EUROPEAN COMMISSION
DIRECTORATE GENERAL JRC
JOINT RESEARCH CENTRE
Institute for Health and Consumer Protection
European Centre for the Validation of Alternative Methods (ECVAM)

# ESAC Statement on the Reduced Local Lymph Node Assay (rLLNA)

At its 26th Meeting, held on 26-27 April 2007 at the European Centre for the Validation of Alternative Methods (ECVAM), Ispra, Italy, the non-Commission members of the ECVAM Scientific Advisory Committee (ESAC)[1] unanimously endorsed the following statement:

Skin sensitisation is an important toxicological endpoint with respect to human safety.

Having reviewed the final report of the independent peer review evaluation co-ordinated by ICCVAM and NICEATM[2], the report by the EMEA[3], the pre-report of the SCCNFP[4], and evidence made available since the original submissions to ICCVAM, in March 2000 the 14th meeting of ESAC stated:

> "Following a review of the scientific report and publications on the local lymph node assay (LLNA) it is concluded that the LLNA is a scientifically validated test which can be used to assess the skin sensitisation potential of chemicals. The LLNA should be the preferred method, as it uses fewer animals and causes less pain and distress than the conventional guinea-pig methods. In some instances and for scientific reasons, the conventional methods can be used."

Since its acceptance for regulatory purposes, the LLNA has proved suitable for the purposes of satisfying a range of EU and other regulatory requirements[5].

The developers of the LLNA have now undertaken a retrospective analysis of published data obtained with the LLNA[6].

They conclude that within a tiered testing strategy in the context of REACH a "reduced" version of the LLNA (rLLNA), using only a negative control group and the equivalent of the high-dose group from the full LLNA, can be used as a screening test to distinguish between sensitisers and non-sensitisers.

ESAC established a peer review panel to evaluate if there was the potential to minimise animal use by employing the rLLNA as a screening test as part of a tiered-testing strategy for chemicals.

Mindful that with the rLLNA:

- When compared with the full LLNA the rLLNA cannot and will not result in additional false positives.
- When compared with the full LLNA the rLLNA may produce a few false negatives (3:169 in the reference document, reducing to 2:169 when negative results obtained with concentrations of <10% are considered invalid)
- The test results provided by the rLLNA do not allow the determination of the potency of a sensitising chemical.

ESAC states that the peer reviewed and published information is of a quality and nature to support the use of the rLLNA within tiered-testing strategies to reliably distinguish between chemicals that are skin sensitisers and non-sensitisers, and that animal use can be minimised providing:

- The concentration used to evaluate sensitisation potential is the maximum consistent with solubility and the need to avoid local and other systemic adverse effects, and that this principle rather than strict adherence to the specific recommended absolute concentrations as in OECD TG 429 should be used.
- Negative test results associated with testing using concentrations of less than 10%, should undergo further evaluation.
- Positive and negative (vehicle) control groups are used, as appropriate, per OECD TG 429.
- The full LLNA should be performed when it is known that an assessment of sensitisation potency is required.

ESAC recommends that further work should be undertaken to determine if the 10% concentration threshold referenced above is optimal.

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27 April 2007

1. The ESAC was established by the European Commission, and is composed of nominees from the EU Members States, industry, academia and animal welfare, together with representatives of the relevant Commission services.

   This statement was endorsed by the following members of the ESAC:

   Ms Sonja Beken (Belgium)
   Ms Dagmar Jírová (Czech Republic)
   Mr Tõnu Püssa (Estonia)
   Mr Lionel Larue (France)
   Mr Manfred Liebsch (Germany)
   Ms Annalaura Stammati (Italy)
   Mr Jan van der Valk (The Netherlands)
   Mr Constantin Mircioiu (Romania)
   Mr Albert Breier (Slovakia)
   Ms Argelia Castaño (Spain)
   Mr Patric Amcoff (Sweden)
   Mr Jon Richmond (UK)
   Mr Carl Westmoreland (COLIPA)
   Ms Vera Rogiers (ECOPA)
   Ms Nathalie Alépée (EFPIA)
   Mr Robert Combes (ESTIV)
   Mr Hasso Seibert (European Science Foundation)

   The following Commission Services and Observer Organisations were involved in the consultation process, but not in the endorsement process itself.

   Mr Thomas Hartung (ECVAM; chairman)
   Mr Jens Linge (ECVAM; ESAC secretary)
   Ms Elke Anklam (Director of IHCP)
   Ms Susanna Louhimies (DG Environment)
   Ms Barbara Mentré (DG ENTR)
   Ms Grace Patlewicz (ECB, DG JRC)
   Mr Christian Wimmer (DG Research)
   Mr Hajime Kojima (JACVAM)
   Ms Laurence Musset (OECD)
   Mr Barry Philips (Eurogroup for Animal Welfare)
   Mr William Stokes (NICEATM, USA)

2. NIH (1999). The murine local lymph node assay. The results of an independent peer review evaluation coordinated by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and the National Toxicology Program Center for the Evaluation of Alternative Toxicological Methods (NICEATM). NIH Publication n.99-4494.
   (http://iccvam.niehs.nih.gov/methods/immunotox/immunotox.htm)

3. EMEA (2000). Report from the ad-hoc expert meeting on testing for immunohypersensitivity (11/01/2000). European Agency for the Evaluation of Medicinal Products.

4. SCCNFP (2000). Opinion adopted by the SCCNFP during the 11 th plenary meeting, 17 February 2000. (http://ec.europa.eu/health/ph_risk/committees/sccp/docshtml/sccp_out114_en.htm)

5. A Cockshott, P Evans, CA Ryan, GF Gerberick, CJ Betts, RJ Dearman, I Kimber and DA Basketter (2006). The local lymph node assay in practice: a current regulatory perspective. Human & Experimental Toxicology **25**, 387-394.

6. I Kimber, RJ Dearman, CJ Betts, GF Gerberick, CA Ryan, PS Kern, GY Patlewicz and DA Basketter (2006.) The local lymph node assay and skin sensitisation: a cut-down screen to reduce animal requirements? Contact Dermatitis **54**, 181-185.