5.0 rLLNA Test Method Data and Results

5.1 Description of the rLLNA Test Method Protocol Used to Generate Data

No specific rLLNA studies were conducted for this evaluation; rather, data from traditional LLNA studies were evaluated retrospectively. The only difference in the test method protocols between the traditional LLNA and the rLLNA is the number of dose levels tested. In the traditional LLNA, at least three test-substance dose levels are tested, with the highest dose level based on maximum solubility and the avoidance of systemic toxicity and/or excessive local irritation. In contrast, only the highest dose level of a substance is tested in the rLLNA (Kimber et al. 2006). This retrospective evaluation assumes that the top dose level tested in the traditional LLNA studies was in fact the maximum soluble concentration that did not cause overt systemic toxicity and/or excessive local irritation. Because the criteria for choosing the top dose in the traditional LLNA and in the rLLNA are the same, the maximum dose level tested should be the same for both. However, it is important to consider that the highest possible dose level selected in a prospective validation study may differ between the two versions of the LLNA. Thus, the accuracy analysis of these same substances in a prospective rLLNA study may differ from the accuracy analysis obtained in this retrospective rLLNA analysis.

5.2 Availability of Original rLLNA Data Used to Evaluate Accuracy and Reliability

While original study records were not obtained for any of the previously conducted studies, compiled *in vivo* reports and/or transcribed results were obtained and/or available for all studies included in this evaluation.[47]

5.3 Description of the Statistical Procedure Used to Evaluate rLLNA Data

The performance analysis in this BRD focuses on the ability of the rLLNA to identify potential skin sensitizers as determined by the calculated SI for each test substance (see **Section 2.1**).

5.4 Summary of Results

The data evaluated here were obtained from 12 sources (**Table D-1**). Where available, the specific information extracted for each substance includes its name, CASRN, physicochemical properties (e.g., form tested, Log K_{ow}), and chemical class[48] (**Annex II**). Dose levels tested, along with calculated SI and/or EC3 values, sensitizing hazard classification, and the data source are provided in **Annex III**. If EC3 values were not included in the data source, they were calculated, where possible, using either interpolation or extrapolation (Dearman et al. 2007). Other than the information provided in the submitted data, no additional attempt was made to identify the source or purity of the test substance.

[47] The LLNA data for several of the substances evaluated for this report were included in the database that was submitted to ICCVAM in 1998 for the initial evaluation of LLNA (ICCVAM 1999). Therefore, some of the original data for these substances were available for review.

[48] Chemical classes were assigned by NICEATM based on the classification of the National Library of Medicine's Medical Subject Heading (available at http://www.nlm.nih.gov/mesh/meshhome.html).

5.5 Use of Coded Substances

Neither the previous evaluation of these 211 substances (ICCVAM 1999) nor any additional studies used in this evaluation describe coding of substances to avoid potential scoring bias.

5.6 Lot-to-Lot Consistency of Test Substances

Ideally, a single lot of each substance is used during the validation of a test method. In situations where multiple lots of a chemical must be used, the lot-to-lot consistency of a test substance must be evaluated to ensure the consistency of the substance evaluated over the course of the study. The procedures used to evaluate lot-to-lot consistency are described in the published reports. No attempt was made to review original records to assess the procedures used to evaluate different batches.

Data submitted by P. Botham/ECPA, P. Ungheuer/EFfCI, and D. Germolec/NIEHS included the source and the batch number of each tested substance.

5.7 Availability of Original Data for External Audit

The LLNA data included in the ICCVAM (1999) database were reviewed during the original evaluation. The original data for the other studies included in this evaluation were not available.