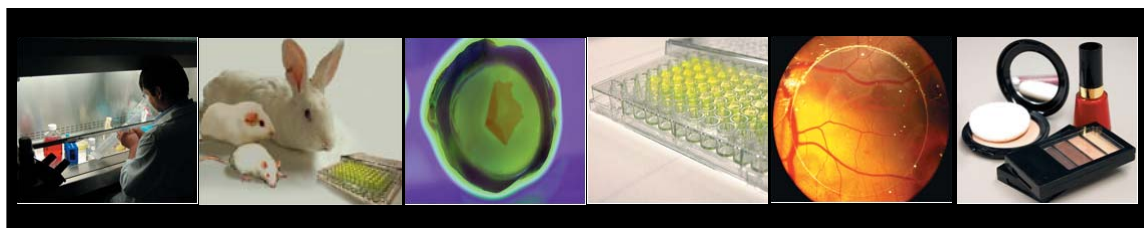




What's New @ MB?



- MB Expands Capabilities - Ames, PK, MTD, Range Finding
- New! - PorCORA - Non-Animal Ocular Irritation/Reversibility Test
- Under Development - PorFocal - Low-Level Ocular Irritation Screening using Confocal Microscopy
- A Glimpse Into The Future - ROBatt - Development of Tiered Testing Strategy using Alternatives to replace Draize Rabbit Eye Test

MB Expands Capabilities – Adds Ames Test, MTD and Range Finding

Bacterial Reverse Mutation Assay

Adapting to changes in testing demand, MB Research has added genotoxicity testing, pharmacokinetic, maximum tolerated dose and range finding capabilities. The inclusion of these new capabilities has allowed us to meet your testing needs.

For over a year, MB has been performing the Bacterial Reverse Mutation Assay (Ames Assay). The Ames assay evaluates the mutagenic potential of a test article based on the reversion of selective growth mutations in several strains of *Salmonella typhimurium* bacteria and in *Escherichia coli* WP2 uvrA bacteria, in the presence and absence of S9 activation. The MB protocol is based on OECD Guidelines for Testing of Chemicals: No. 471 – Bacterial Reverse Mutation Test (July 1997) and the U.S. EPA Health Effects Test Guidelines OPPTS 870.5100 – Bacterial Reverse mutation Test (August 1998).



More information about the Ames Test (MB Protocol #820), please contact Client Services.

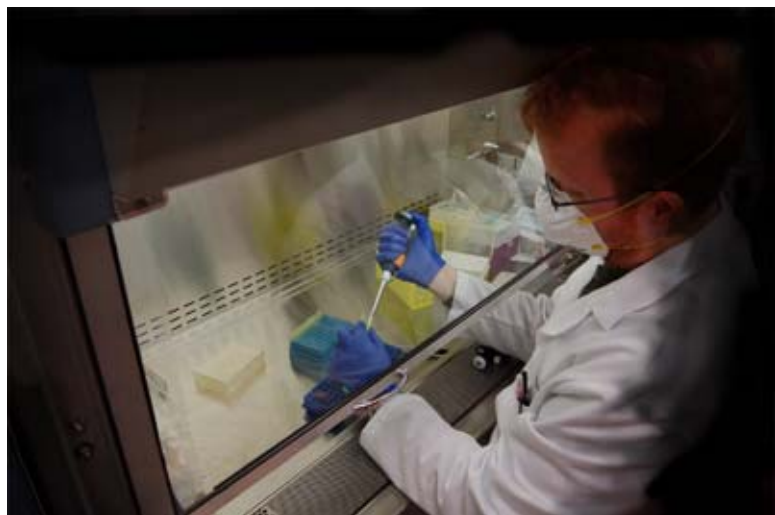
Maximum Tolerated Dose and Range Finding

Maximum Tolerated Dose/Dose Range Finding, subchronic and chronic toxicology studies are routinely conducted at MB Research in various species using a variety of routes of administration. These studies are individually designed for the sponsor in order to comply with specific regulatory requirements and intended use of the test substance.

Studies are performed by highly trained and qualified personnel and are closely supervised by a senior Toxicologist at every phase.

Our staff of Study Directors communicate actively with each client. Preliminary results are often available within a day of study completion. Draft reports for acute studies are normally available within 3-4 weeks of study completion.

MB Research has partnered with several laboratories to offer other testing services such as Pathology, which are conducted under the direction of a Board Certified Veterinary Pathologist. Ophthalmology services are available through a leading Veterinary Ophthalmologist, and analytical chemistry.

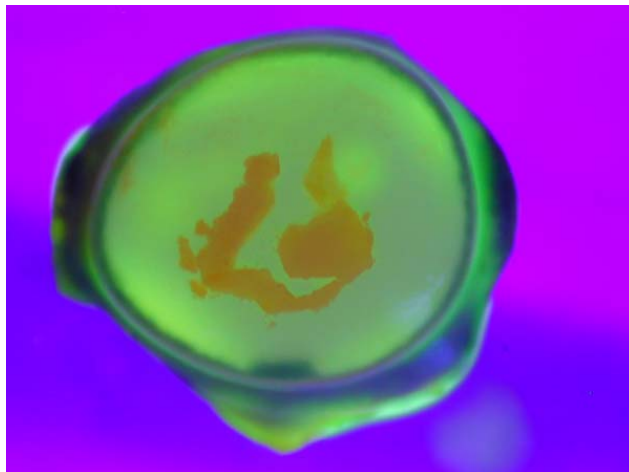


Seeing is Believing - PorCORA – Porcine Cornea Opacity and Reversibility Assay

Regulatory agencies require manufacturers to characterize the risk of eye irritation/damage, and mandate the use of animals. Eye irritation is still characterized using a rabbit test (Draize et al., 1944), which evaluates the effects of a single exposure of the test substance on eye tissues for a period of up to three weeks. This extended period allows the evaluation of reversibility of damage.

Due to ethical issues in animal testing, there has been an effort to modify current practices in toxicology that **reduce, refine and replace** the number of animals used in product safety testing. Although several alternative methods exist to characterize aspects of eye irritation and damage, no established method can model recovery after injury as in a Draize test.

Since some regulatory classification methods of ocular irritancy depend upon the time for an ocular injury to completely heal (OECD, 1967; WHMIS, 1988; HMIS, 1996; EPA, 1997), MB Research has developed an alternative assay that measures corneal damage and recovery for extended periods in excised porcine corneas – PorCORA. When paired with other assays that quantify the severity (such as the BCOP, Epi-Ocular, and Chorioallantoic membrane-based assays) of ocular irritancy, PorCORA significantly reduces the need for using live animals in eye irritation studies. Histopathology can also be used to evaluate the depth of injury and amount of recovery.



Ex-vivo porcine cornea stained with fluorescein to reveal damage caused by 3% SDS.

PorCORA uses a special culturing method that allows us to maintain excised porcine corneas for over 21 days. These corneas can be treated with a test material (solids and liquids) and stained with a fluorescent dye to view damage to the corneal epithelium.

Recently, MB has completed an in depth validation of the PorCORA method. Validation studies included over 32 chemicals with recovery information taken from the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) *in vivo* eye irritation data bank.

We have demonstrated that re-epithelialization of the corneal surface (reversibility) can be measured with fluorescein stain, which is representative of actual tissue damage determined by fluorescence confocal microscopy (live/dead stain), reflective confocal microscopy (no stain), and histology. This model system is representative of corneal healing in the rabbit eye, as comparison of average days to clear in PorCORA to average days to clear in the ECETOC historical Draize database yields a correlation coefficient of 0.84.

Results from a 32-reference compound validation of the PorCORA yielded very favorable results predicting reversibility correctly for 30 of 32 compounds tested. In addition to the validation studies, a 6-product mixture test set was also assayed and has also indicated promising results yielding representative amounts of damage and reversibility.

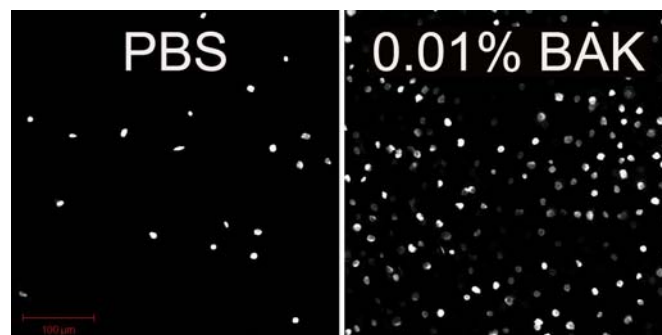
More information about PorCORA can be found at <http://www.porcora.com/> or by contacting Client Services at 1-215-536-4110, email: mbinfo@mbresearch.com.

Under Development: PorFocal – Alternative **Low Level** Ocular Irritation Screening Test

MB Research Labs is in the early stages of developing the Porcine Confocal assay to screen slight, ultra-mild ocular irritants.

The PorFocal Assay is an artifact of the development of PorCORA. While conducting preliminary experiments with confocal microscopy to characterize the mechanistic properties of PorCORA, we noted that Live/Dead staining and quantification of dead cells in the corneal tissue was possible. Consequently, low-level amounts of corneal damage can be quantified as the number of dead cells per volume of corneal tissue. Due to the extreme sensitivity, the PorFocal Assay is highly applicable to the categorizing of low-level irritants. This optical histology assay can be conducted within 48hrs of corneal treatment, a significant improvement compared with standard histology and subsequent light-microscopic evaluation.

To test proof of concept and feasibility of the PorFocal assay, we used a total of 8 cultured porcine corneas: 4 were treated with PBS, 4 were treated with 0.01% BAK. These two test materials are extremely mild and separation of the irritation potential of these two compounds would clearly demonstrate the resolution and sensitivity of PorFocal. Corneas were incubated for 30 minutes/37°C with 2µM Ethidium homodimer from the Live/Dead staining kit (Molecular Probes) in culture medium. Each corneal tissue was imaged and equivalent volumes of tissue were quantified for dead cell number. We examined six 450µm x 450 µm x 56µm volumes of tissue per cornea for a total of 24 volumes per condition. Most tissue damage did not extend beyond the first few layers of epithelial cells.



The total dead cell number for the 24 volumes (6 per cornea) from the 4 PBS-treated corneas was 1659 cells. The total dead cell number for the 24 volumes from the 4 BAK-treated corneas is 3591 cells. When we conducted an ANOVA analysis on the data, the two groups are significantly different ($p=0.000631$). The mean number of dead cells per 450 x 450 x 56µm tissue volume for PBS is 70 ± 16 (see image above) and for BAK is 150 ± 15 .

Clearly, the measure of cytotoxicity and irritability within a tissue by quantifying *individual cell death* cannot be improved upon regarding sensitivity and resolution. Due to the extreme sensitivity of the PorFocal assay, this assay is highly applicable to the function of categorizing low-level ocular irritants. Finally, this cell death measurement can be conducted on the corneas immediately upon termination of the corneal treatment period with results within 24 hours, a significant improvement on the turn-around time compared to classical histopathology.

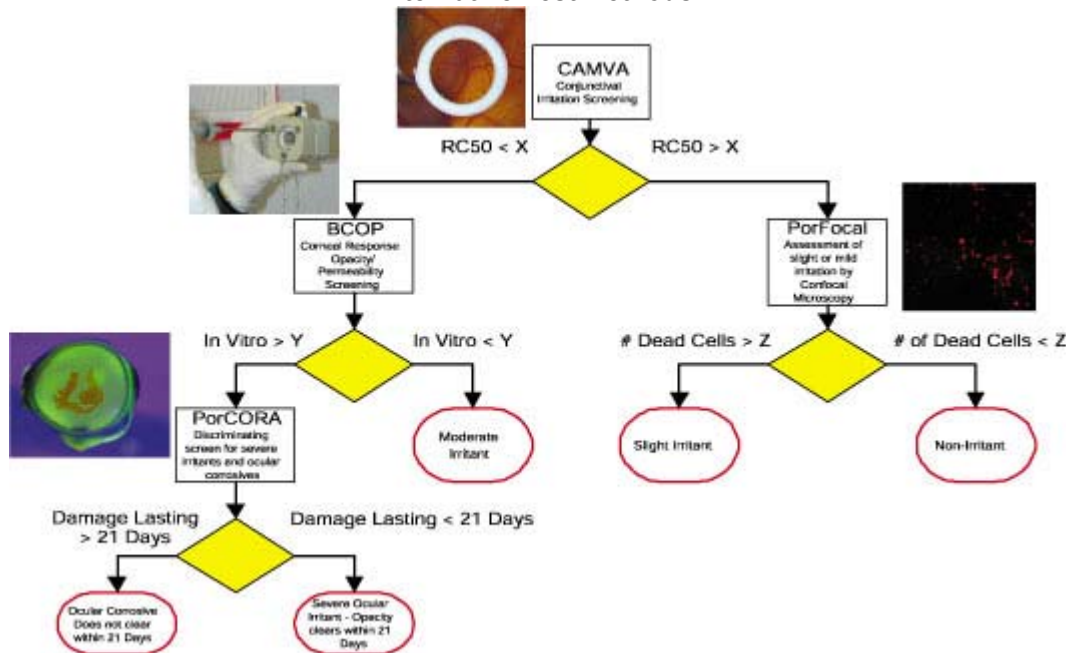
We are very excited with these results and have submitted grant applications to the NIH for possible funding to develop this assay further.

A Glimpse Into The Future: ROBatt – Replacement Ocular Battery

The negative public attitude regarding the use of animals in toxicity testing has created a societal as well as commercial need for the development of a non-animal replacement for the Draize rabbit eye test – a principal target for critics of animal use. The Draize rabbit eye test has been used since 1944, and has proven to be a reliable method for safety determinations. However, this test has been criticized for its lack of reproducibility and use of live animals (Weil and Scala, 1991; Bruner, 1992).

The ROBatt is a tiered testing strategy consisting of the Chorioallantoic Membrane Vascular Assay (CAMVA), the Bovine Cornea Opacity/Permeability (BCOP) test, the PorCORA and PorFocal. Having worked with alternatives for Draize since 1988, we feel confident that the ROBatt will fulfill the public and scientific requirements as a standalone replacement strategy for ocular irritation. ROBatt provides classification of ocular irritancy and, critically, an estimation of recovery potential. Time to recovery after exposure to a possible irritant is an endpoint of particular interest to regulatory bodies, such as the US EPA, OECD, WHMIS and HMIS regulations. These agencies classify ocular irritancy not by severity but by the time for the injury to heal (OECD, 1967; WHMIS, 1988; HMIS, 1996; EPA, 1997).

Replacement Ocular Battery – Tiered Testing Strategy for Ocular Irritancy Classification using Alternative Test Methods.



Test materials (Solids and Liquids) are first assayed with CAMVA. Depending on the results, the test material is either tested using PorFocal for slight to non-irritants OR tested with the BCOP if the test material is moderately or severely irritating. If the test material shows severe irritation potential, it will be tested using PorCORA. By using PorCORA, classification of severe irritant or ocular corrosive can be determined.

Determining the X, Y and Z values of this battery is the initial priority of the ROBatt project. After which, full validation studies will be performed.

We have taken this concept and requested funding through the NIH Challenge grant program as part of the Economic Stimulus package. Funding this research would allow MB to pursue the development and validation of the ROBatt and be able to offer you a full alternative to the Draize Rabbit Eye Test. If you would like to know more about ROBatt or possibly participate in the validation of ROBatt, please feel free to contact Client Services.

Recent Posters and Presentations

A 3D skin model for cosmetic, chemical and medical device phototoxicity testing. Lisa F. Pratt, Daniel R. Cerven, and George L. DeGeorge, 48th Society of Toxicology Annual Meeting, Baltimore, MD.

Assessment Of Ultraviolet Light- And Chemical-Induced UDS In Various Cell Lines Using Flow Cytometry. Jianming Tao, Will Newhard, Daniel R. Cerven and George L. DeGeorge, 48th Society of Toxicology Annual Meeting, Baltimore, MD.

Alternative Porcine Corneal Ocular Reversibility Assay Correlates with Draize Reversibility Data Piehl, M., Soda, R., Carathers, M., Donovan, A., and Cerven, D., 48th Society of Toxicology Annual Meeting, Baltimore, MD.

In Vitro Phototoxicity Methods Compared: 3T3NRU PT vs. Phototoxicity Assay in Reconstituted Skin. L. Pratt, G. DeGeorge, 29th Annual Meeting American College of Toxicology, Tucson, AZ.

An Improved Flow Cytometry-Based Unscheduled DNA Synthesis (UDS) Assay. J. Tao, G. DeGeorge, 29th Annual Meeting American College of Toxicology, Tucson, AZ.

Copies of these posters are available upon request.