2.0 rLLNA Protocol Components

2.1 Overview

The technical aspects of the rLLNA are identical to those of the traditional LLNA; the two methods differ only in the number of test substance dose levels tested (Kimber et al. 2006). In the traditional LLNA, each test substance is tested at a minimum of three dose levels. The highest dose level is the maximum soluble concentration that does not cause systemic toxicity and/or excessive local irritation (ICCVAM 1999). In the rLLNA, in addition to the concurrent vehicle-control group, each test substance is tested at only the highest testable dose level (Kimber et al. 2006).

A Stimulation Index (SI) is calculated as the ratio of radioactivity incorporated into the cells of draining auricular lymph nodes of the treated animals to that of the vehicle-control animals. In both the traditional LLNA and the rLLNA, the threshold for classifying a substance as a skin sensitizer is an SI $\geq$ 3.

2.2 Basis for Test Method Selection

The rLLNA was proposed by Kimber et al. (2006) in an effort to reduce the number of animals used for skin sensitization testing and as a means of streamlining the LLNA for testing that will be required under the Registration, Evaluation and Authorisation of Chemicals regulations (Kimber et al. 2006).

2.3 Proprietary Test Method Components

The rLLNA does not employ any proprietary components.

2.4 Basis for the Number of Mice per Dose Group

The basis for the number of mice per dose group in the rLLNA is the same as that for the traditional LLNA (ICCVAM 1999).

2.5 Study Acceptance Criteria

Similar to the traditional LLNA, in order for an rLLNA study to be considered acceptable, the positive control must yield an SI $\geq$ 3 (ICCVAM 1999).

2.6 Basis for Selection of the Test Substance Dose

As noted in **Section 2.1**, the rLLNA tests each substance at only the highest testable dose level, in addition to the concurrent vehicle control. Consistent with the criteria for selecting the highest dose level in the traditional LLNA (ICCVAM 1999), the dose level used to evaluate sensitization potential in the rLLNA should be the maximum soluble concentration that does not cause systemic toxicity and/or excessive local irritation (ICCVAM 1999).