

Poster: current activities regarding the EU-chemicals policy (REACH)

Alternative endpoints in the LLNA in order to reduce false positive results

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Background: According to the EU-chemicals policy (REACH), the murine Local Lymph Node Assay (LLNA) is the first-choice method for *in vivo* testing of skin sensitization properties. Compounds inducing lymphocyte proliferation in lymph nodes draining the site of topical exposure are regarded as sensitizers in the traditional LLNA. However, some non-sensitizing irritants have been shown to induce false positive results in the traditional LLNA. In this study, we investigated if a method using alternative endpoints is able to discriminate between sensitizers and irritants in order to reduce the number of false positive results.

Methods: Mice were treated with vehicle (actone:olive oil, 4:1 v/v), a sensitizer (0.5% DNCB) or an irritant (20% SDS or 1.0% BZC) on the dorsal surface of both ears for 3 consecutive days. Both traditional LLNA endpoints (ear irritation, lymph node (LN) size and cell count) and alternative endpoints (ear weight, LN weight and B220+ cell population in the draining LN) were analyzed one day after the last treatment.

Results: DNCB induced an increase in LN weight, size and cell count. Furthermore, a trend towards an increased B220+ cell population was observed after DNCB treatment. SDS induced slight erythema and increased ear weight and LN cell counts. BZC caused severe erythema and increased ear weight, LN weight and cell count. Both irritants did not induce an increase in the B220+ cell population.

Discussion: The traditional lymphocyte proliferation, measured by LN size and cell counts, was not able to discriminate between the sensitizer and irritants. Although analysis of the B220+ cell population was suboptimal and further optimization is necessary, all three compounds were correctly classified as sensitizer or irritant using B220+ cell populations and ear weights.

In conclusion, analysis of B220+ cell populations and ear weights proved to be promising alternative endpoints in the LLNA in order to reduce the number of false positive results of irritants.

References

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