3.0 Substances Used for Validation of the rLLNA

3.1 Rationale for the Substances or Products Included in the Evaluation

Data from 471 LLNA studies were obtained from 12 sources (**Table D-1**), including published reports and unpublished data submitted to NICEATM in response to 72 FR 27815.[40]

3.2 Rationale for the Number of Substances Included in the Evaluation

The database from the 471 traditional LLNA studies included 457 unique substances,[41] 211 of which were included in the original ICCVAM evaluation of the traditional LLNA (ICCVAM 1999). Fourteen of the 457 unique substances were tested two to five times each in different LLNA studies. Specifically, nine of the 14 substances were evaluated two to five times in different vehicles, and five of the 14 substances were evaluated two to five times in the same vehicle. Two of the five substances evaluated in the same vehicle (hexyl cinnamic aldehyde [HCA] and potassium dichromate) were also tested using different vehicles (one study for HCA and two studies for potassium dichromate). Due to the small number of repeated studies (5% of total studies), all were treated independently for accuracy evaluation. When the studies for the substances repeated in the same vehicle were considered together to yield an overall skin sensitization classification, there were 465 studies with unique substance–vehicle combinations.

3.3 Detailed Description of Substances Included in the Evaluation

Annex II provides information on the physicochemical properties (e.g., physical form tested), Chemical Abstracts Service Registry Number (CASRN), and chemical class for each substance tested. This information was obtained from the published reports, submitted data, or literature searches.

When available, chemical classes for each substance were retrieved from the National Library of Medicine's ChemIDplus® database. If chemical class information was not located, chemical classes were assigned for each test substance using a standard classification scheme based on the National Library of Medicine Medical Subject Headings.[42] A substance could be assigned to more than one chemical class; however, no substance was assigned to more than three classes. Certain complex pharmaceuticals and pharmaceutical intermediates were simply identified as pharmaceutical substances. Chemical class information is presented only to indicate the variety of structural elements present in the substances evaluated in this analysis; it is not intended to evaluate the impact of structure on skin sensitization activity or potency.

[40] May 17, 2007, available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_9544.pdf

[41] Some substances were tested in more than one vehicle. In such instances, each substance–vehicle combination was considered separately, thus a total of 465 unique substance–vehicle combinations were evaluated.

[42] Available at http://www.nlm.nih.gov/mesh/meshhome.html

Table D-1 Summary of Traditional LLNA Data Sources and Rationale for Substance Selection

Data Source	Number of Studies	Primary Data Source and Substance Selection Rationale
Gerberick et al. (2005)[1]	210	Compiled from previously conducted studies (published literature and unpublished sources) on substances with varying skin sensitization potential
M.J. Olson/GlaxoSmithKline	124	Pharmaceuticals, pharmaceutical intermediates
Basketter, Gerberick, and Kimber[2]	31	Compiled from previously conducted studies (published literature and unpublished sources) on substances with varying skin sensitization potential
K. Skirda/CESIO (TNO Report V7217)	18	Data were provided by CESIO member companies for use in a paper titled "Limitations of the Local Lymph Node Assay (LLNA) as preferred test for skin sensitisation: concerns about false positive and false negative test results" (TNO report V7217)
Lalko and Api (2006)	17	Original research conducted on essential oils, which were representative of the oils commonly used in perfumery. Each contains significant amounts of one or more known skin sensitizers.
H.W. Vohr/BGIA	16	Original research with epoxy resin components as part of a validation effort for non-radioactive versions of the local lymph node assay
Ryan et al. (2002)	15	Original research with known water-soluble haptens and known skin sensitizers to assess the usefulness of a novel vehicle
D. Germolec/NIEHS	15	Substances evaluated by the National Toxicology Program for skin sensitization potential
E. Debruyne/Bayer CropScience SA	10	Original research on different pesticide types and formulations
P. Ungeheur/EFfCI	9	Data for selected unsaturated chemicals were provided in the report entitled "Comparative Experimental Study on the Skin Sensitising Potential of Selected Unsaturated Chemicals as Assessed by the Murine Local Lymph Node Assay (LLNA) and the Guinea Pig Maximisation Test (GPMT)"
P. Botham/ECPA	6	Plant protection products (i.e., pesticides) were evaluated in the local lymph node assay with a novel vehicle to assess its usefulness
Basketter et al., 2007	1	Original research that re-evaluated resorcinol in the local lymph node assay, which identified resorcinol as a sensitizer.
Total	**471**[3]	

Abbreviations: BGIA = Berufsgenossenschaftliches Institut fur Arbeitsschutz; CESIO = Comite Europeen des Agents de Surface et de Leurs Intermediaires Organiques; ECPA = European Crop Protection Association; EFfCI = European Federation for Cosmetic Ingredients; NIEHS = National Institute for Environmental Health Sciences: TNO = TNO Nutrition and Food Research

[1] These data were submitted to ICCVAM in 1998 for the original evaluation of the validation status of the LLNA (ICCVAM 1999) and were evaluated by the European Centre for the Validation of Alternative Methods (ECVAM) Scientific Advisory Committee in its evaluation of the rLLNA (Gerberick et al. 2005).

[2] Data were included in a submission to ECVAM for the validation of the traditional LLNA as a stand-alone assay for potency determination.

[3] The total number of studies does not take into account the fact that some substances were tested more than once (see **Section 3.2**)

Table D-2 provides chemical class information for the test substances in this rLLNA evaluation. The table distinguishes the chemical classifications of the 211 substances in the original evaluation of the rLLNA (Kimber et al. 2006; ESAC 2007) and the chemical classifications of the additional substances received in response to 72 FR 27815.[43] Of the 211 substances initially evaluated by Kimber et al. (2006), the known chemical classes with the greatest number of substances were carboxylic acids (29) and halogenated hydrocarbons (27). Of the additional 246 substances in this evaluation, the known chemical classes with the greatest number of substances tested were pharmaceutical chemicals (125), carboxylic acids (15), and lipids (14). Ten of the substances included in this evaluation were formulations. Seventy substances could not be assigned to a specific chemical class due to incomplete information (e.g., the lack of a CASRN or structure).

3.4 Coding Procedures

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.

Table D-2 Chemical Classes[1] Represented in the Current Traditional LLNA Database

Chemical Class	Number of Substances - Original[2]	Number of Substances - Additional[2]	Chemical Class	Number of Substances - Original	Number of Substances - Additional
Alcohols	9	4	Inorganic Chemicals	0	2
Aldehydes	21	4	Isocyanates	1	0
Amides	4	0	Ketones	5	0
Amidines	1	0	Lactones	2	2
Amines	14	7	Lipids	7	14
Anhydrides	1	0	Macromolecular Substances[3]	0	5
Carbohydrates	3	2	Nitriles	1	1
Carboxylic Acids	29	15	Nitro Compounds	2	0
Esters	3	0	Nitroso Compounds	3	0
Ethers	14	2	Onium Compounds	1	0
Formulations[3]	0	10	Pharmaceutical chemicals[4]	0	125
Heterocyclic Compounds	18	4	Phenols	18	2
Hydrocarbons, Acyclic	2	1	Polycyclic Compounds	5	3
Hydrocarbons, Cyclic	14	7	Quinones	1	1
Hydrocarbons, Halogenated	27	1	Sulfur Compounds	20	2
Hydrocarbons, Other	7	8	Urea	3	0
Imines	0	1	Unknown	28	42

[1] Total number of substances assigned to chemical classes does not equal the total number of substances evaluated because some substances were assigned to more than one class and some substances were not assigned to a specific chemical class.
[2] Number of substances - original represents the substances evaluated in Kimber et al. (2006).
Number of substances - additional represents the substances received in response to 72 FR 27815 (May 17, 2007) (see below)
[3] No chemical class could be assigned. The terms "formulation" or "macromolecular substance" was used to identify these substances.
[4] The chemical classification of "pharmaceutical chemicals" for the GlaxoSmithKline (GSK) substances was suggested by Dr. Michael Olson of GSK to capture three types of pharmaceutical substances (actives, intermediates, and starting materials).

[43] May 17, 2007, available at http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/FR/FR_E7_9544.pdf