7.0 Reliability of the rLLNA

An assessment of test method reliability (intralaboratory repeatability and intra- and interlaboratory reproducibility) is essential to evaluate the performance of an alternative test method (ICCVAM 2003). *Repeatability* refers to the closeness of agreement between test results obtained within a single laboratory when the procedure is performed on the same substance under identical conditions within a given time period (ICCVAM 1997, 2003). *Intralaboratory reproducibility* refers to the determination of the extent to which qualified personnel within the same laboratory can replicate results using a specific test protocol at different times. *Interlaboratory reproducibility* refers to the determination of the extent to which different laboratories can replicate results using the same protocol and test substances, and indicates the extent to which a test method can be transferred successfully among laboratories.

In the data review, interlaboratory reproducibility of the rLLNA could be assessed with traditional LLNA data available for only five substances that had been tested in the same vehicle at multiple labs (**Annex III**). These are dinitrochlorobenzene (DNCB), HCA, linalool alcohol, methyl salicylate, and potassium dichromate. **Table D-6** provides a summary of the responses obtained by the rLLNA. Among these five substances, tested independently in two to three laboratories, DNCB, methyl salicylate, and potassium dichromate (3/5 = 60%) were classified as sensitizers or non-sensitizers in all studies (i.e., 100% concordance). For the other two substances, HCA and linalool alcohol, tested independently in two laboratories, one traditional LLNA study indicated each substance as a sensitizer and the other traditional LLNA study indicated each substance as a non-sensitizer.

Review of the studies indicates that the discordant results were due to differences in the highest dose levels tested. However, because the rLLNA and traditional LLNA use identical protocols and use similar data sets to evaluate the accuracy of the rLLNA and traditional LLNA, the reliability of the two methods would be expected to be similar. That is, the intra- and interlaboratory reliability of the rLLNA would be expected to be similar to that of the traditional LLNA (see ICCVAM 1999 for these statistics).

Table D-6 rLLNA Responses for Repeated Studies

Substance	Data Source	Vehicle	Traditional LLNA Response in Multiple Studies						rLLNA Classification [1]
			Dose (%)/SI	Dose (%)/SI	Dose (%)/SI	Dose (%)/SI	Dose (%)/SI	Dose (%)/SI	
1-Chloro-2-dinitrobenzene	Gerberick et al. (2005)	AOO	0.01/1.50	0.025/1.80	0.05/2.40	0.1/8.90	0.25/38.00	NA	+
	Data submitted by D. Germolec		0.01/1.17	0.025/1.12	0.05/1.93	0.1/1.95	0.25/7.10	NA	+
Hexyl cinnamic aldehyde	Gerberick et al. (2005)	AOO	2.5/1.30	5/1.10	10/2.50	25/10.00	50/17.00	NA	+
	Data Submitted by H.W. Vohr		2.5/1.10	5/1.20	10/2.84	NA	NA	NA	-
Linalool alcohol	Gerberick et al. (2005)	AOO	NA	NA	NA	25/2.50	50/4.80	100/8.30	+
	Data Submitted by D. Basketter, I. Kimber, and F. Gerberick		1/1.00	10/1.30	30/1.30	NA	NA	NA	-
Methyl salicylate	Gerberick et al. (2005)	AOO	1/1.00	2.5/1.10	5/1.60	10/1.40	20/0.90	NA	-
	Data submitted by D. Germolec		1/0.86	2.5/1.19	5/1.16	10/1.41	20/1.72	NA	-
Potassium dichromate	Gerberick et al. (2005)	DMSO	0.025/1.60	0.05/1.40	0.1/3.80	0.25/5.30	0.5/16.10	NA	+
	Data submitted by D. Germolec		0.025/1.21	0.05/1.84	0.1/2.22	0.25/3.39	NA	NA	+
	Ryan et al. (2002)		0.025/1.40	0.05/2.50	0.1/9.50	0.25/25.90	0.5/10.10	NA	+

Abbreviations: AOO = acetone: olive oil; DMSO = dimethyl sulfoxide; NA = not applicable because dose level was not tested; SI = stimulation index

[1] - = non-sensitizer, + = sensitizer