

Multiple Comparisons

Suppose you reject the overall hypothesis $H_0: \mu_1 = \mu_2 = \mu_3 = \dots = \mu_t$

What next? Which means differ?

Linear combination: $\ell = \sum_{i=1}^t a_i \mu_i$ which is estimated by $\hat{\ell} = \sum_{i=1}^t a_i \bar{y}_i$. This linear combination is called a CONTRAST if $\sum_{i=1}^t a_i = 0$

Suppose $t=4$ populations

Example 1: The contrast for comparing two means, $\mu_1 = \mu_2$, is the contrast of the difference of the two means, $\mu_1 - \mu_2$, $\ell = \sum_{i=1}^t a_i \mu_i = (1)\mu_1 + (-1)\mu_2 + (0)\mu_3 + (0)\mu_4$

Example 2: Contrast for comparing the mean of one population with the average of two other population means $\frac{\mu_1 + \mu_2}{2} = \mu_3$. This can be written as a the contrast

$$\ell = \sum_{i=1}^t a_i \mu_i = (-1)\mu_1 + (-1)\mu_2 + (2)\mu_3 + (0)\mu_4 = \left(-\frac{1}{2}\right)\mu_1 + \left(-\frac{1}{2}\right)\mu_2 + (1)\mu_3 + (0)\mu_4$$

You can test hypotheses about contrasts.

$$H_0: \ell = \sum_{i=1}^t a_i \mu_i = 0$$

$$H_a: \ell = \sum_{i=1}^t a_i \mu_i \neq 0$$

$$TS: F_{obs} = \frac{SSC}{MSE} \text{ where } SSC = \frac{\hat{\ell}^2}{\sum_{i=1}^t a_i^2 / n_i} \quad \text{with } p\text{-value} = \Pr(F_{1, n_T - t} > F_{obs})$$

Two contrasts: $\ell_1 = \sum_{i=1}^t a_i \mu_i$ and $\ell_2 = \sum_{i=1}^t b_i \mu_i$ are ORTHOGONAL if $\sum_{i=1}^t \frac{a_i b_i}{n_i} = 0$

Treatment SS can be partitioned into $t-1$ mutually orthogonal contrasts

Example: The Meat study with using logCounts

Using R

Consider the following contrasts:

1. Compare the mean log Bacteria count for CO₂ to the mean log Bacteria count for the plastic condition.

What would this contrast look like?

2. Compare the mean log Bacteria count for CO₂ to the mean log Bacteria count for the mixed condition.

What would this contrast look like?

3. Compare the mean log Bacteria count for CO₂ to the mean log Bacteria count for the vacuum condition.

What would this contrast look like?

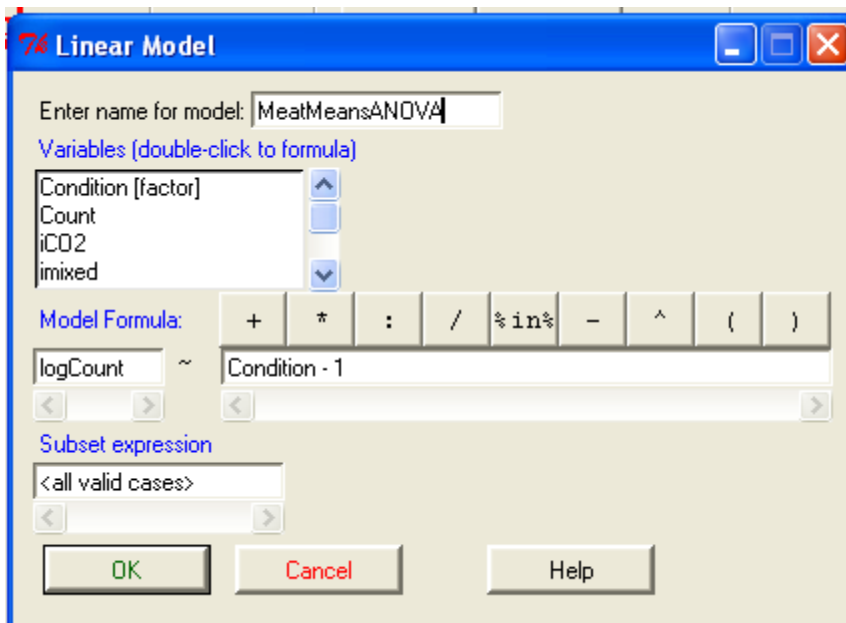
4. Compare the mean log Bacteria count for plastic to the average mean log Bacteria count for the other three conditions.

What would this contrast look like?

OK, so how do we do this on R? We first need to fit the Means Model, so:

With *MeatBacteria* the active data set, using RCommander:

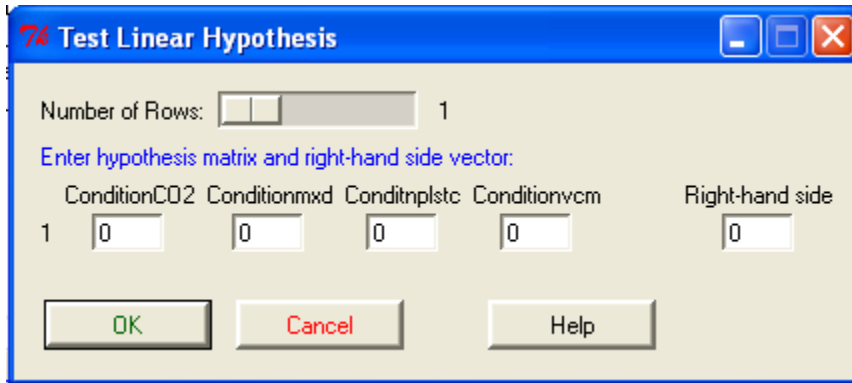
Statistics > Fit Models > Linear model ...



Now we can obtain contrasts of the means using this Means model.

With *MeatMeansANOVA* the active model, using RCommander:

Models > Hypothesis tests > Linear hypothesis ...

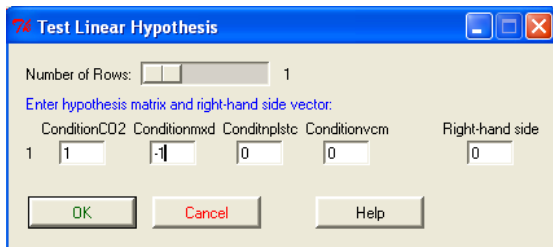


For each of the four contrasts we just plug in the constants as follows.

The four contrasts of interest were:

1. Compare the mean log Bacteria count for CO2 to the mean log Bacteria count for the plastic condition.

this contrast was: $\ell_1 = +1*\mu_{CO2} + -1*\mu_{plas} + 0*\mu_{mixed} + 0*\mu_{vac} = 0$.



```
> linear.hypothesis(MeatMeansANOVA, .Hypothesis,
rhs=.RHS)
Linear hypothesis test

Hypothesis:
ConditionCO2 - Conditionmixed = 0

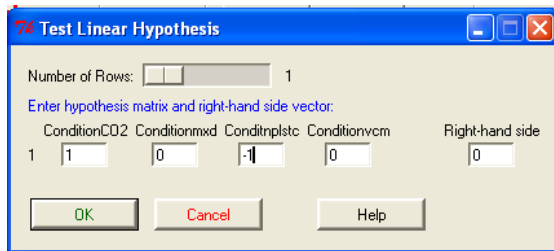
Model 1: logCount ~ Condition - 1
Model 2: restricted model

  Res.Df      RSS Df Sum of Sq      F      Pr(>F)
1       8   0.9268  0      0.0000    NA      NA
2       9  23.7418 -1  -22.8150 196.94 6.451e-07 ***
---
```

Conclusion?

- Compare the mean log Bacteria count for CO2 to the mean log Bacteria count for the mixed condition.

this contrast was: $\ell_2 = +1*\mu_{CO2} + 0*\mu_{plas} + -1*\mu_{mixed} + 0*\mu_{vac} = 0$.



```
> linear.hypothesis(MeatMeansANOVA, .Hypothesis,
rhs=.RHS)
```

Linear hypothesis test

Hypothesis:
ConditionCO2 - Conditionplastic = 0

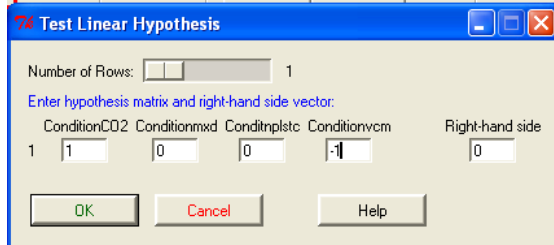
Model 1: logCount ~ Condition - 1
Model 2: restricted model

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
1	8	0.9268				
2	9	26.3884	-1	-25.4616	219.78	4.22e-07 ***

Conclusion?

- Compare the mean log Bacteria count for CO2 to the mean log Bacteria count for the vacuum condition.

this contrast was: $\ell_3 = +1*\mu_{CO2} + 0*\mu_{plas} + 0*\mu_{mixed} + -1*\mu_{vac} = 0$.



```
> linear.hypothesis(MeatMeansANOVA, .Hypothesis,
rhs=.RHS)
```

Linear hypothesis test

Hypothesis:
ConditionCO2 - Conditionvacuum = 0

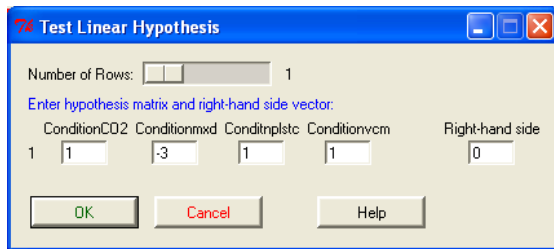
Model 1: logCount ~ Condition - 1
Model 2: restricted model

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
1	8	0.9268				
2	9	7.7962	-1	-6.8694	59.296	5.742e-05 ***

Conclusion?

- Compare the mean log Bacteria count for plastic to the average mean log Bacteria count for the other three conditions.

this contrast was: $\ell_4 = +1*\mu_{CO2} + -3*\mu_{plas} + 1*\mu_{mixed} + 1*\mu_{vac} = 0$.



```
> linear.hypothesis(MeatMeansANOVA, .Hypothesis,
rhs=.RHS)
Linear hypothesis test

Hypothesis:
ConditionCO2 - 3 Conditionmixed + Conditionplastic +
Conditionvacuum = 0

Model 1: logCount ~ Condition - 1
Model 2: restricted model
```

	Res.Df	RSS	Df	Sum of Sq	F	Pr(>F)
1	8	0.9268				
2	9	8.3252	-1	-7.3984	63.862	4.401e-05 ***

Conclusion?

Can we obtain estimates and CI's for these contrasts using R?

```
> library(multcomp)
Loading required package: mvtnorm
> glht(MeatMeansANOVA, linfct=matrix(c(1, -1, 0, 0,
+ 1, 0, -1, 0,
+ 1, 0, 0, -1,
+ 1, -3, 1, 1), byrow=TRUE, ncol=4))

General Linear Hypotheses

Linear Hypotheses:
Estimate
1 == 0 -3.90
2 == 0 -4.12
3 == 0 -2.14
4 == 0 -5.44
> summary(glht(MeatMeansANOVA, linfct=matrix(c(1, -1, 0, 0,
+ 1, 0, -1, 0,
+ 1, 0, 0, -1,
+ 1, -3, 1, 1), byrow=TRUE, ncol=4)))

Simultaneous Tests for General Linear Hypotheses

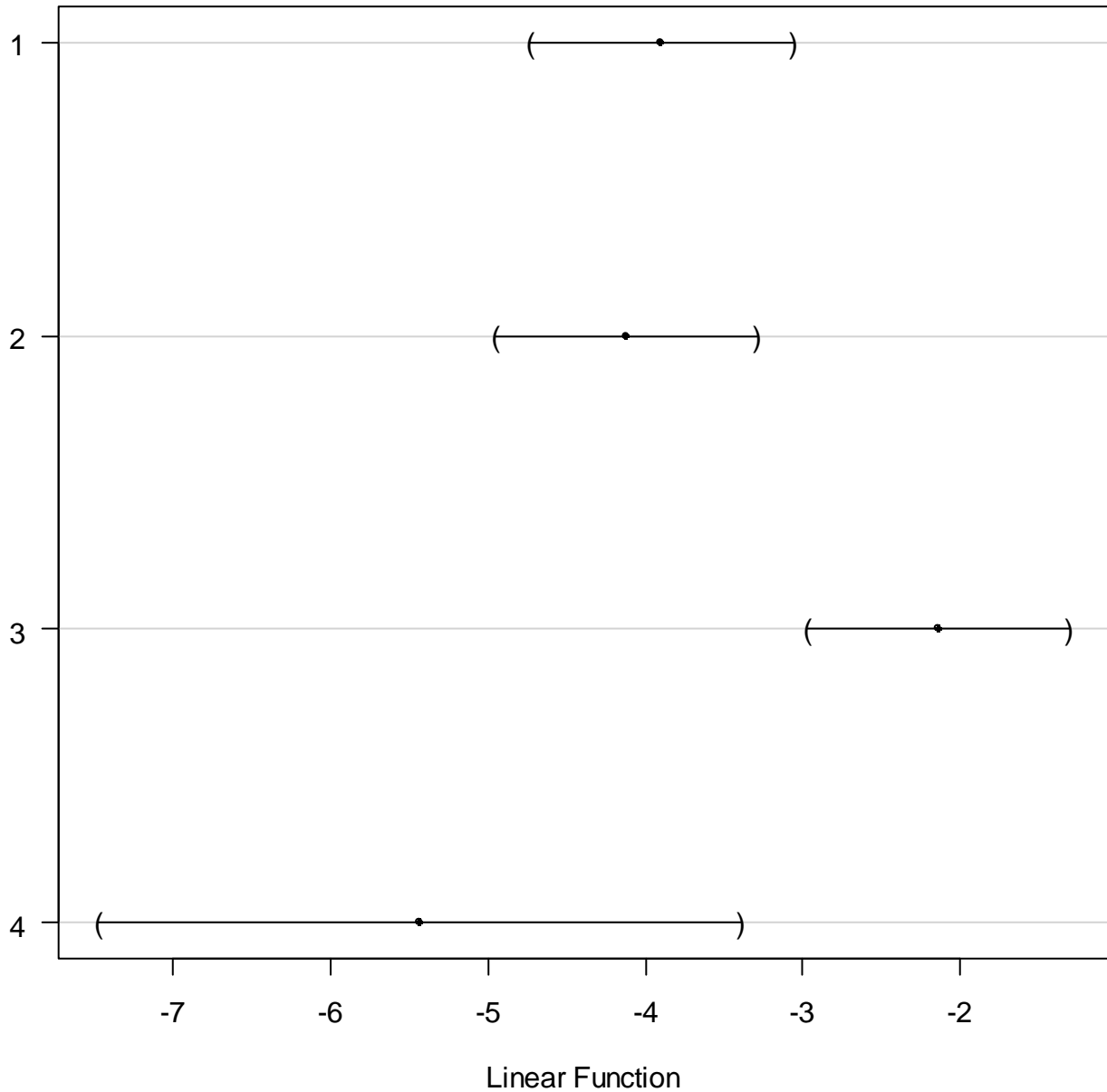
Fit: lm(formula = logCount ~ Condition - 1, data = MeatBacteria)

Linear Hypotheses:
Estimate Std. Error t value p value
1 == 0 -3.9000 0.2779 -14.033 <0.001 ***
2 == 0 -4.1200 0.2779 -14.825 <0.001 ***
3 == 0 -2.1400 0.2779 -7.700 <0.001 ***
4 == 0 -5.4400 0.6807 -7.991 <0.001 ***
---
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported)
```

And a plot of these contrast estimates and CI's is

```
> plot(glht(MeatMeansANOVA, linfct=matrix(c(1, -1, 0, 0,
+                                           1, 0, -1, 0,
+                                           1, 0, 0, -1,
+                                           1, -3, 1, 1), byrow=TRUE, ncol=4)))
```

95% family-wise confidence level



```

title "One-way ANOVA/ CRD example + contrasts";
title2 "Bacteria in meat data";
data meat;
  input condition $ logcount @@;
  datalines;
plastic 7.66 plastic 6.98 plastic 7.80
vacuum 5.26 vacuum 5.44 vacuum 5.80
mixed 7.41 mixed 7.33 mixed 7.04
CO2 3.51 CO2 2.91 CO2 3.66
;
* ORDER=DATA says plastic < vacuum < mixed < CO2 in Labels otherwise sorts
proc glm data=meat order=data;
class condition;
model logcount=condition;
output out=new p=yhat r=resid;
contrast 'plastic vs. rest' condition 3 -1 -1 -1;
estimate 'plastic vs. rest' condition 3 -1 -1 -1;
contrast 'CO2 vs. plastic' condition -1 0 0 1;
estimate 'CO2 vs. plastic' condition -1 0 0 1;
contrast 'CO2 vs. vacuum' condition 0 -1 0 1;
estimate 'CO2 vs. vacuum' condition 0 -1 0 1;
contrast 'CO2 vs. mixed' condition 0 0 -1 1;
estimate 'CO2 vs. mixed' condition 0 0 -1 1;
run;

```

* output (edited)

The GLM Procedure

Dependent Variable: logcount

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	32.87280000	10.95760000	94.58	<.0001
Error	8	0.92680000	0.11585000		
Corrected Total	11	33.79960000			

R-Square	Coeff Var	Root MSE	logcount Mean
0.972580	5.768940	0.340367	5.900000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
condition	3	32.87280000	10.95760000	94.58	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
condition	3	32.87280000	10.95760000	94.58	<.0001

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
plastic vs. rest	1	9.98560000	9.98560000	86.19	<.0001
CO2 vs. plastic	1	25.46160000	25.46160000	219.78	<.0001
CO2 vs. vacuum	1	6.86940000	6.86940000	59.30	<.0001
CO2 vs. mixed	1	22.81500000	22.81500000	196.94	<.0001

Dependent Variable: logcount

Parameter	Estimate	Standard Error	t Value	Pr > t
plastic vs. rest	6.32000000	0.68073490	9.28	<.0001
CO2 vs. plastic	-4.12000000	0.27790886	-14.83	<.0001
CO2 vs. vacuum	-2.14000000	0.27790886	-7.70	<.0001
CO2 vs. mixed	-3.90000000	0.27790886	-14.03	<.0001

Individual versus Experimentwise Error Rates

α_I = **Individual** Type I error rate

= Pr(Type I Error on a PARTICULAR comparison)

α_E = **Experimentwise** Type I error rate

= Pr(at least one Type I error when conducting a collection of comparisons)

If you have "m" independent comparisons, $\alpha_E = 1 - (1 - \alpha_I)^m$

So, α_E increases with the number of comparisons.

Bonferroni inequality: $\alpha_E \leq m \alpha_I$ so ...

set $\alpha_I = \alpha_E / m$ for individual tests to protect overall at fixed α_E

Procedure		
Bonferroni	t-based with Bonferroni adjustment for α_I	
Fisher's LSD	t-based ("protected" if require overall F to reject first)	-MCA
Tukey's HSD	Based on Studentized Range distribution -	-MCA -Can also form simultaneous CIs - harmonic mean often substituted if n_i not same
Dunnett's Procedure	Comparing all groups to control (i.e. "t-1" comparisons)	-MCC
Scheffe' S	General procedure for comparing all possible contrasts	

Example: The Meat study with using logCounts

Using R

```
> library(multcomp)
Loading required package: mvtnorm
> confint(MeatANOVA)
              2.5 %   97.5 %
(Intercept)    2.906844 3.813156
Condition[T.mixed]  3.259141 4.540859
Condition[T.plastic] 3.479141 4.760859
Condition[T.vacuum]  1.499141 2.780859
> print(confint(glht(MeatANOVA, linfct=mcp(Condition="Tukey"))))

      Simultaneous Confidence Intervals for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

Fit: lm(formula = logCount ~ Condition, data = MeatData)

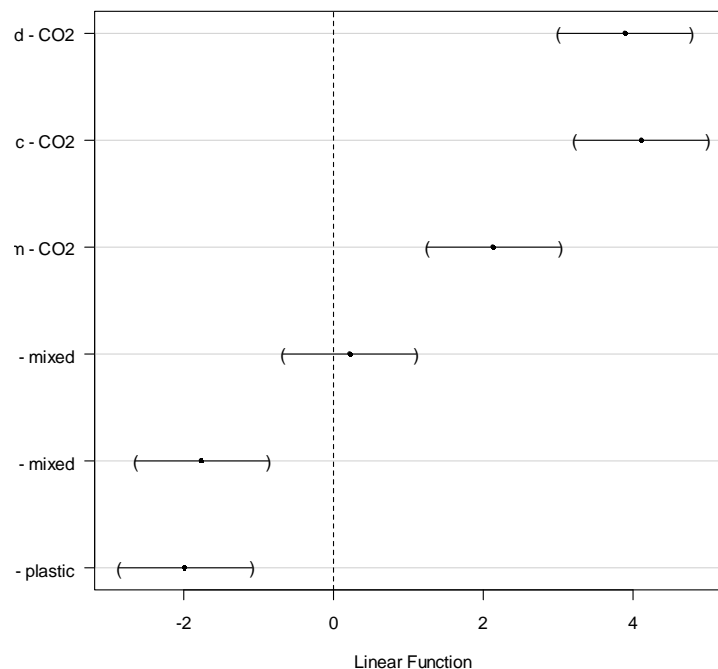
Estimated Quantile = 3.2024

Linear Hypotheses:
              Estimate lwr      upr
mixed - CO2 == 0    3.9000  3.0100  4.7900
plastic - CO2 == 0   4.1200  3.2300  5.0100
vacuum - CO2 == 0   2.1400  1.2500  3.0300
plastic - mixed == 0  0.2200 -0.6700  1.1100
vacuum - mixed == 0  -1.7600 -2.6500 -0.8700
vacuum - plastic == 0 -1.9800 -2.8700 -1.0900

95% family-wise confidence level

> plot(print(confint(glht(MeatANOVA, linfct=mcp(Condition="Tukey")))))
```

95% family-wise confidence level



Using SAS

```

title "One-way ANOVA/ CRD example + contrasts";
title2 "Bacteria in meat data";
data meat;
  input condition $ logcount @@;
  datalines;
plastic 7.66 plastic 6.98 plastic 7.80
vacuum 5.26 vacuum 5.44 vacuum 5.80
mixed 7.41 mixed 7.33 mixed 7.04
CO2 3.51 CO2 2.91 CO2 3.66
;
/*
ORDER=DATA says plastic < vacuum < mixed < CO2 in
Labels otherwise sorts
*/
proc glm data=meat order=data;
  class condition;
  model logcount=condition;
  lsmeans condition / stderr pdiff;
  means condition / lsd clm; * Fisher LSD;
  means condition / bon scheffe tukey; * Bonferroni, Scheffe, Tukey;
  means condition / bon tukey cldiff; * pairwise CIs generated;
run;

```

The GLM Procedure
Least Squares Means

condition	logcount LSMEAN	Standard Error	Pr > t	LSMEAN Number
plastic	7.48000000	0.19651124	<.0001	1
vacuum	5.50000000	0.19651124	<.0001	2
mixed	7.26000000	0.19651124	<.0001	3
CO2	3.36000000	0.19651124	<.0001	4

Least Squares Means for effect condition

Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: logcount

i/j	1	2	3	4
1		<.0001	0.4514	<.0001
2	<.0001		0.0002	<.0001
3	0.4514	0.0002		<.0001
4	<.0001	<.0001	<.0001	

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used

t Confidence Intervals for logcount

Alpha 0.05
Error Degrees of Freedom 8
Error Mean Square 0.11585
Critical Value of t 2.30600
Half Width of Confidence Interval 0.453156

condition	N	Mean	95% Confidence	
			Limits	
plastic	3	7.4800	7.0268	7.9332
mixed	3	7.2600	6.8068	7.7132
vacuum	3	5.5000	5.0468	5.9532
CO2	3	3.3600	2.9068	3.8132

means condition / bon scheffe tukey;

Tukey's Studentized Range (HSD) Test for logcount

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha 0.05
Error Degrees of Freedom 8
Error Mean Square 0.11585
Critical Value of Studentized Range 4.52880
Minimum Significant Difference 0.89

Means with the same letter are not significantly different.

	Mean	N	condition
A	7.4800	3	plastic
A			
A	7.2600	3	mixed
B	5.5000	3	vacuum
C	3.3600	3	CO2

Bonferroni (Dunn) t Tests for logcount

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha 0.05
Error Degrees of Freedom 8
Error Mean Square 0.11585
Critical Value of t 3.47888
Minimum Significant Difference 0.9668

Means with the same letter are not significantly different.

	Mean	N	condition
A	7.4800	3	plastic
A			
A	7.2600	3	mixed
B	5.5000	3	vacuum
C	3.3600	3	CO2

Scheffe's Test for logcount

NOTE: This test controls the Type I experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 8
Error Mean Square 0.11585
Critical Value of F 4.06618
Minimum Significant Difference 0.9706

Means with the same letter are not significantly different.

	Mean	N	condition
A	7.4800	3	plastic
A			
A	7.2600	3	mixed

B	5.5000	3	vacuum
C	3.3600	3	CO

means condition / bon tukey cldiff;

Tukey's Studentized Range (HSD) Test for logcount

NOTE: This test controls the Type I experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	0.11585
Critical Value of Studentized Range	4.52880
Minimum Significant Difference	0.89

Comparisons significant at the 0.05 level are indicated by ***.

condition Comparison	Difference		Simultaneous 95% Confidence Limits	
	Between Means			
plastic - mixed	0.2200	-0.6700	1.1100	
plastic - vacuum	1.9800	1.0900	2.8700	***
plastic - CO2	4.1200	3.2300	5.0100	***
mixed - plastic	-0.2200	-1.1100	0.6700	
mixed - vacuum	1.7600	0.8700	2.6500	***
mixed - CO2	3.9000	3.0100	4.7900	***
vacuum - plastic	-1.9800	-2.8700	-1.0900	***
vacuum - mixed	-1.7600	-2.6500	-0.8700	***
vacuum - CO2	2.1400	1.2500	3.0300	***
CO2 - plastic	-4.1200	-5.0100	-3.2300	***
CO2 - mixed	-3.9000	-4.7900	-3.0100	***
CO2 - vacuum	-2.1400	-3.0300	-1.2500	**

Bonferroni (Dunn) t Tests for logcount

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than Tukey's for all pairwise comparisons.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	0.11585
Critical Value of t	3.47888
Minimum Significant Difference	0.9668

Comparisons significant at the 0.05 level are indicated by ***.

condition Comparison	Difference		Simultaneous 95% Confidence Limits	
	Between Means			
plastic - mixed	0.2200	-0.7468	1.1868	
plastic - vacuum	1.9800	1.0132	2.9468	***
plastic - CO2	4.1200	3.1532	5.0868	***
mixed - plastic	-0.2200	-1.1868	0.7468	
mixed - vacuum	1.7600	0.7932	2.7268	***
mixed - CO2	3.9000	2.9332	4.8668	***
vacuum - plastic	-1.9800	-2.9468	-1.0132	***
vacuum - mixed	-1.7600	-2.7268	-0.7932	***
vacuum - CO2	2.1400	1.1732	3.1068	***
CO2 - plastic	-4.1200	-5.0868	-3.1532	***
CO2 - mixed	-3.9000	-4.8668	-2.9332	***
CO2 - vacuum	-2.1400	-3.1068	-1.1732	***