Attachment10f:_Statistical analysis of the quantitative data for BLR

Givaudan Statistical Report

Sensory Platform - Ashford

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SUMMARY

The Ring study is a project to assess the within and between laboratory reproducibility (WLR and BLR) of a biological assay to test skin sensitizing chemicals.
Assays were run in five laboratories in three repetitions (each repetition being the result of three replicates). One of the laboratories is Givaudan. Of interest was evaluating the reliability of the data of the other labs, in particular compared to the Givaudan data.
By calculating for each {parameter*chemical} data set the ratio {variance with the lab excluded / variance with all labs included}, the contributions to error variance (variance attributable to the measurement procedure) of the individual labs could be assessed. The ratio summarises contributions of the lab to error variance where low values of the ratio are indicative of large contributions. The error variance consists of the three components:

- Lab effect – the lab estimate differs from the other labs
- Replicate effect – variability in the replicate scores
- Lab*Replicate interaction – differences in variability of replicate scores between labs

One laboratory (lab 1) could be identified which has a ratio < 1 ($p<0.0001$) so has a relatively large contribution to the overall error variance. For the other three labs the ratio is not statistically significantly different from 1.

1. INTRODUCTION

The Ring study is a project to assess the within and between laboratory reproducibility (WLR and BLR) of a biological assay to test skin sensitizing chemicals.
In this assay 12 concentrations of test chemicals in two-fold dilutions are added to a cell line and the induction of a specific gene activity is measured. The concentration for a 1.5 fold induction of gene activity is then extracted from these curves by linear interpolation of the value above and below the threshold.
This assay was run in five laboratories in three repetitions (each repetition being the result of three replicates).
Three parameters have been measured (EC1.5, IC50, Imax) for a set of 33 chemicals. EC1.5 was measured for 16 chemicals, IC50 for 18 chemicals, and Imax for 28 chemicals, as shown in the table below.

	Parameter			
	EC1.5	IC50	Imax	OVERALL
Chemical				
(5-chloro)-Methyl-isothiazolinone	1	1	1	3
2,4-Dinitro-chlorobenzene	1	1	1	3
2-Mercapto-benzothiazole	1	1	1	3
4-Phenylenediamine	1	1	1	3
4-nitrobenzylbromide	1	1	1	3
Chlorobenzene	.	.	1	1
Cinnamic aldehyde	1	1	1	3
Cinnamyl alcohol	1	.	1	2
Citral	1	1	1	3
Diethyl phthalate	.	.	1	1
Ethylene glycol dimethacrylate	1	1	1	3
Eugenol	.	1	1	2
Glycerol	.	.	1	1
Glyoxal	1	1	1	3
Hexyl cinnamic aldehyde	.	1	1	2
Imidazolidinyl urea	1	1	1	3
Isoeugenol	1	1	1	3
Isopropanol	.	.	1	1

	Parameter			
	EC1.5	IC50	Imax	OVERALL
Lactic acid	.	.	1	1
Methyl salicylate	.	.	1	1
Methyldibromo glutaronitrile	1	1	1	3
Metol	1	1	1	3
Oxazolone	1	.	1	2
Phenyl benzoate	.	1	1	2
Salicylic acid	.	.	1	1
Sodium lauryl sulfate	.	1	1	2
Sulfanilamide	.	.	1	1
tetramethyl-thiuramdisulfide	1	1	1	3
OVERALL	16	18	28	62

2. METHOD

A total of five laboratories have been studied one of which is Givaudan. The aim is to assess the within and between laboratory reproducibility (WLR and BLR).
For each parameter and chemical, data are available of each of the five labs and three replications. Therefore the following *variance components* contribute to the total variance in the data for each parameter*chemical combination:

1. The between laboratories variance: to what extent do the scores of the laboratories differ when averaged over the replications.
2. The replications variance: to what extent do the scores differ over the replications for all labs taken together.
3. The laboratories*replications interaction: differences in variablility across replications for the respective labs.

In order to assess the contributions of the individual laboratories to the total variance for each parameter*chemical combination, these variance components were estimated under two conditions:

- With all five laboratories included,
- With one laboratory (not Givaudan) excluded.

It was evaluated what happens to the variance if one lab is excluded by calulating the ratio {variance with one lab excluded / variance with all labs included}. Since basically we want labs to be similar to others and consistent over time, we would like the error variance to be as low as

possible. Accordingly, if a lab is excluded from the set of all labs we want the error variance to go up and not down. The ratio {variance with one lab excluded / variance with all labs included} should therefore be as high as possible, and not < 1.

3. RESULTS

Laboratory	Ratio*	P-value
lab 1	0.825	<0.0001
lab 2	1.012	0.7398
lab 3	0.972	0.5542
lab 4	0.990	0.8397

*Ratio {variance with lab excluded / variance with all labs included}

It can be seen in the table above that for lab 1, the ratio {variance with the lab excluded / variance with all labs included} is < 1. The difference is statistically significant ($p<0.05$). For the other three labs, the null hypothesis that he ratio equals 1 cannot be rejected.
This means that labs 2, 3 and 4 in general provide reliable data with relatively low error variance, and lab 1 less so. The best performing lab is lab 2 and the worst perfoming lab is lab 1.
The table below provides the ratios for the respective labs and parameters.

	Lab				
	1	**2**	**3**	**4**	**OVERALL**
parameter					
EC1.5	0.874	1.002	0.988	1.023	0.970
IC50	0.848	0.963	1.028	0.937	0.942
Imax	0.757	1.086	0.905	1.014	0.932
OVERALL	0.825	1.012	0.972	0.990	0.944

This table shows that the poor overall result for lab 1 is caused by high error variance for all three parameters.

A more detailed analysis relating these results back to the source data is possible by looking at the ingredient data. In the three tables below in appendix 1 (pages 7-9) the ratios {variance with the lab excluded / variance with all labs included} are given for each lab and ingredient. Values < 0.5 have been indicated in red. Very low values mean that the lab is a big contributor to the total error variance.

For lab 1 the first red number is for Glyoxal, parameter EC1.5 (page 6: ratio=0.331). The Givaudan estimate for this is 134.16 with SD=5.17 (see appendix 2, page 10 below). The estimate of Lab 1 is 99.35 with SD=65.42. So the estimate is quite different from Givaudan's, and the variability in the scores is considerable.

A second example concerning lab 1 is Isoeugenol, parameter EC1.5 (ratio=0.470). The Givaudan estimate for this is 19.64 with SD=8.89. The estimate of Lab 1 is 210.45 with SD=310.16. So the estimate is very different from Givaudan's indeed, and the variability in the scores is huge.

These two examples demonstrate the meaning of the ratio {variance with the lab excluded / variance with all labs included}: it summarises contributions of the lab to error variance where low values of the ratio are indicative of large contributions. The error variance constists of the three components:

- Lab effect – the lab estimate differs from the other labs
- Replicate effect – variability in the replicate scores
- Lab*Replicate interaction – differences in variability of replicate scores between labs.

4. DISCUSSION AND CONCLUSIONS

By calculating for each parameter*chemical data set the ratio {variance with the lab excluded / variance with all labs included}, the contributions to error variance (= variance attributable to the measurement procedure) of the individual labs can be assessed. The ratio summarises contributions of the lab to error variance where low values of the ratio are indicative of large contributions. The error variance consists of the three components:

- Lab effect – the lab estimate differs from the other labs
- Replicate effect – variability in the replicate scores
- Lab*Replicate interaction – differences in variability of replicate scores between labs

One laboratory (lab 1) could be identified which has a ratio < 1 ($p<0.0001$) so has a relatively large contribution to the overall error variance. For the other three labs the ratio is not statistically significantly different from 1.

5. APPENDIX 1: RATIOS PER PARAMETER, INGREDIENT AND LAB

parameter EC1.5

	lab			
	1	**2**	**3**	**4**
ingredient				
(5-chloro)-Methyl-isothiazolin	0.912	1.206	0.861	1.286
2,4-Dinitro-chlorobenzene	1.169	1.057	0.580	1.314
2-Mercapto-benzothiazole	0.854	0.928	1.252	0.940
4-Phenylenediamine	1.221	0.885	1.322	0.346
4-nitrobenzylbromide	1.146	0.825	1.085	1.085
Cinnamic aldehyde	0.946	1.007	1.257	0.851
Cinnamyl alcohol	0.543	1.308	1.140	1.260
Citral	1.032	0.911	0.854	1.307
Ethylene glycol dimethacrylate	0.948	1.317	0.243	1.325
Glyoxal	0.331	1.293	1.182	1.034
Imidazolidinyl urea	1.312	0.605	1.233	1.327
Isoeugenol	0.470	1.225	0.843	1.296
Methyldibromo glutaronitrile	0.982	1.023	1.244	1.130
Metol	0.956	0.985	1.263	0.876
Oxazolone	1.075	0.966	1.295	0.664
tetramethyl-thiuramdisulfide	0.879	0.817	1.244	1.112

parameter IC50

	lab			
	1	2	3	4
ingredient				
(5-chloro)-Methylisothiazolino	0.865	1.169	1.225	1.168
2,4-Dinitrochlorobenzene	0.417	1.181	1.124	0.986
2-Mercaptobenzothiazole	0.933	1.100	1.057	1.092
4-Methylaminophenol sulphate (	1.055	1.314	0.615	0.767
4-Phenylenediamine	1.275	1.102	1.253	0.680
4-nitrobenzylbromide	0.604	0.915	1.142	1.131
Cinnamic aldehyde	1.192	1.208	0.338	1.026
Citral	1.238	1.114	1.330	0.796
Ethylene glycol dimethacrylate	0.946	1.058	1.206	0.851
Eugenol	0.637	0.978	0.967	1.256
Glyoxal	0.321	1.224	1.021	1.202
Hexyl cinnamic aldehyde	1.125	1.002	1.108	0.672
Imidazolidinyl urea	0.786	0.373	1.276	1.295
Isoeugenol	0.766	0.779	1.206	1.039
Methyldibromo glutaronitrile	0.764	0.760	1.186	1.131
Phenyl benzoate	0.981	0.837	1.068	0.957
Sodium lauryl sulfate	1.091	0.984	0.946	0.745
tetramethylthiuramdisulfide	1.175	0.812	1.171	0.546

parameter Imax

	lab			
	1	2	3	4
ingredient				
(5-chloro)-Methyl-isothiazolin	0.991	1.039	0.525	1.183
2,4-Dinitro-chlorobenzene	0.266	1.254	1.017	1.212
2-Mercapto-benzothiazole	0.507	1.079	0.992	1.202
4-Phenylenediamine	1.043	1.132	1.120	0.998
4-nitrobenzylbromide	0.677	0.978	1.024	1.167
Chlorobenzene	1.084	1.066	1.254	0.597
Cinnamic aldehyde	0.483	1.176	0.887	1.215
Cinnamyl alcohol	0.249	0.971	1.290	1.279
Citral	0.719	0.892	0.899	1.309
Diethyl phthalate	1.135	1.056	1.097	0.741
Ethylene glycol dimethacrylate	0.458	1.152	0.903	1.232
Eugenol	1.116	0.900	0.970	1.026
Glycerol	1.307	0.854	1.054	1.104
Glyoxal	0.607	1.242	0.682	1.245
Hexyl cinnamic aldehyde	1.033	1.112	1.008	0.716
Imidazolidinyl urea	1.245	1.225	0.891	1.030
Isoeugenol	0.996	1.117	0.512	1.118
Isopropanol	1.255	0.788	0.790	1.143
Lactic acid	1.212	1.146	1.238	1.245
Methyl salicylate	0.164	1.279	1.280	1.242
Methyldibromo glutaronitrile	0.589	1.113	0.870	1.218
Metol	0.828	1.112	0.671	1.165
Oxazolone	1.045	1.142	0.774	1.010
Phenyl benzoate	1.288	1.243	1.290	0.118
Salicylic acid	0.824	1.099	1.230	1.317
Sodium lauryl sulfate	0.750	0.985	1.173	0.954
Sulfanilamide	1.282	1.169	0.378	1.226
tetramethyl-thiuramdisulfide	0.616	1.310	0.621	1.229

6. APPENDIX 2: MEANS AND STANDARD DEVIATIONS OF LAB SOURCE DATA

parameter EC1.5

	lab														
	GIV			Lab1			Lab2			Lab3			Lab4		
	Result			Result			Result			Result			Result		
	N	Mean	STD	N	Mean	STD	N	Mean	STD	N	Mean	STD	N	Mean	STD
ingredient															
(5-chloro)-Methyl-isothiazolin	3	8.67	4.76	3	1.23	0.84	3	4.51	1.82	3	0.93	0.48	3	2.94	1.15
2,4-Dinitro-chlorobenzene	3	3.35	0.78	3	2.17	0.62	3	3.05	0.76	3	1.47	0.54	3	2.12	0.03
2-Mercapto-benzothiazole	3	44.96	9.19	3	287.27	301.93	3	74.45	51.66	3	65.56	3.38	3	234.26	68.83
4-Phenylenediamine	3	9.98	1.26	3	10.37	2.78	3	9.44	5.63	3	6.04	0.43	3	13.85	11.27
4-nitrobenzylbromide	3	1.45	0.44	3	0.68	0.31	3	1.48	0.72	3	0.50	0.00	3	0.50	0.00
Cinnamic aldehyde	3	14.33	1.44	3	14.77	0.89	3	6.14	1.60	3	7.73	2.22	3	7.56	5.07
Cinnamyl alcohol	3	104.98	15.90	2	125.24	92.89	3	87.81	20.42	3	133.30	29.58	3	118.50	9.98
Citral	3	17.27	1.99	3	13.81	6.19	3	22.10	10.92	3	18.07	9.03	3	16.35	1.36
Ethylene glycol dimethacrylate	3	82.29	14.47	3	113.67	21.27	3	54.78	5.10	3	11.01	3.44	3	42.24	5.89
Glyoxal	3	134.16	5.77	3	99.35	65.42	3	120.13	6.52	3	96.29	14.14	3	174.01	49.54
Imidazolidinyl urea	2	50.00	0.00	3	35.94	2.93	3	31.58	11.74	3	31.34	2.03	3	32.19	0.89
Isoeugenol	3	19.64	8.89	3	204.45	310.16	3	11.26	4.82	3	5.38	4.25	3	24.20	10.46
Methyldibromo glutaronitrile	3	12.80	3.56	3	7.58	3.05	3	11.33	3.98	3	7.41	0.88	3	6.72	1.00
Metol	3	9.41	4.88	3	3.45	1.52	3	6.84	3.97	3	6.62	1.06	3	4.36	3.30
Oxazolone	3	191.22	6.04	3	213.78	142.95	3	264.25	120.46	3	154.63	28.82	3	89.72	34.68
tetramethyl-thiuramdisulfide	3	0.50	0.00	3	3.60	5.37	3	4.21	1.02	3	2.18	1.22	3	0.87	0.64

parameter IC50

	lab														
	GIV			Lab1			Lab2			Lab3			Lab4		
	Result			Result			Result			Result			Result		
	N	Mean	STD	N	Mean	STD	N	Mean	STD	N	Mean	STD	N	Mean	STD
ingredient															
(5-chloro)-Methylisothiazolino	3	41.45	31.58	3	10.35	5.85	3	25.58	0.36	3	15.40	6.89	3	13.70	6.68
2,4-Dinitrochlorobenzene	3	10.11	0.92	3	7.28	3.99	3	9.71	1.99	3	8.69	2.42	3	12.53	0.20
2-Mercaptobenzothiazole	3	950.10	20.33	3	1131.9	311.27	3	1718.6	252.12	3	1773.2	225.63	3	1632.9	399.07
4-Methylaminophenol sulphate (	3	56.18	26.57	3	36.08	18.56	3	53.62	1.13	3	139.61	32.69	3	29.87	22.08
4-Phenylenediamine	3	393.15	11.12	3	396.42	3.22	3	597.66	180.04	3	672.99	211.71	3	509.63	342.18
4-nitrobenzylbromide	3	6.36	0.90	3	7.57	5.19	3	15.20	8.84	3	11.20	3.26	3	9.14	5.27
Cinnamic aldehyde	3	158.17	19.77	3	183.17	69.06	3	133.80	21.38	3	366.25	126.83	3	113.24	6.27
Citral	3	103.81	3.70	3	162.22	36.47	3	178.61	56.07	3	167.08	10.65	3	239.04	11.54
Ethylene glycol dimethacrylate	3	897.87	40.29	3	967.90	545.13	3	1247.9	651.82	3	996.92	333.88	3	1923.4	132.69
Eugenol	3	1343.4	20.61	3	1315.5	364.96	3	1664.4	290.78	3	1658.3	297.69	3	1538.5	48.22
Glyoxal	3	615.75	12.57	3	541.00	162.52	3	723.20	54.27	3	800.00	0.00	3	733.61	57.67
Hexyl cinnamic aldehyde	3	32.29	4.50	3	42.16	19.04	3	32.68	13.20	3	65.66	25.71	3	91.01	6.52
Imidazolidinyl urea	3	92.85	1.67	3	82.01	18.46	3	132.24	56.13	3	103.08	4.62	3	94.71	0.88
Isoeugenol	3	526.86	46.77	3	645.06	346.87	3	1045.6	411.75	3	795.89	101.56	3	533.31	172.93
Methyldibromo glutaronitrile	3	31.62	7.07	3	25.21	5.85	3	53.58	3.98	3	43.57	2.66	3	32.47	7.93
Phenyl benzoate	3	186.32	2.13	3	279.85	110.74	3	392.87	345.03	3	251.73	100.29	3	466.36	296.95
Sodium lauryl sulfate	3	56.64	5.56	3	76.10	18.15	3	57.34	23.59	3	46.27	1.76	3	91.21	4.64
tetramethylthiuramdisulfide	3	23.24	0.49	3	29.26	15.50	3	53.69	0.86	3	37.56	10.03	3	11.96	2.01

parameter Imax

	lab														
	GIV			Lab1			Lab2			Lab3			Lab4		
	Result			Result			Result			Result			Result		
	N	Mean	STD	N	Mean	STD	N	Mean	STD	N	Mean	STD	N	Mean	STD
ingredient															
(5-chloro)-Methyl-isothiazolin	3	7.63	1.46	3	4.95	0.97	3	4.79	0.17	3	13.54	2.21	3	7.83	3.32
2,4-Dinitro-chlorobenzene	3	12.88	2.60	3	4.28	1.29	3	12.24	5.37	3	19.53	4.79	3	15.64	1.28
2-Mercapto-benzothiazole	3	24.55	17.47	3	3.87	1.97	3	54.23	26.84	3	64.05	25.14	3	21.53	14.42
4-Phenylenediamine	3	45.16	27.75	3	23.19	11.76	3	19.38	3.12	3	39.00	12.05	3	19.97	8.70
4-nitrobenzylbromide	3	7.03	2.37	3	4.68	1.73	3	13.60	6.28	3	14.02	0.78	3	9.69	4.64
Chlorobenzene	3	1.26	0.21	3	1.51	0.26	3	1.35	0.30	3	1.32	0.06	3	1.82	0.41
Cinnamic aldehyde	3	22.39	9.34	3	9.83	5.15	3	32.35	9.08	3	44.71	12.32	3	22.24	11.81
Cinnamyl alcohol	3	10.85	6.24	3	1.71	0.25	3	17.10	2.30	3	9.08	1.99	3	8.63	3.37
Citral	3	60.19	30.13	3	22.34	16.61	3	39.98	40.22	3	104.45	27.94	3	50.15	10.55
Diethyl phthalate	3	1.19	0.21	3	1.21	0.11	3	1.44	0.53	3	1.73	0.31	3	1.90	0.54
Ethylene glycol dimethacrylate	3	176.86	51.31	3	25.79	6.30	3	102.13	72.89	3	362.97	51.86	3	204.93	21.05
Eugenol	3	1.36	0.27	3	1.39	0.19	3	2.50	0.90	3	2.53	0.32	3	1.65	0.80
Glycerol	3	1.35	0.24	3	1.19	0.04	3	1.29	0.25	3	1.21	0.17	3	1.13	0.09
Glyoxal	3	67.11	40.79	3	14.49	3.48	3	58.30	24.36	3	195.02	73.05	3	69.70	44.95
Hexyl cinnamic aldehyde	3	1.75	0.53	3	1.40	0.11	3	1.54	0.36	3	4.21	1.22	3	5.26	1.20
Imidazolidinyl urea	3	1.85	0.35	3	3.58	1.61	3	5.07	3.53	3	11.53	9.53	3	9.16	1.69
Isoeugenol	3	13.20	5.66	3	8.44	2.58	3	23.23	13.48	3	56.80	36.28	3	14.38	10.33
Isopropanol	3	1.26	0.06	3	1.32	0.08	3	1.39	0.43	3	1.54	0.37	3	1.13	0.10
Lactic acid	3	1.72	0.61	3	1.31	0.14	3	1.34	0.36	3	1.25	0.06	3	1.12	0.06
Methyl salicylate	3	1.41	0.26	3	2.74	1.00	3	1.48	0.13	3	1.50	0.09	3	1.53	0.20
Methyldibromo glutaronitrile	3	5.20	1.17	3	2.07	0.22	3	6.10	3.08	3	7.83	1.72	3	3.30	0.34
Metol	3	5.91	0.11	3	4.48	0.40	3	9.39	2.33	3	12.15	0.48	3	6.35	2.27
Oxazolone	3	24.29	27.44	3	6.48	4.43	3	19.28	12.83	3	46.28	55.63	3	6.38	5.00
Phenyl benzoate	3	1.02	0.07	3	1.19	0.05	3	1.21	0.19	3	1.23	0.09	3	2.36	0.50
Salicylic acid	3	1.08	0.05	3	1.39	0.17	3	1.29	0.17	3	1.29	0.11	3	1.26	0.05
Sodium lauryl sulfate	3	5.94	2.02	3	1.44	0.43	3	5.14	4.27	3	2.66	0.89	3	8.15	4.44
Sulfanilamide	3	1.05	0.03	3	1.15	0.06	3	1.10	0.03	3	1.53	0.18	3	1.29	0.09
tetramethyl-thiuramdisulfide	3	22.27	13.14	3	6.08	6.23	3	17.70	2.01	3	67.90	25.87	3	9.28	0.47