Subject: FW: ICCVAM/NICEATM FR Notice related to the murine LLNA
Date: Monday, October 22, 2007 11:34 AM
From: Henk van Loveren <Henk.van.Loveren@rivm.nl>

Dear dr. Allen

Thank you for giving us the opportunity to respond to the draft ICCVAM performance standards for the murine LLNA: methods for assessing lymphocyte proliferation.

We have discussed the draft in my group (Janine Ezendam, Rob vandebriel, Wim de Jong) and have the following comments:

Add to line 316 after LLNA: "Especially for the latter category of products to be investigated adaptations may be possible to overcome this problem. See ASTM protocol F2148-01.

ASTM F2148-01. Standard practice for evaluation of delayed contact hypersensitivity using the murine local lymph node assay. ASTM F2148-01, West Conshohocken, PA, USA.

Add to line 337 after proliferation: Should perhaps possible other endpoints be mentioned here? In any case, also modifications in determination of cellular proliferation exist that use ex vivo DNA labeling with tritium-thymidine and should be mentioned here (Kimber and Weisberger 1989, Van Och et al 2000).

Kimber, I., Weisenberger, C. A murine local lymph node assay for the identification of contact allergens. Assay development and results of an initial validation study. Arch. Toxicol. 63, 274?282, 1989.

Van Och, F.M.M., Slob, W., De Jong, W.H., Vandebriel, R.J., Van Loveren, H. A quantitative method for assessing the sensitizing potency of low molecular weight chemicals
using a local lymph node assay: employment of a regression method that includes determination of the uncertainty margins. Toxicology 146, 49?59, 2000.

Add to note 5 at page 6: An alternative mice strain that is frequently used is the BALB/c strain which shows similar responses as the CBA mice (Woolhiser et al 2000).

Woolhiser MR, Munson AE, Meade BJ. Comparison of mouse strains using

the local lymph node assay. Toxicology 146, 221-227, 2000.

Line 441: Delete 20. This gives the impression that you need to validate each alternative assay with these 20 compounds. Or is this the intention?

Line 532:
Why is the CV limited to 30% ? This looks reasonable but in table 2-3 for DNCB two out of 6 laboratories have a CV above 30%, of 35 and 46% respectively.

Line 636: A comparison of the performance of several mouse strains in the LLNA is presented in Woolhiser et al 2000.

Line 659: An example is presented in ASTM protocol F2148-01.

Line 717: The pooling approach should be discouraged as a statistic evaluation is not possible and non responding outliers cannot be detected. Also in the ICCVAM evaluation and proposed protocol pooling is not recommended. Include in text preference for individual sampling an d determination of cell proliferation.

Line 743: Add text: For this reason individual sampling should be recommended.

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