The Chemical Company

May 13, 2009

Dr. William S. Stokes,
Director,
NICEATM,
NIEHS,
P.O. Box 12233,
Mail Stop: K2– 16,
Research Triangle Park, NC 27709

Dear Dr. Stokes,

BASF SE through BASF Corporation is pleased to provide comments on the draft ICCVAM test method recommendations for alternative methods to evaluate eye irritation. BASF SE has extensive experience validating one of these methods and comparing the results to current methods. In summary, we provide comments on the HET-CAM assay based on our retrospective analysis of HET-CAM results generated during in-house routine testing. We are not providing comment on the BCOP assay because is just being established in the Laboratory for Acute Toxicology and has not yet been evaluated. My colleague, Dr. Arnhild Schrage, will attend to provide additional comment at the meeting.

Regards,

Raymond M. David, Ph.D., DABT
Manager, Toxicology

BASF Corporation
100 Campus Drive
Florham Park N.J. 07932
Tel: (800) 526-1072
www.basf.com/usa

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Comments on the Draft BRD for the HET-CAM method

a. General comment:

There exist various protocols, endpoints and prediction models, especially for the HET-CAM method, making a comparison of different studies difficult. This observation is reflected in the ICCVAM report 2006[1] where information was collected on roughly 260 substances in 383 HET-CAM studies (Draft HET-CAM BRD, line 1118ff). So many substances were tested that for the analysis of one single HET-CAM protocol, only 25 % of all studies could be used because of the differences in protocols and endpoints. However, the results could be compared to *in vivo* data, using a specific analysis of one protocol with its specific endpoints and fewer substances, e.g only 63 substances from 4 publications for the IS(A) analysis method (Draft HET-CAM BRD, line 1112 ff). Therefore, as recommended by ICCVAM, we emphasize the importance of determining one specific protocol and specific irritant endpoints.

b. Specific comments

- *Line 877-879: development of irritant endpoints (hemorrhage [bleeding], vascular lysis [blood vessel disintegration], and coagulation [intra-and extravascular protein denaturation]*
  In our hands, distinguishing between hemorrhage and lysis during microscopic observation is difficult, as both effects result in blood vessel leakage. We recommend either a detailed description of the observed effects within the protocol that helps to distinguish between both effects, or combine both effects in one endpoint, which would then be considered as part of the the calculation of the irritation score (line 897).

- *Line 975ff:* in vivo *data*
  In addition to the *in vivo* classification, including an *in vivo* score from the results of the rabbit eye studies would facilitate the comparison of *in vitro* and *in vivo* data, e.g. the MMAS = modified maximum average score used by Balls *et al.* (1995)[2].

- *Line 1162ff.: HET-CAM Test Method Accuracy*
  (1182-1186: overall or for specific chemical and physical classes)
  To improve the predictability of the HET-CAM method, we recommend an analysis after grouping the substances by their solubility in water or oil.
  Our retrospective analysis of 145 routinely tested substances (manuscript submitted to *Alternatives to Laboratory Animals* in April 2009)[3] revealed that the HET-CAM´s overall accuracy and the overall rates of false negatives or

[1] http://iccvam.niehs.nih.gov/methods/ocutox/ivocutox/ocu_brd_hetcam.htm
[2] Balls *et al.*, Toxic. In Vitro Vol. 9, No. 6, pp. 871-929, 1995).
[3] Schrage A, Gamer AO, van Ravenzwaay B, Landsiedel R. Experiences with the HET-CAM method in the routine testing of a broad variety of chemicals and formulations. Submitted.

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false positives made this assay inadequate. However, the HET-CAM was sufficiently specific (few false positives) for water soluble substances, and highly sensitive (no false negatives) for non-water- and oil-soluble substances. Therefore, the HET-CAM might be applicable for excluding severe ocular irritation among water-insoluble substances. A copy of the abstract is attached.

Abstract of the manuscript, submitted to ATLA in April 2009

Experiences with the HET-CAM method in the routine testing of a broad variety of chemicals and formulations

Arnhild Schrage, Armin O. Gamer, Bennard van Ravenzwaay, and Robert Landsiedel
BASF SE, Experimental Toxicology and Ecology, 67056 Ludwigshafen, Germany

Data on eye irritation are generally needed for hazard identification of chemicals. For the routine testing of a broad variety of chemicals and formulations we used the Hen´s Egg Test - chorioallantoic membrane (HET-CAM) method. In the course of a tiered testing strategy and due to the lack of regulatory acceptance we also performed the Rabbit Eye Irritation test according to the OECD Test Guideline 405.
76 % of the 145 tested substances were non to mild irritating and 13 % were identified as irritating *in vivo* according to the EU classification system (GHS: 61% or 28 %, respectively). The remaining 11 % were severe irritants *in vivo*, which was based on the irreversibility of effects and not due to sufficiently high irritation scores in the three days after application.
The retrospective analysis revealed, that the HET-CAM´s overall accuracy was 65 % and the overall rate of false negatives (FN) and false positives (FP) was 50 % or 33 %, respectively. The HET-CAM was sufficiently specific (few FP) for water solubles, but failed to identify nearly all severe irritants within this group. In contrast, it was highly sensitive (no FN) for non- and oil-soluble substances, but the specificity for this group was rather low.
Therefore, the HET-CAM is not useful in our tiered-testing strategy for eye irritation testing. But for water-insoluble substances it might be applicable in combination with another *in vitro* method, provided that the regulatory acceptance is given.