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Review

The murine local lymph node assay: Regulatory and potency considerations under REACH

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Abstract

From June 2007, new chemicals legislation on the registration, evaluation, authorisation and restriction of chemicals (REACH) will come into force across the European Union. This will require the submission of data on human health effects of chemicals, including chemical safety assessments which will require measurements of potency. For skin sensitization hazard identification, REACH states that the first-choice *in vivo* assay is the local lymph node assay (LLNA). This test has also been the UK competent authority's preferred test for skin sensitization since 2002, and has now replaced guinea pig tests in dossiers submitted to it under the Notification of New Substances Regulations. Advantages of the LLNA over guinea pig tests include improvements in animal welfare, a more scientific approach to hazard identification, and the inclusion of a dose–response element in the endpoint, which enables an estimation of potency. However, notifiers to the UK competent authority have sometimes been reluctant to use the assay because of concerns over false-positive reactions. Across Europe, these concerns have been heightened in the lead-up to the introduction of REACH, since the use of *in vivo* alternatives to the LLNA will require scientific justification. This review will address some of these concerns from a regulatory perspective.

Keywords: Local lymph node assay (LLNA); Skin sensitization; REACH; Potency; Vehicle effects; Irritants

Contents

Abbreviations: ACS, allergic contact sensitization; AOO, acetone/olive oil (4:1); CA, competent authority; DEP, diethyl phthalate; DMF, dimethyl formamide; DMSO, dimethyl sulphoxide; DNCB, 2,4-dinitrochlorobenzene; DNELs, derived no-effect levels; ECETOC, European Centre for Ecotoxicology and Toxicology; FITC, fluorescein isothiocyanate; FCA, Freund's complete adjuvant; GPT, guinea pig tests; GPMT, guinea pig maximization test; LLNA, local lymph node assay; LNC, lymph node cells; LOAEL, low-observed adverse effect level; MCI/MI, methylchloroisothiazolinone/methylisothiazolinone; MEK, methyl ethyl ketone; MEST, mouse ear swelling test; NOAEL, no-observed adverse effect level; NOEL, no-observed effect level; NONS, Notification of New Substances Regulations; PG, propylene glycol; SI, stimulation index; SLS, sodium lauryl sulphate

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1. Background

Since 2002, the murine local lymph node assay (LLNA) (Kimber et al., 1994), as performed in accordance with OECD test guideline 429 (OECD, 2002), has been the UK competent authority's (CA) preferred test for skin sensitization. Extensive validation (Kimber et al., 1994, 1998; Kimber and Basketter, 1992) has shown that this assay is a suitable substitute for guinea pig tests (GPT) for skin sensitization, the most commonly used of which, the Guinea Pig Maximization Test (GPMT) and the Buehler Test, are described in OECD test guideline 406 (OECD, 1992). Importantly, it also has many advantages over GPT in terms of both scientific progress and animal welfare (Gerberick et al., 2000; National Institutes of Health, 1999; Sailstad, 2002). The assay provides information on a substance's ability to induce sensitization, unlike the GPT, which measure responses to the elicitation phase, and, importantly, delivers quantifiable data that enable dose response assessment. Although animals are not replaced, the numbers used are usually reduced (although a modified OECD guideline allowed half the original number of animals to be used for the GPMT) and the procedures are substantially refined. Since responses to the induction phase are measured, the LLNA does not require the elicitation of challenge-induced dermal hypersensitivity reactions, thus reducing animal discomfort. Additionally, as no adjuvant is required, the severity of the procedure and thus animal distress are reduced compared with the GPMT. The use of mice rather than guinea pigs, and the shorter experimental time involved, also greatly reduce the costs associated with testing for skin sensitization potential. For all these reasons, in 2002 the UK CA advised its notifiers of new substances that the LLNA was now accepted as a stand-alone test, and indeed was the preferred test for skin sensitization.

However, despite its obvious advantages, reservations about the suitability of the LLNA in certain circumstances have emerged and recently it has become apparent that notifiers of new substances are reverting to the use of GPT for the testing of particular substances. Specifically, there have been concerns that some vehicles may augment or dampen lymph node cell (LNC) proliferative responses; that the assay has not been validated for the testing of formulations, including emulsions, suspensions and mixtures; and that some irritant substances give false positive responses. These concerns have become heightened with the imminent implementation of the new European chemicals legislation on the registration, evaluation, authorisation and restriction of chemicals (REACH), which specifies that the LLNA must be used for new *in vivo* testing for skin sensitization hazards: only under 'exceptional circumstances' should another *in vivo* method be used, and only when this can be scientifically justified (EC, 2006). Once REACH comes into force, the LLNA will be much more widely used throughout Europe than it currently is. REACH also requires that a chemical safety assessment be undertaken which includes dose (concentration)–response (effect) relationships for human health hazards for chemicals marketed in quantities of ten tonnes or more per annum. In view of these points, this review will address some of the concerns mentioned above and, in addition, examine the evidence for the LLNA's ability to provide information on dose–response relationships, and thus on the skin sensitization potency, of substances.