4.0 Comparative *In Vivo* Reference Data – the Traditional LLNA

4.1 The Traditional LLNA Protocol Used to Generate Comparative *In Vivo* Reference Data

As described in **Section 2.1**, the traditional LLNA protocol was consistent with the original ICCVAM-recommended protocol (ICCVAM 1999). That original LLNA test method protocol was accepted by U.S. regulatory agencies (e.g., 2003 EPA Health Effects Test Guidelines) and is itself consistent with procedures described in OECD TG 429, having served as the basis for development of the test guideline. Still, TG 429 allows for more procedural variation than the ICCVAM-recommended protocol (ICCVAM 1999).

4.2 Comparative Traditional LLNA Reference Data Used

The traditional LLNA data used to evaluate the rLLNA were obtained from 12 sources (**Table D-1)**. In addition to calculated SI values for each of the tested dose levels, the vehicle tested and values for the estimated concentration needed to produce an SI of 3 (EC3) for substances classified as sensitizers were provided in Gerberick et al. (2005). The data received in response to 72 FR 27815 (May 17, 2007[44]) included calculated SI values for each of the dose levels tested and the vehicle used. If EC3 values were not included in the data source, they were calculated, where possible, using either interpolation or extrapolation (Dearman et al. 2007). This information and the database (by each source) follow in **Annex III**.

4.3 Availability of Original Records for Comparative Traditional LLNA Reference Data

An attempt was made to obtain the original records for the traditional LLNA data through the *FR* notice (72 FR 27815, May 17, 2007[44]) and requests to specific stakeholders. Although the original study records were not obtained for any of the studies, compiled *in vivo* reports and/or transcribed results were obtained and/or are available for all studies included in this evaluation.

4.4 Quality of Comparative Traditional LLNA Reference Data

Good Laboratory Practice (GLP) guidelines are internationally recognized rules designed to produce high-quality laboratory records (OECD 1998; EPA 2006a, 2006b; U.S. Food and Drug Administration [FDA] 2007a). They provide an internationally standardized procedure for the conduct of studies, reporting requirements, archiving of study data and records, and information about the test protocol to ensure the integrity, reliability, and accountability of a study.

Ideally, all data supporting the validity of a test method should be obtained from studies reported and conducted in accordance with GLP guidelines. The extent to which the traditional LLNA studies complied with GLP guidelines is based on the information provided in published and submitted reports. Based on the available information, the following papers and data submissions were identified as originating from studies that followed GLP guidelines or used data obtained according to GLP guidelines:

- H.W. Vohr/Berufsgenossenschaftliches Institut fur Arbeitsschutz (BGIA)

[44] Available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_9544.pdf

- P. Ungeheuer/European Federation for Cosmetic Ingredients (EFfCI)
- E. Debruyne/Bayer CropScience SA
- P. Botham/European Crop Protection Association (ECPA)
- M.J. Olson/GlaxoSmithKline (GSK)
- D. Germolec/National Institute for Environmental Health Sciences (NIEHS)

The publication by Gerberick et al. (2005) does not address the GLP compliance of any of the studies discussed. Several of the substances listed in Gerberick et al. (2005) were included in the original LLNA submission to ICCVAM (ICCVAM 1999). According to the submission, "Much of the data used here to support this submission and much of the data contained within the publications cited in this document have been derived from audited Good Laboratory Practice (GLP) compliant studies. Where this is not the case all investigations have been conducted to the spirit of GLP or Good Research Practice in GLP compliant facilities" (reproduced in ICCVAM 1999). Furthermore, in response to requests from ICCVAM, records were provided indicating compliance with GLP guidelines for some of the studies.

4.5 Accuracy and Reliability of the Traditional LLNA

4.5.1 Accuracy

ICCVAM (1999) reviewed the performance of the traditional LLNA with comparisons to (1) the Guinea Pig Maximization Test and the Buehler Test (EPA 2003) and (2) human results obtained from the human maximization test[45] and human patch test allergen[46] panels. The evaluation concluded that the LLNA demonstrated adequate accuracy (ICCVAM 1999).

4.5.2 Reliability

ICCVAM (1999) also reviewed the reliability of the traditional LLNA as assessed by intra- and interlaboratory reproducibility. The evaluation concluded that the LLNA demonstrated adequate intra- and interlaboratory repeatability and reproducibility (ICCVAM 1999).

[45] The human maximization test involves application of occluded patches on the same skin site with a rest period between each reapplication. Two weeks after the last induction patch, sensitization is evaluated using a 48-hour occluded patch test. The site is scored 24 and 48 hours after patch removal.

[46] Allergen patch tests are diagnostic tests applied to the surface of the skin to identify the cause of contact dermatitis. Chemicals and substances included in these tests (e.g., nickel, rubber, and fragrance mixes) are known to cause contact dermatitis (i.e., skin sensitization) (http://www.fda.gov/cber/allergenics.htm).