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Subject

Expert Panel Report ICCVAM/NICEATM

Dear Sir,

In reaction to the request for comments made public via the Federal Register notice, Volume 70, No. 142, Tuesday, July 26, 2005/Notices, 43149, the Dutch Research Organization TNO would like to forward the following comments and remarks concerning the ICE test method described in the Addendum to “*In Vitro* Ocular Toxicity Draft Background Review Documents”.

The inclusion of the additional data, forwarded by TNO beginning of this year, is highly appreciated. Because TNO has a longstanding experience with the screening of severe eye irritants for contract research, the additional data was forwarded to substantiate this particular application of the ICE. The data contained the full set of chemicals and/or formulations that was tested *in vitro* and *in vivo* over a period of several years. As we have experienced over the last 20 years of contract *in vivo* eye and skin irritation testing, about 10% of the compounds consist of eye/skin corrosives, of which almost all were screened by the ICE as indeed severely eye irritating. Therefore, we found it rather peculiar that eight severe eye irritating compounds were excluded from the reanalysis on the basis that insufficient *in vivo* data was provided to classify the compound according to the GHS classification system. All these compounds were corrosive in the *in vivo* skin irritation test, which was performed after the outcome of the ICE. The individual *in vivo* skin irritation data of these compounds were provided to ICCVAM. In full agreement with the guidelines, TNO decided to waive the *in vivo* eye irritation test in rabbits in these cases.

As the main purpose of the ICE (and any other *in vitro* eye irritation method) in contract research is to prevent severe irritants to be tested in the Draize eye test, it is rather paradoxical to see that all these actual cases of correctly identified severe eye irritants are not taken into account for the evaluation of this method.

Date

August 8, 2005

Our reference

TAP-2005

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The Standard Conditions for Research Instructions given to TNO, as filed at the Registry of the District Court and the Chamber of Commerce in The Hague shall apply to all instructions given to TNO; the Standard Conditions will be sent on request.

Attachments

Article TIV (pdf : 4 pages)
ECETOC data (3 pages)



In contrast to what is taken by ICCVAM as the reason for exclusion, the GHS criteria clearly mentions “corrosive to skin” as one of the criteria for class I “irreversible eye effects”. For the EPA classification, I cannot imagine that a skin corrosive is not assumed to be a category I compound for eye irritation. Furthermore, two compounds from the EC/HO validation study study, i.e. *p*-fluoroaniline and 2,2-dimethyl butanoic acid, were excluded from the reanalysis. Both compounds were identified by the management of the EC/HO validation study (Balls et al., 1995) as R41 severely eye irritating on the basis of the individual *in vivo* data (ECETOC, Technical document no. 48: 2, June 1998; data attached). These two compounds were also correctly identified by the ICE and most other *in vitro* methods participating. The *in vivo* classification was based on sound scientific judgment and it is unclear on which basis ICCVAM refuted this expert judgment. The probable reason for ICCVAM to exclude the compounds, may be that a 21-day observation period was not completed, is inadequate and, if so, demonstrates insufficient knowledge of the eye irritation process in rabbits. Moreover, the guidelines (at the time of testing) specified that the observation period should be long enough to evaluate the reversibility or irreversibility of the lesions. The six rabbits treated with 2,2-dimethyl butanoic acid still showed slight to severe corneal opacity and neovascularization of the cornea at 14 days after treatment. Clearly, these lesions are not reversible within a 21-day observation period. The same applies to *p*-fluoroaniline, causing moderate to severe corneal opacity and iritis score 2 (highest score possible; no reaction to light, haemorrhage, gross destruction). The test was terminated on day 3, which is in agreement with the current guidelines which mention that animals may be humanely sacrificed if the severity of the effects is considered too high.

On the basis of the above, TNO strongly requests ICCVAM to revise the present analyses with respect to the screening of severe irritants by inclusion of these ten cases. We have also concern about the fact that the data of the various studies performed with the ICE in different time periods are pooled for analyses and that the outcome of the individual studies is not discussed individually. Success or failure of *in vitro* methods has much to do with the setting in which the method is used and the way the *in vivo* data was obtained. Therefore, we advise ICCVAM to also mention and comment the successful use and strategy of the ICE by TNO for screening severe irritants.

With respect to the proposed Candidate substances (appendix V-A1), TNO would like to express reservations about the usefulness/appropriateness of such a list, containing only summary data. By now, after all the validation studies already carried out, we know that quite a different approach for validation is needed, including a meticulous test substance selection. With respect to this issue, we would like to draw your attention to the discussion article attached to this letter and which is currently in press in Toxicology In Vitro. This article deals, among other things, with the problem of using historical *in vivo* eye data for validation of *in vitro* test systems. We hope this article will contribute to the discussion concerning the validation process, i.e. not starting a new validation process before dealing with the basic issues of the *in vivo* test.

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We are looking forward ICCVAM's official reaction to our comments. In the mean time, TNO is, as always, available for additional information and discussion, if needed. In that respect, we were rather disappointed that we were not asked to comment on the above mentioned issue and, moreover, we were not informed of the availability of the reanalysis on the ICCVAM website.

Yours faithfully,

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August 8, 2005

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ECETOC data (3 pages)

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Article Reference

Prinsen MK. In Press. The Draize Eye Test and in vitro alternatives; a left-handed marriage? Toxicology in Vitro.

This copyrighted article was accepted 20 June 2005. The corrected proof has been available online since August 1, 2005, at www.sciencedirect.com.