Appendix E

Supplementary Analysis of Pesticide Formulations in the LLNA

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1.0 TESTING OF PESTICIDE FORMULATIONS

1.1 Testing of Pesticide Formulations: LLNA vs. GP with Available Reference Data for the Entire Formulation

For the 22 formulations that had associated GP data for the formulation itself, 14% (3/22) were classified as sensitizers and 86% (19/22) as non-sensitizers according to the GP results (**Figure E-1**). These results are based on a positive overall GP call for formulation EXP 10810[1]. The LLNA classified 59% (13/22) of the formulations as sensitizers and 41% (9/22) as non-sensitizers (**Figure E-1**). All three of the pesticide formulations identified as sensitizers in the GP test were also identified as sensitizers in the LLNA. The LLNA also identified an additional six substances as sensitizers that were classified as non-sensitizers in the GP test (**Table E-1**). There were no comparative human data with which to determine the actual human sensitization potential.

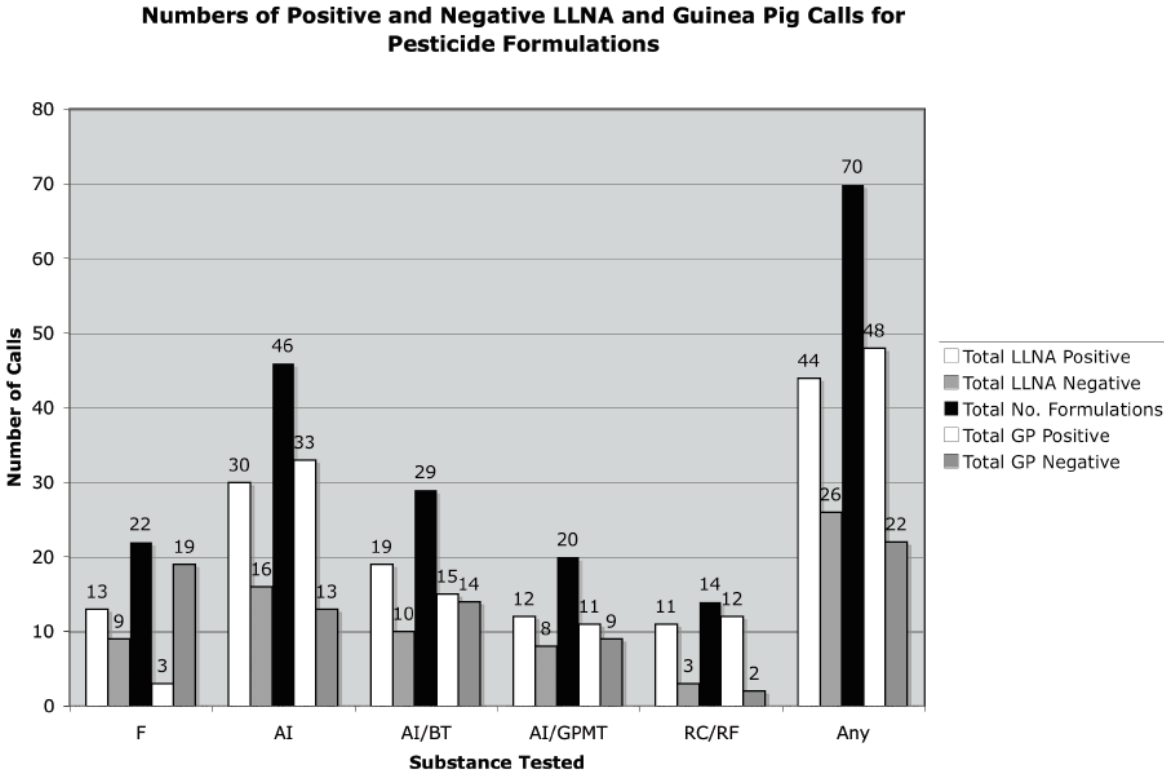
1.2 Testing of Pesticide Formulations: LLNA vs. GP with Any Available Reference Data for Relevant Substances

Of the 70 formulations, 69% (48/70) were classified as sensitizers and 31% (22/70) as non-sensitizers on the basis of various types of GP data (**Figure E-1**). To assign these classifications, a most conservative approach was used; i.e., if a GP result for the formulation, any active ingredient, a substance related to an active ingredient, or a related formulation indicated sensitization, the formulation was classified as a sensitizer. Additionally, a GP result for the formulation itself was given priority over a result for an active ingredient; a result for an active ingredient was given priority over results for a substance related to an active ingredient, or a related formulation. Based on the LLNA result with the entire formulation for these same 70 pesticide formulations, 63% (44/70) were classified as sensitizers and 37% (26/70) as non-sensitizers (**Figure E-1**). Sixty-five percent (31/48) of the pesticide formulations classified as sensitizers by a GP test, based on the

[1] Formulation EXP 10810 A (submitted by E. Debruyne, Bayer Crop Science), the only formulation for which there was data in both the GPMT and the BT, showed equivocal results in the guinea pig. This formulation tested positive in the GPMT (sensitization incidence 100%), and negative in the BT (sensitization incidence 10%). The patch concentration in the GPMT was the same as the induction concentration in the BT (50%).

criteria given above would also have been classified as sensitizers in the LLNA (**Table E-1**). The LLNA also identified an additional 14 formulations as sensitizers that would have been classified as non-sensitizers by a GP test based on these criteria. However, the LLNA failed to identify an additional 36% (17/48) formulations as sensitizers, which would have been classified as such by a GP test, based on the criteria given above.

Figure E-1 Numbers of Positive and Negative LLNA (All Mouse Strains) and GP Calls for Pesticide Formulations



Abbreviations: AI - Active Ingredient Test: BT= Buehler test; F - Formulation Test; GP = Guinea pig; ; GPMT = Guinea Pig Maximization Test; RC/RF - Related Substance or Related Formulation Test

Table E-1 Evaluation of the Performance of the LLNA in Testing Pesticide Formulations

Comparison[1]	n[2]	Accuracy		Sensitivity		Specificity		False Positive Rate		False Negative Rate	
		%	No.[3]	%	No.[3]	%	No.[3]	%	No.[3]	%	No.[3]
LLNA vs. GP[4] (Formulation[5])	22	54	12/22	100	3/3	53	10/19	47	9/19	0	0/3
LLNA vs. GP[4] (Any[6])	70	56	39/70	65	31/48	36	8/22	64	14/22	35	17/48
LLNA vs. GP[4] (Active Ingredient[7])	46	72	33/46	76	25/33	62	8/13	38	5/13	24	8/33
LLNA vs. BT (Active Ingredient[7])	29	59	17/29	73	11/15	43	6/14	57	8/14	27	4/15
LLNA vs. GPMT (Active Ingredient[7])	20	55	11/20	64	7/11	44	4/9	56	5/9	36	4/11
LLNA vs. GP[4] (Related Substance or Formulation[8])	14	64	9/14	75	9/12	0	0/2	100	2/2	25	3/12
ICCVAM 1999 Database: Evaluation of LLNA Data vs. GP Data or Human Data[9]											
LLNA vs. GP[4]	126	86	108/126	87	81/93	82	27/33	18	6/33	13	12/93
LLNA vs. Human[10]	74	72	53/74	72	49/68	67	4/6	33	2/6	28	19/68
GP[4] vs. Human[10]	62	73	45/62	71	42/59	100	3/3	0	0/3	29	17/59

Abbreviations: GP = Guinea pig skin sensitization outcomes; LLNA = Local Lymph Node Assay; No. = Number.
Accuracy (concordance) = the proportion of correct outcomes (positive and negative) of a test method; Sensitivity = the proportion of all positive substances that are classified as positive; Specificity = the proportion of all negative substances that are classified as negative; False negative rate: the proportion of all positive substances that are falsely identified as negative; False positive rate = the proportion of all negative substances that are falsely identified as positive.
[1]This accuracy analysis is only for formulations that have LLNA data and some type of associated GP data; none of the pesticide formulations analyzed had human data, so a comparison between LLNA vs. human and LLNA vs. GP is not included.
[2]n = Number of substances included in this analysis.
[3]The data on which the percentage calculation is based.
[4]GP refers to outcomes obtained by studies conducted using either the Guinea Pig Maximization Test , the Buehler Test or the McGuire Test.
[5]Formulation refers to associated GP data for the formulation itself.
[6]Any refers to associated GP data for the formulation itself, any active ingredient in the formulation, a substance related to an active ingredient, or a related formulation.
[7]Active ingredient refers to associated GP data for any active ingredient in the formulation
[8]Related substance or formulation refers to associated GP data for a substance related to an active ingredient, or a related formulation.
[9]For comparison purposes, an excerpt from the ICCVAM evaluation report (ICCVAM 1999; **Appendix A**) showing the overall performance of the LLNA vs. GP and human, and GP versus human is included here.
[10]Human refers to outcomes obtained by studies conducted using the Human Maximization Test or the inclusion of the test substance in a Human Patch Test Allergen Kit.

1.3 Testing of Pesticide Formulations: LLNA vs. GP with Available Reference Data for Active Ingredients

Of the 46 formulations that had associated GP data for one or more of the active ingredients, 72% (33/46) were classified as sensitizers and 28% (13/46) as non-sensitizers on the basis of

an active ingredient in a GP test. Based on the LLNA result with the entire formulation for these same 46 pesticide formulations, 65% (30/46) were classified as sensitizers and 35% (16/46) as non-sensitizers (**Figure E-1**). Seventy-six percent (25/33) of the pesticide formulations identified as sensitizers based on a GP test of an active ingredient were identified as sensitizers in the LLNA (**Table E-1**). The LLNA also identified an additional five substances as sensitizers that were classified as non-sensitizers in the GP test. However, the LLNA failed to identify 24% (8/33) of the formulations as sensitizers that would have been classified as such by a GP test on an active ingredient (**Table E-1**).

Among these same 46 formulations with available GP data for one or more of the active ingredients, 29 had BT data and 20 had GPMT data (**Figure E-1**).

Of the 29 pesticide formulations with BT data for the active ingredient, 52% (15/29) were classified as sensitizers and 48% (14/29) as non-sensitizers. By comparison, LLNA results with the complete formulation for each of these products identified 66% (19/29) as sensitizers and 34% (10/29) as non-sensitizers (**Figure E-1**). Eleven of the pesticide formulations identified as sensitizers based on a BT of an active ingredient were identified as sensitizers in the LLNA (**Table E-1**). The LLNA also identified an additional eight substances as sensitizers that would have been classified as non-sensitizers in a BT on an active ingredient. However, the LLNA failed to identify 27% (4/15) formulations as sensitizers that would have been classified as such by a BT on an active ingredient.

Similarly, of the 20 pesticide formulations with GPMT data for the active ingredient, 55% (11/20) were classified as sensitizers and 45% (9/20) as non-sensitizers. The proportion of formulations classified as sensitizers was similar that classified as sensitizers by the BT done on an active ingredient. By comparison, LLNA results with the complete formulation for each of these products identified 60% (12/20) as sensitizers and 40% (8/20) as non-sensitizers. Sixty-four percent (7/11) of the pesticide formulations identified as sensitizers based on a GPMT of an active ingredient were identified as sensitizers in the LLNA (**Table E-1**). The LLNA also identified an additional five formulations as sensitizers that would have been classified as non-sensitizers by GPMT on an active ingredient. However, the LLNA failed to identify 36% (4/11) formulations as sensitizers that would have been classified as such by a GPMT based on an active ingredient (**Table E-1**).

1.4 Testing of Pesticide Formulations: LLNA vs. GP with Available Reference Data for a Related Substance

Of the 14 formulations that had associated GP data for a substance related to an active ingredient, or a related formulation, 86% (12/14) were classified as sensitizers and 14% (2/14) as non-sensitizers on the basis of the related substance or formulation in a GP test. By comparison, LLNA results with the complete formulation identified 79% (11/14) as sensitizers and 21% (3/14) as non-sensitizers (**Figure E-1**). Nine of the pesticide formulations identified as sensitizers based on a GP test on a substance related to an active ingredient, or a related formulation, were identified as sensitizers in the LLNA (**Table E-1**). The LLNA also identified an additional two formulations as sensitizers that would have been classified as non-sensitizers by a GP test on a substance related to an active ingredient, or a related formulation.. However, the LLNA failed to identify an additional three formulations as sensitizers that would have been classified as by a GP test on a substance related to an active ingredient, or a related formulation (**Table E-1**).

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