how the various eye parts *in vivo* can be modelled by more-and-more reductionist models *in vitro*. These *in vitro* surrogates can be dosed in a realistic and reproducible manner, and then interrogated for a variety of endpoints in an intelligent fashion.

Are *In Vitro* Ocular Irritation Tests Actually Being Used?

Our affiliation with the Institute for *In Vitro* Sciences (IIVS) and its important programme of performing *in vitro* assays for diverse segments of industry, gives us a rather privileged view of at least a portion of industry's routine use of alternative methods. It is sad that a large portion of the positive trends toward *in vitro* ocular irritation testing are invisible, not only to the general public, but also to the general scientific community (and even to ECVAM). This is for several reasons. One is because industry treats the majority of its ocular safety and efficacy studies as confidential. The studies are conducted mainly on proprietary materials, and there is a significant competitive advantage in keeping confidential even the fact that such studies were conducted, let alone the results obtained. The second reason is that there is not a convenient forum for communicating the results of routine, unexciting ocular safety studies.

What we witness at IIVS on a daily basis, however, is very encouraging. We see many companies making an extraordinary effort to use *in vitro* methods within their ocular safety or product development programmes. Recently, several of these efforts have been presented to the toxicology community through poster presentations at annual meetings (9–21). I estimate that thousands of new products and materials are tested worldwide each year in *in vitro* eye irritation studies, with only a tiny fraction of the results being reported. This is a silent success story.

How do companies succeed in reducing or eliminating the Draize test from their safety testing programmes? Their strategies vary considerably, depending on their types of product and on existing government regulations. For example, in areas such as occupational health, where toxicologists need to determine potential for eye irritation so that adequate eye protection regulations for their plant workers can be determined, there are no specific regulations requiring animal testing. In such a case, many companies have performed in-house or consortia evaluations of *in vitro* eye irritation tests to determine their effectiveness when used with test sets of specific compounds that represent the chemical classes likely to be encountered in future tests (22, 23). If *in vitro* tests are found reliable, they are then substituted for the existing animal test in subsequent routine testing. Some companies have used this approach to completely eliminate animal use, while others have been able to significantly reduce their animal use by adopting a tiered approach (24, 25), in which *in vitro*-negative materials, or materials suspected of falling outside the chemical classes previously evaluated, are tested on a single animal. These approaches are also useful in related areas, such as qualifying new lots of raw materials for use in manufacturing a product, or doing periodic checks on the safety of products after changes in the manufacturing process.

Some industrial toxicologists find the *in vitro* methods extremely helpful in prioritising products or ingredients for future development or use. In the vast majority of such situations, there is no regulatory requirement to use the Draize eye test. Indeed, the Draize test is so imprecise (see previous discussion) that it provides little, if any, value to the investigator who is looking for small differences between products or materials. When making decisions on various levels of mildness, or whether a new material is less irritating than a benchmark existing material, a sensitive test, such as one of the three-dimensional human tissue constructs, is often far more valuable than the conventional animal test. When this alternative approach is used, both

time and animals are saved, giving the industrial toxicologist the ability to move products to the market much faster.

Which *In Vitro* Ocular Irritation Tests Are Being Used?

A large number of *in vitro* ocular irritation models are currently in common use worldwide. The tests are generally mechanistic, in that data that they provide can be directly related to some animal or human measurement of ocular irritation, as is shown in Figure 1. At the primary level, tests such as the enucleated rabbit eye test (26, 27) or chicken eye test (28, 29) provide endpoints such as opacity, corneal swelling and histopathology after dosing directly on the surface of the isolated eye. At the next level, the bovine corneal opacity and permeability (BCOP) assay, for example, utilises just the isolated cornea, with opacity, permeability, and histopathology as endpoints (25, 30). A still lower level is represented by the 3-dimensional tissue constructs that are representative of only the epithelial layer of the cornea (31, 32). These models generally have cytotoxicity as an endpoint that can be correlated with the extent of injury to the epithelium. Finally, at the currently lowest level of reduction, are found monolayer cultures of human or animal cells (33), which can be monitored for cytotoxicity and which model superficial damage to the cornea or conjunctiva.

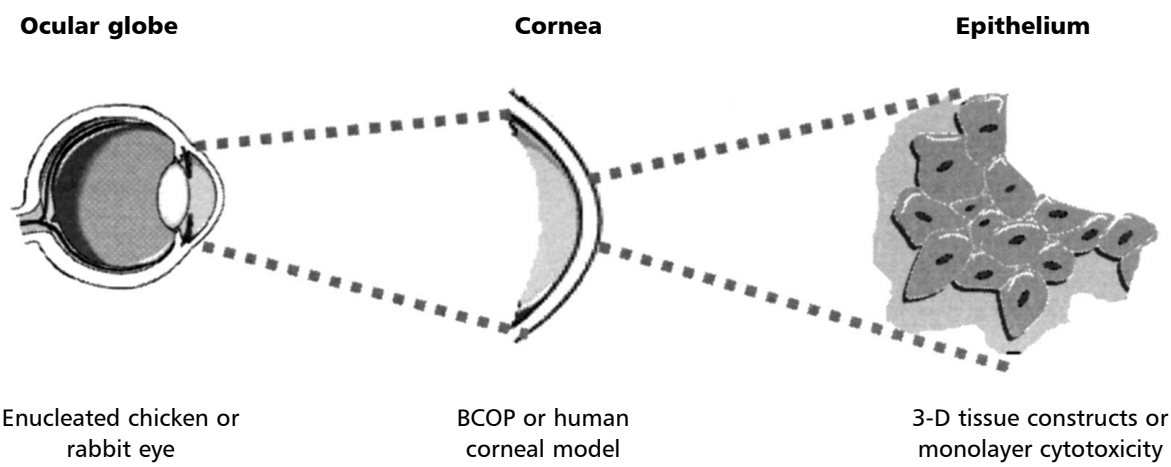
Other assays have been developed to look at some of the endpoints other than direct tissue damage. The hen's egg test-chorioallantoic membrane (HET-CAM; 34) and the chorioallantoic membrane vascular assay (CAMVA; 34, 35), for example, attempt to add vascular endpoints such as haemorrhage and coagulation to the *in vitro* armamentarium, by using the chorioallantoic membrane of the chick embryo as a target tissue.

However, in addition to the models themselves and the specific endpoints that they address, the toxicologist must consider the relevant exposure kinetics, in order to obtain the greatest value from the *in vitro* system. *In vitro* investigations should not be restricted by the rather inflexible exposure that effectively limits the information that can be gained from a conventional Draize rabbit test. The Draize test, as it is currently employed, makes use of the minimum number of animals to provide a single point estimate of ocular irritation hazard after a single exaggerated acute exposure. Although there are certainly ethical reasons for using only a single standard exposure with the minimum number of animals, there can be compelling scientific arguments for investigating parameters such as dose–response, time–response, effect of repeat application, and dilution effects, to name but a few. The beauty of most *in vitro* systems is that all of the above parameters can be evaluated without the associated ethical quandary. A toxicologist can intelligently review the physicochemical parameters of the test chemical, the duration of use, frequency of reapplication, etc., and can then design an *in vitro* protocol that takes these important parameters into consideration. Each of these can be assessed in the appropriate *in vitro* system, so that an explicit picture of the hazard or safety of a specific material under defined conditions can be assessed.

In Vitro Tests Are Not "Stand Alone"

In conclusion, it should be remembered that ocular safety decisions, like any toxicological conclusions, should not be based only on the numerical result of a single test. Just as should be done when using

Figure 1: *In vitro* ocular irritation assay systems are designed to model incrementally smaller portions of the ocular globe



conventional animal tests, a knowledge base of all available toxicological information should be incorporated into any safety assessment involving *in vitro* models. Information from existing toxicity databases, from structure–activity analysis, from in-use history, etc., should be considered, along with the *in vitro* data, when making a safety decision. When these data are intelligently combined, we can feel comfortable that our final decisions when using *in vitro* ocular methods, are well supported.

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