SELECTING ERROR MEAN SQUARES IN NON-REPLICATED AND COMPLEX DESIGN SITUATIONS

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ABSTRACT

It is demonstrated how to select error mean squares for setting confidence intervals and testing for significance for various effects. Often a complete or fractional factorial without replication is used. Often some function of the factors such as orthogonal polynomials can be used to explain most or all of the variation in the factor effects. In other situations, the complexity of the experimental design dictates that different error mean squares are needed for different factors in the experiment. Two such cases are a split-block experiment design and a repeated measures experiment design. Example 1 could be considered as a split-block designed experiment within runs and units or as a repeated measures experiment design. Example 2 is a non-replicated three-factor factorial treatment design with one combination missing. This makes the treatment design a fractional factorial. The data obtained from conducting the experiment are also presented. Analyses of variance partitioning of degrees of freedom are given for both examples. A SAS/GLM code and output from the program are given for Example 1 and for Example 2.

Keywords: Factorial, Fractional factorial, Orthogonal polynomial regression, Interaction, Split-block, Repeated measures.

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INTRODUCTION

Experiments are often conducted in such a manner that it is not obvious what to use as an error mean square for an effect for testing for significance and constructing confidence intervals. It may be necessary to have several error mean squares for the various effects present in an experiment. Sometimes a split-block or split-plot experiment design involving few to many error mean squares may be used. Sometimes a design can be considered either as a split-block or repeated measures experiment design. In other cases, a single replicate of a complete or fractional factorial may be used with no apparent error mean square for testing significance of effects. It is demonstrated how to select appropriate error mean squares for the various effects in an experiment. (See Effect of Microbial Activity on Trace Metals Released from Sewage Sludge, by Shabnam Qureshi, *et. al.*)

Example 1 may be considered to be designed either as a split-block or as a repeated measures experiment design. An analysis is given for each situation. A SAS/GLM program is given for an analysis of this experiment along with the output from the code. Example 2 was designed as a three-factor fractional factorial without replication and one missing combination. Two of the factors, time and temperature, lend themselves to orthogonal polynomial regression and interactions of these regressions. Data for this example are given. A SAS/GLM code is presented performing the statistical analyses and the output for one of the response variables is presented. Finally some comments relative to the examples are presented.

EXAMPLE 1 -- SPLIT-BLOCK

It was desired to know if given an option, fish would stay in water containing zinc or would migrate to water without zinc. Four units were set up. Each unit had three compartments. In one of the compartments fresh water was entered. In another compartment water containing zinc was entered. The water from the two end compartments flowed into and out of a middle compartment. The two treatments were designated as ZnYes and ZnNo. Five fish were introduced into the middle compartment of the tank (unit) and could choose to swim into either of the side compartments. Note that fish could choose to stay in the middle compartment and were not counted so that the counts in the side compartments did not necessarily have to add up to five. The number of fish in the two treatments was counted at three-minute intervals starting at 21 minutes. The counts continued up to 60 minutes or 14 time periods. The time periods and treatments are crossed as in a split-block experiment design. There were four units in each of three runs (replicates). Thus, a total of 2 treatments × 14 time periods × 4 units × three runs equals 336 observations. A possible analysis of variance table (ANOVA) is:

Source of variation	Degrees of freedom
Total	336
Correction for the mean	1
Runs	2

Units	3	
Runs × units	6	
Treatment	1	
Treatment within runs and units	11	(Error mean square for treatments)
Time	13	
Time within runs and units	143	(Error mean square for time)
Treatment × time	13	
Treatment × time within runs and units	143	(Error mean square for treatment × time)

There are three different error mean squares required for this analysis, one for treatments, one for times, and one for the interaction of times and treatments. Since this is an orthogonal design, little is to be gained by considering runs and units as random variables and using a mixed-model analysis, such as, e. g., the SAS/MIXED procedure.

Instead of considering time as levels of a fourth factor, the above may be considered as a three-factor factorial arrangement of treatment, unit, and run with repeated measurements over time. The response variables analyzed would then be the 2 × 4 × 3 = 24 linear regression coefficients, the 24 quadratic regression coefficients, etc. Also, one could conduct an analysis of the 24 predicted responses using linear regression, using linear plus quadratic regression, etc.

The data obtained are presented below with the SAS/GLM code following. Three separate models are considered. The second model arose from the fact that there are no discernable time effects, meaning that the experimenter could have stopped counting with the first time period, 21 minutes. The third model was an attempt to discern any possible time trends in the data. The various error terms used are given following the computer output.

```
DATA GEORGE;
INPUT Run Unit Time Treatment $ Group Number;
newtime=time;
CARDS;
53      1      21      ZnYes      1      1
53      1      21      ZnNo      1      3
53      2      21      ZnYes      2      1
53      2      21      ZnNo      2      4
53      3      21      ZnYes      3      2
53      3      21      ZnNo      3      2
53      4      21      ZnYes      4      1
53      4      21      ZnNo      4      2
53      1      24      ZnYes      1      1
53      1      24      ZnNo      1      2
53      2      24      ZnYes      2      1
53      2      24      ZnNo      2      4
53      3      24      ZnYes      3      1
53      3      24      ZnNo      3      3
53      4      24      ZnYes      4      0
53      4      24      ZnNo      4      4
53      1      27      ZnYes      1      1
53      1      27      ZnNo      1      2
```

53	2	27	ZnYes	2	2
53	2	27	ZnNo	2	3
53	3	27	ZnYes	3	1
53	3	27	ZnNo	3	1
53	4	27	ZnYes	4	0
53	4	27	ZnNo	4	2
53	1	30	ZnYes	1	1
53	1	30	ZnNo	1	1
53	2	30	ZnYes	2	2
53	2	30	ZnNo	2	2
53	3	30	ZnYes	3	0
53	3	30	ZnNo	3	4
53	4	30	ZnYes	4	1
53	4	30	ZnNo	4	2
53	1	33	ZnYes	1	1
53	1	33	ZnNo	1	2
53	2	33	ZnYes	2	1
53	2	33	ZnNo	2	3
53	3	33	ZnYes	3	1
53	3	33	ZnNo	3	3
53	4	33	ZnYes	4	0
53	4	33	ZnNo	4	3
53	1	36	ZnYes	1	1
53	1	36	ZnNo	1	2
53	2	36	ZnYes	2	1
53	2	36	ZnNo	2	3
53	3	36	ZnYes	3	1
53	3	36	ZnNo	3	3
53	4	36	ZnYes	4	1
53	4	36	ZnNo	4	2
53	1	39	ZnYes	1	1
53	1	39	ZnNo	1	2
53	2	39	ZnYes	2	2
53	2	39	ZnNo	2	2
53	3	39	ZnYes	3	0
53	3	39	ZnNo	3	2
53	4	39	ZnYes	4	1
53	4	39	ZnNo	4	3
53	1	42	ZnYes	1	1
53	1	42	ZnNo	1	2
53	2	42	ZnYes	2	1
53	2	42	ZnNo	2	3
53	3	42	ZnYes	3	0
53	3	42	ZnNo	3	1
53	4	42	ZnYes	4	0
53	4	42	ZnNo	4	4
53	1	45	ZnYes	1	1
53	1	45	ZnNo	1	2
53	2	45	ZnYes	2	1
53	2	45	ZnNo	2	3
53	3	45	ZnYes	3	1
53	3	45	ZnNo	3	2
53	4	45	ZnYes	4	3
53	4	45	ZnNo	4	2
53	1	48	ZnYes	1	1
53	1	48	ZnNo	1	2
53	2	48	ZnYes	2	1

53	2	48	ZnNo	2	2
53	3	48	ZnYes	3	0
53	3	48	ZnNo	3	3
53	4	48	ZnYes	4	1
53	4	48	ZnNo	4	2
53	1	51	ZnYes	1	1
53	1	51	ZnNo	1	2
53	2	51	ZnYes	2	0
53	2	51	ZnNo	2	3
53	3	51	ZnYes	3	0
53	3	51	ZnNo	3	3
53	4	51	ZnYes	4	3
53	4	51	ZnNo	4	2
53	1	54	ZnYes	1	1
53	1	54	ZnNo	1	2
53	2	54	ZnYes	2	1
53	2	54	ZnNo	2	3
53	3	54	ZnYes	3	0
53	3	54	ZnNo	3	2
53	4	54	ZnYes	4	2
53	4	54	ZnNo	4	1
53	1	57	ZnYes	1	1
53	1	57	ZnNo	1	2
53	2	57	ZnYes	2	0
53	2	57	ZnNo	2	3
53	3	57	ZnYes	3	0
53	3	57	ZnNo	3	2
53	4	57	ZnYes	4	3
53	4	57	ZnNo	4	1
53	1	60	ZnYes	1	2
53	1	60	ZnNo	1	1
53	2	60	ZnYes	2	0
53	2	60	ZnNo	2	3
53	3	60	ZnYes	3	0
53	3	60	ZnNo	3	2
53	4	60	ZnYes	4	3
53	4	60	ZnNo	4	2
54	1	21	ZnYes	5	0
54	1	21	ZnNo	5	5
54	2	21	ZnYes	6	0
54	2	21	ZnNo	6	4
54	3	21	ZnYes	7	2
54	3	21	ZnNo	7	3
54	4	21	ZnYes	8	3
54	4	21	ZnNo	8	2
54	1	24	ZnYes	5	2
54	1	24	ZnNo	5	2
54	2	24	ZnYes	6	1
54	2	24	ZnNo	6	3
54	3	24	ZnYes	7	2
54	3	24	ZnNo	7	2
54	4	24	ZnYes	8	0
54	4	24	ZnNo	8	3
54	1	27	ZnYes	5	1
54	1	27	ZnNo	5	3
54	2	27	ZnYes	6	2
54	2	27	ZnNo	6	1

54	3	27	ZnYes	7	2
54	3	27	ZnNo	7	1
54	4	27	ZnYes	8	1
54	4	27	ZnNo	8	1
54	1	30	ZnYes	5	1
54	1	30	ZnNo	5	2
54	2	30	ZnYes	6	0
54	2	30	ZnNo	6	1
54	3	30	ZnYes	7	3
54	3	30	ZnNo	7	1
54	4	30	ZnYes	8	1
54	4	30	ZnNo	8	3
54	1	33	ZnYes	5	1
54	1	33	ZnNo	5	2
54	2	33	ZnYes	6	0
54	2	33	ZnNo	6	3
54	3	33	ZnYes	7	2
54	3	33	ZnNo	7	3
54	4	33	ZnYes	8	0
54	4	33	ZnNo	8	4
54	1	36	ZnYes	5	1
54	1	36	ZnNo	5	2
54	2	36	ZnYes	6	1
54	2	36	ZnNo	6	4
54	3	36	ZnYes	7	2
54	3	36	ZnNo	7	2
54	4	36	ZnYes	8	0
54	4	36	ZnNo	8	2
54	1	39	ZnYes	5	2
54	1	39	ZnNo	5	2
54	2	39	ZnYes	6	0
54	2	39	ZnNo	6	4
54	3	39	ZnYes	7	1
54	3	39	ZnNo	7	4
54	4	39	ZnYes	8	0
54	4	39	ZnNo	8	3
54	1	42	ZnYes	5	2
54	1	42	ZnNo	5	2
54	2	42	ZnYes	6	0
54	2	42	ZnNo	6	4
54	3	42	ZnYes	7	2
54	3	42	ZnNo	7	3
54	4	42	ZnYes	8	1
54	4	42	ZnNo	8	1
54	1	45	ZnYes	5	1
54	1	45	ZnNo	5	3
54	2	45	ZnYes	6	1
54	2	45	ZnNo	6	3
54	3	45	ZnYes	7	1
54	3	45	ZnNo	7	2
54	4	45	ZnYes	8	1
54	4	45	ZnNo	8	3
54	1	48	ZnYes	5	0
54	1	48	ZnNo	5	4
54	2	48	ZnYes	6	0
54	2	48	ZnNo	6	5
54	3	48	ZnYes	7	2

54	3	48	ZnNo	7	1
54	4	48	ZnYes	8	0
54	4	48	ZnNo	8	1
54	1	51	ZnYes	5	0
54	1	51	ZnNo	5	3
54	2	51	ZnYes	6	1
54	2	51	ZnNo	6	4
54	3	51	ZnYes	7	2
54	3	51	ZnNo	7	2
54	4	51	ZnYes	8	0
54	4	51	ZnNo	8	3
54	1	54	ZnYes	5	0
54	1	54	ZnNo	5	4
54	2	54	ZnYes	6	0
54	2	54	ZnNo	6	4
54	3	54	ZnYes	7	3
54	3	54	ZnNo	7	1
54	4	54	ZnYes	8	0
54	4	54	ZnNo	8	2
54	1	57	ZnYes	5	0
54	1	57	ZnNo	5	4
54	2	57	ZnYes	6	0
54	2	57	ZnNo	6	5
54	3	57	ZnYes	7	1
54	3	57	ZnNo	7	2
54	4	57	ZnYes	8	0
54	4	57	ZnNo	8	3
54	1	60	ZnYes	5	0
54	1	60	ZnNo	5	3
54	2	60	ZnYes	6	0
54	2	60	ZnNo	6	5
54	3	60	ZnYes	7	1
54	3	60	ZnNo	7	0
54	4	60	ZnYes	8	0
54	4	60	ZnNo	8	4
55	1	21	ZnYes	9	1
55	1	21	ZnNo	9	1
55	2	21	ZnYes	10	1
55	2	21	ZnNo	10	3
55	3	21	ZnYes	11	0
55	3	21	ZnNo	11	5
55	4	21	ZnYes	12	2
55	4	21	ZnNo	12	3
55	1	24	ZnYes	9	2
55	1	24	ZnNo	9	2
55	2	24	ZnYes	10	1
55	2	24	ZnNo	10	2
55	3	24	ZnYes	11	2
55	3	24	ZnNo	11	3
55	4	24	ZnYes	12	3
55	4	24	ZnNo	12	1
55	1	27	ZnYes	9	2
55	1	27	ZnNo	9	1
55	2	27	ZnYes	10	1
55	2	27	ZnNo	10	3
55	3	27	ZnYes	11	1
55	3	27	ZnNo	11	4

55	4	27	ZnYes	12	1
55	4	27	ZnNo	12	4
55	1	30	ZnYes	9	4
55	1	30	ZnNo	9	1
55	2	30	ZnYes	10	1
55	2	30	ZnNo	10	2
55	3	30	ZnYes	11	0
55	3	30	ZnNo	11	5
55	4	30	ZnYes	12	1
55	4	30	ZnNo	12	4
55	1	33	ZnYes	9	4
55	1	33	ZnNo	9	0
55	2	33	ZnYes	10	1
55	2	33	ZnNo	10	4
55	3	33	ZnYes	11	3
55	3	33	ZnNo	11	2
55	4	33	ZnYes	12	2
55	4	33	ZnNo	12	3
55	1	36	ZnYes	9	1
55	1	36	ZnNo	9	1
55	2	36	ZnYes	10	1
55	2	36	ZnNo	10	4
55	3	36	ZnYes	11	0
55	3	36	ZnNo	11	5
55	4	36	ZnYes	12	1
55	4	36	ZnNo	12	2
55	1	39	ZnYes	9	0
55	1	39	ZnNo	9	4
55	2	39	ZnYes	10	0
55	2	39	ZnNo	10	5
55	3	39	ZnYes	11	0
55	3	39	ZnNo	11	5
55	4	39	ZnYes	12	1
55	4	39	ZnNo	12	4
55	1	42	ZnYes	9	0
55	1	42	ZnNo	9	4
55	2	42	ZnYes	10	0
55	2	42	ZnNo	10	5
55	3	42	ZnYes	11	0
55	3	42	ZnNo	11	5
55	4	42	ZnYes	12	0
55	4	42	ZnNo	12	5
55	1	45	ZnYes	9	1
55	1	45	ZnNo	9	4
55	2	45	ZnYes	10	0
55	2	45	ZnNo	10	5
55	3	45	ZnYes	11	1
55	3	45	ZnNo	11	4
55	4	45	ZnYes	12	0
55	4	45	ZnNo	12	4
55	1	48	ZnYes	9	0
55	1	48	ZnNo	9	5
55	2	48	ZnYes	10	1
55	2	48	ZnNo	10	2
55	3	48	ZnYes	11	0
55	3	48	ZnNo	11	4
55	4	48	ZnYes	12	2

```
55      4       48      ZnNo      12      3
55      1       51      ZnYes      9      1
55      1       51      ZnNo      9      3
55      2       51      ZnYes      10      0
55      2       51      ZnNo      10      3
55      3       51      ZnYes      11      2
55      3       51      ZnNo      11      3
55      4       51      ZnYes      12      0
55      4       51      ZnNo      12      4
55      1       54      ZnYes      9      0
55      1       54      ZnNo      9      5
55      2       54      ZnYes      10      0
55      2       54      ZnNo      10      5
55      3       54      ZnYes      11      2
55      3       54      ZnNo      11      2
55      4       54      ZnYes      12      0
55      4       54      ZnNo      12      4
55      1       57      ZnYes      9      0
55      1       57      ZnNo      9      5
55      2       57      ZnYes      10      0
55      2       57      ZnNo      10      5
55      3       57      ZnYes      11      0
55      3       57      ZnNo      11      4
55      4       57      ZnYes      12      0
55      4       57      ZnNo      12      4
55      1       60      ZnYes      9      1
55      1       60      ZnNo      9      4
55      2       60      ZnYes      10      0
55      2       60      ZnNo      10      5
55      3       60      ZnYes      11      0
55      3       60      ZnNo      11      5
55      4       60      ZnYes      12      2
55      4       60      ZnNo      12      3
;
RUN;

proc iml;
      opn14=orpol(1:14,4);
      opn14[,1]=(1:14)`;
      op14=opn14;print opn14;
      create opn14 from opn14[colname={'time' 'T1' 'T2' 'T3' 'T4'}];
      append from opn14;
      close opn14;
run;
data new14;
      input time newtime ;
cards;
1   21
2   24
3   27
4   30
5   33
6   36
7   39
8   42
9   45
10  48
```

```
11  51
12  54
13  57
14  60
;
run;
data opn14new;
   merge opn14 new14;
   by time;
   drop time;
proc print data=opn14new;
run;

data big;
      set george;
      idx=_n_;
proc sort data=big;
      by newtime;
proc sort data=opn14new;
   by newtime;
data big;
      merge big opn14new;
      by newtime;

proc sort data=big;
      by idx;
      run;

proc glm data = george;
      class treatment  time run unit;
      model number = run unit run*unit treatment
      treatment*run treatment*unit treatment*run*unit
      time time*run time*unit time*run*unit
      time*treatment ;
      lsmeans treatment;
run;

proc glm data = george;
   class treatment run unit;
   model number = run unit run*unit treatment treatment*run
   treatment*unit treatment*run*unit;
   lsmeans treatment;
run;

proc glm data = big;
   class treatment run time unit;
   model number = run unit run*unit treatment treatment*run
   treatment*unit treatment*run*unit T1 T2 T1*run T2*run
   T1*unit T2*unit T1*run*unit T2*run*unit;
   lsmeans treatment;
run;

QUIT;
```

The output from the above SAS/GLM procedure code is given below:

OPN14

1	-0.430946	0.4818121	-0.458578	0.3875695
2	-0.364646	0.2594373	-0.035275	-0.208691
3	-0.298347	0.0741249	0.2116516	-0.357756
4	-0.232048	-0.074125	0.3142705	-0.249345
5	-0.165748	-0.185312	0.30465	-0.035234
6	-0.099449	-0.259437	0.2148584	0.1707474
7	-0.03315	-0.2965	0.0769642	0.2927098
8	0.0331497	-0.2965	-0.076964	0.2927098
9	0.099449	-0.259437	-0.214858	0.1707474
10	0.1657484	-0.185312	-0.30465	-0.035234
11	0.2320477	-0.074125	-0.31427	-0.249345
12	0.2983471	0.0741249	-0.211652	-0.357756
13	0.3646464	0.2594373	0.0352753	-0.208691
14	0.4309458	0.4818121	0.4585784	0.3875695

Obs	T1	T2	T3	T4	newtime
1	-0.43095	0.48181	-0.45858	0.38757	21
2	-0.36465	0.25944	-0.03528	-0.20869	24
3	-0.29835	0.07412	0.21165	-0.35776	27
4	-0.23205	-0.07412	0.31427	-0.24935	30
5	-0.16575	-0.18531	0.30465	-0.03523	33
6	-0.09945	-0.25944	0.21486	0.17075	36
7	-0.03315	-0.29650	0.07696	0.29271	39
8	0.03315	-0.29650	-0.07696	0.29271	42
9	0.09945	-0.25944	-0.21486	0.17075	45
10	0.16575	-0.18531	-0.30465	-0.03523	48
11	0.23205	-0.07412	-0.31427	-0.24935	51
12	0.29835	0.07412	-0.21165	-0.35776	54
13	0.36465	0.25944	0.03528	-0.20869	57
14	0.43095	0.48181	0.45858	0.38757	60

The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	2	ZnNo ZnYes
Time	14	21 24 27 30 33 36 39 42 45 48 51 54 57 60
Run	3	53 54 55
Unit	4	1 2 3 4

Number of observations 336

Dependent Variable: Number

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	192	514.7261905	2.6808656	1.84	<.0001
Error	143	207.8333333	1.4533800		

Corrected Total	335	722.5595238

R-Square	Coeff Var	Root MSE	Number Mean
0.712365	63.49042	1.205562	1.898810

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Run	2	16.7916667	8.3958333	5.78	0.0039
Unit	3	2.5833333	0.8611111	0.59	0.6209
Run*Unit	6	8.8273810	1.4712302	1.01	0.4198
Treatment	1	320.1904762	320.1904762	220.31	<.0001
Treatment*Run	2	21.7916667	10.8958333	7.50	0.0008
Treatment*Unit	3	19.9761905	6.6587302	4.58	0.0043
Treatment*Run*Unit	6	34.3988095	5.7331349	3.94	0.0011
Time	13	4.3928571	0.3379121	0.23	0.9975
Time*Run	26	7.8750000	0.3028846	0.21	1.0000
Time*Unit	39	17.5833333	0.4508547	0.31	1.0000
Time*Run*Unit	78	30.5059524	0.3911020	0.27	1.0000
Treatment*Time	13	29.8095238	2.2930403	1.58	0.0980

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Run	2	16.7916667	8.3958333	5.78	0.0039
Unit	3	2.5833333	0.8611111	0.59	0.6209
Run*Unit	6	8.8273810	1.4712302	1.01	0.4198
Treatment	1	320.1904762	320.1904762	220.31	<.0001
Treatment*Run	2	21.7916667	10.8958333	7.50	0.0008
Treatment*Unit	3	19.9761905	6.6587302	4.58	0.0043
Treatment*Run*Unit	6	34.3988095	5.7331349	3.94	0.0011
Time	13	4.3928571	0.3379121	0.23	0.9975
Time*Run	26	7.8750000	0.3028846	0.21	1.0000
Time*Unit	39	17.5833333	0.4508547	0.31	1.0000
Time*Run*Unit	78	30.5059524	0.3911020	0.27	1.0000
Treatment*Time	13	29.8095238	2.2930403	1.58	0.0980

Least Squares Means

Treatment	Number LSMEAN
ZnNo	2.87500000
ZnYes	0.92261905

The GLM Procedure

Dependent Variable: Number

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	424.5595238	18.4591097	19.33	<.0001
Error	312	298.0000000	0.9551282		
Corrected Total	335	722.5595238			

R-Square	Coeff Var	Root MSE	Number Mean
0.587577	51.46944	0.977307	1.898810

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Run	2	16.7916667	8.3958333	8.79	0.0002
Unit	3	2.5833333	0.8611111	0.90	0.4407
Run*Unit	6	8.8273810	1.4712302	1.54	0.1645
Treatment	1	320.1904762	320.1904762	335.23	<.0001
Treatment*Run	2	21.7916667	10.8958333	11.41	<.0001
Treatment*Unit	3	19.9761905	6.6587302	6.97	0.0001
Treatment*Run*Unit	6	34.3988095	5.7331349	6.00	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Run	2	16.7916667	8.3958333	8.79	0.0002
Unit	3	2.5833333	0.8611111	0.90	0.4407
Run*Unit	6	8.8273810	1.4712302	1.54	0.1645
Treatment	1	320.1904762	320.1904762	335.23	<.0001
Treatment*Run	2	21.7916667	10.8958333	11.41	<.0001
Treatment*Unit	3	19.9761905	6.6587302	6.97	0.0001
Treatment*Run*Unit	6	34.3988095	5.7331349	6.00	<.0001

Least Squares Means

Treatment	Number LSMEAN
ZnNo	2.87500000
ZnYes	0.92261905

The GLM Procedure

Dependent Variable: Number

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	47	444.4613095	9.4566236	9.79	<.0001
Error	288	278.0982143	0.9656188		
Corrected Total	335	722.5595238			

R-Square	Coeff Var	Root MSE	Number Mean
0.615121	51.75132	0.982659	1.898810

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Run	2	16.7916667	8.3958333	8.69	0.0002
Unit	3	2.5833333	0.8611111	0.89	0.4458
Run*Unit	6	8.8273810	1.4712302	1.52	0.1701
Treatment	1	320.1904762	320.1904762	331.59	<.0001
Treatment*Run	2	21.7916667	10.8958333	11.28	<.0001

Treatment*Unit	3	19.9761905	6.6587302	6.90	0.0002
Treatment*Run*Unit	6	34.3988095	5.7331349	5.94	<.0001
T1	1	0.1648352	0.1648352	0.17	0.6798
T2	1	0.1607715	0.1607715	0.17	0.6835
T1*Run	2	1.8002747	0.9001374	0.93	0.3949
T2*Run	2	0.2583562	0.1291781	0.13	0.8748
T1*Unit	3	4.9945055	1.6648352	1.72	0.1622
T2*Unit	3	1.1686699	0.3895566	0.40	0.7506
T1*Run*Unit	6	9.4304945	1.5717491	1.63	0.1392
T2*Run*Unit	6	1.9238782	0.3206464	0.33	0.9198

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Run	2	16.7916667	8.3958333	8.69	0.0002
Unit	3	2.5833333	0.8611111	0.89	0.4458
Run*Unit	6	8.8273810	1.4712302	1.52	0.1701
Treatment	1	320.1904762	320.1904762	331.59	<.0001
Treatment*Run	2	21.7916667	10.8958333	11.28	<.0001
Treatment*Unit	3	19.9761905	6.6587302	6.90	0.0002
Treatment*Run*Unit	6	34.3988095	5.7331349	5.94	<.0001
T1	1	0.1648352	0.1648352	0.17	0.6798
T2	1	0.1607715	0.1607715	0.17	0.6835
T1*Run	2	1.8002747	0.9001374	0.93	0.3949
T2*Run	2	0.2583562	0.1291781	0.13	0.8748
T1*Unit	3	4.9945055	1.6648352	1.72	0.1622
T2*Unit	3	1.1686699	0.3895566	0.40	0.7506
T1*Run*Unit	6	9.4304945	1.5717491	1.63	0.1392
T2*Run*Unit	6	1.9238782	0.3206464	0.33	0.9198

Least Squares Means

Treatment	Number LSMEAN
ZnNo	2.87500000
ZnYes	0.92261905

Using the above output, the following ANOVA table was constructed:

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	Pr>F
Total (corrected for mean)	335	722.5595			
Runs	2	16.7917	8.3958		
Units	3	2.5833	0.8611		
Runs × units	6	8.8274	1.4715		
Treatment	1	320.1905	320.1905	46.2	0.0000
Treatment within runs and units	11	76.1667	6.9242		
Time	13	4.3929	0.3379	0.86	0.4036
Time within runs and units	143	55.9643	0.3914		
Treatment × time	13	29.8095	2.2930	1.58	0.0973
Treatment × time within runs and units	143	207.8333	1.4534		

The treatment within runs and units sum of squares is obtained by obtaining the sum of the sums of squares for treatment × run, the treatment × unit, and the treatment × run × unit sums of squares from the above output. The time within runs and units and treatment × time within runs and units sums of squares are similarly obtained. Since there are no discernable time effects, this variable may be omitted from the analysis. It appears that once a fish settles on a treatment, it stays there. Perhaps longer time periods, say days, might show a difference in times. Hence the second ANOVA appears to be the appropriate model for this data set. Note that the counts did not add up to five as some fish were in the middle compartment and were not counted.

EXAMPLE 2--THREE-FACTOR FRACTIONAL FACTORIAL

The purpose of this experiment was to evaluate the effect that microbes have on the solubility of trace metals released from sewage sludge that has been spread over fields. Trace metals in water leached from such fields can be toxic and environmentally destructive. Much of the trace metals in these fields is adsorbed on soil particles and are environmentally inert. It is hypothesized that the activity of soil microbes can release these inert trace metals, making them soluble in rainwater, and are then leached into the environment in rain runoff, becoming environmentally destructive.

To evaluate the effect microbes have on the amount of leached trace metals, 16 grams sewage sludge was thoroughly mixed with 600 g 1.2 mm diameter glass beads. A 5 cm thick layer of this mixture was placed on top of 3 cm of pure glass beads in each of 16 columns. Four such columns were incubated in each of four temperature chambers at temperatures of 4, 16, 28, and 37 °C for a total of six weeks. In each of these temperature chambers, one of the four columns was treated with silver nitrate ($AgNO_2$), and three were not. This treatment was not included for the 37°C temperature, thus making this an incomplete or fractional factorial treatment design. The silver should have virtually eliminated microbial activity in those columns so treated. During this period, at the end of every 6 day 8 hour incubation period, columns were removed and placed in a synthetic rainmaker apparatus and "rained on" for 16 hours. Leached water that drained from these columns was collected and the amount of trace metals (copper, zinc, nickel, phosphorus, molybdenum, cadmium, chromium, lead, and sulfur) contained in the leached water measured. Such analyses were done at the end of weeks 1, 2, 3, 4, and 6 for each column. One of the four columns was treated with silver nitrate and the other three columns were not.

ANOVAS: For each temperature (chamber), note that weeks and columns are crossed. The weeks are repeated measurements on the same column over time. Therefore, an ANOVA for each temperature is:

	Temp. 4	Temp. 16	Temp. 28	Temp.37	Sum
Source of variation	df	df	df	df	df
Total	20	20	20	15	75
Correction for mean	1	1	1	1	4
Week	4	4	4	4	16

Wlinear	1	1	1	1	4
Wquadratiic	1	1	1	1	4
Remainder	2	2	2	2	8
Treatment	1	1	1	0	3
Week × treatment	4	4	4	0	12
Wlinear × treat	1	1	1	0	3
Wquad × treat	1	1	1	0	3
Wres × treat	2	2	2	0	6
Remainder	10	10	10	10	40

For each temperature chamber, an error mean square with 2 + 10 = 12 degrees of freedom (df) would be obtained as the sum of the sums of squares of the two remainders.

The degrees of freedom in the last column under Sum may be partitioned as follows:

Source of variation	Degrees of freedom			
Correction /Temperature	4			("/" means within temperature)
Correction for mean		1		
Temperature		3		
Tlinear			1	(Linear regression on levels)
Tquadratic			1	(Quadratic regression on levels)
Remainder			1	
Week/temperature	16			
Week		4		
Wlinear			1	
Wquadratic			1	
Remainder			2	
Week × temperature		12		
Trearment/temperature	3			
Treatment		1		
Treatment × temperature		2		
Treatment × Tlinear			1	
Treatment × Tquadratic			1	
Week × treatment/temper.	12			
Week × treatment		4		
Wlinear × treatment			1	
Wquadratic × treatment			1	
Remainder			2	
Week × temper. × treat.		8		
Wlinear × Tlinear × treat.			1	
Wlinear × Tquad. × treat,			1	
Wquad. × Tlinear × treat.			1	
Wquad. × Tquad. × treat.			1	
Remainder			4	
Remainder/temperature	40			

Since this is an exponential decay problem, it is assumed that linear and quadratic regression is a good approximation for the data. Alternatively, one could have used log temp and log week. For the above ANOVA partitioning of degrees of freedom, it is assumed that only the linear and quadratic effects of weeks and temperatures are important and that higher degree polynomials are not. Also, one may wish to pool the last four Remainder sums of squares to obtain an error sum of squares with 1 + 2 + 8 + 40 = 51 degrees of freedom. Alternately, instead of pooling, use these terms to test for lack of fit of the quadratic model. Using an error mean square with 40 degrees of freedom should be sufficient for most purposes. It may be that the error variances change with time (week). Perhaps a variance stabilizing transformation such as log response should be used.

<u>SAS/GLM Computer Code and Data:</u> Computer codes for various types of analyses using orthogonal polynomials have been described in a series of papers listed under the literature citations at the end of this paper. A code for analyses of the above type is given below using centered X-variables as regression coefficients as non-centered ones would be difficult to interpret if indeed they are interpretable. The code is adapted from the one given by Federer and Wolfinger (2000), June). Comments are entered in /* … */.

```
Options ls=76;
DATA SHABNAM;
 INPUT Obs Agtrt $ temp $ week $ REP Cu Zn MO CD CR NI PB SE;
 newweek=week;
 newtemp=temp;

/* The above two lines are needed here in order change letter designations to
the numeric ones required for regression. */

 CARDS;
1  NOAG 28C WK1 1 17.7700 36.5900 0.1700 0.0504 0.1598 3.4390 0.2123  1.3880
2  NOAG 28C WK1 2 25.8200 40.2900 0.1957 0.0423 0.3937 3.8760 0.5025  1.5080
3  NOAG 28C WK1 3 11.2700 26.3700 0.2200 0.0611 0.1363 2.4580 0.1577  1.6110
4  AG   28C WK1 4  0.5085  2.8950 0.0028 0.0050 0.0553 0.3192 0.0160  0.1090
5  NOAG 28C WK2 1  4.9080  6.2360 0.0151 0.0319 0.0439 0.4793 0.1164  0.1221
6  NOAG 28C WK2 2  6.7950  8.8030 0.0273 0.0422 0.0763 0.6748 0.0946  0.1997
7  NOAG 28C WK2 3  4.9780  7.3440 0.0262 0.0380 0.0700 0.6056 0.1157  0.1862
8  AG   28C WK2 4  0.3545  1.8450 0.0126 0.0070 0.0003 0.3667 0.0130  0.1265
9  NOAG 28C WK3 1  2.2250  1.2360 0.0000 0.0059 0.0000 0.0753 0.0078  0.0000
10 NOAG 28C WK3 2  2.6790  1.3590 0.0000 0.0141 0.0262 0.1283 0.0117  0.0224
11 NOAG 28C WK3 3  1.8680  1.4290 0.0000 0.0051 0.0000 0.0944 0.0000  0.0000
12  AG  28C WK3 4  0.1903  1.2580 0.0101 0.0001 0.0375 0.0200 0.0000  0.0110
13 NOAG 28C WK4 1  1.5780  0.7236 0.0000 0.0064 0.0000 0.0507 0.0000  0.0000
14 NOAG 28C WK4 2  1.4790  0.5997 0.0000 0.0063 0.0113 0.0611 0.0000  0.0037
15 NOAG 28C WK4 3  1.2710  0.6912 0.0000 0.0040 0.0000 0.0461 0.0000  0.0000
16  AG  28C WK4 4  0.2276  0.7129 0.0123 0.0001 0.0000 0.0220 0.0000  0.0000
17 NOAG 28C WK6 1  0.5514  0.2641 0.0000 0.0017 0.0000 0.0100 0.0000  0.0000
18 NOAG 28C WK6 2  0.4039  0.1896 0.0000 0.0014 0.0000 0.0072 0.0000  0.0000
19 NOAG 28C WK6 3  0.5113  0.2556 0.0000 0.0000 0.0000 0.0032 0.0000  0.0000
20  AG  28C WK6 4  0.1042  0.4769 0.0121 0.0000 0.0000 0.0000 0.0000  0.0000
```

```
21 NOAG 16C WK1 1 24.8000 26.6300 0.1934 0.0039 0.3500 2.6750 0.1300  1.2220
22 NOAG 16C WK1 2 21.5400 35.9900 0.1865 0.0385 0.3532 3.5210 0.3200  1.4120
23 NOAG 16C WK1 3 22.2300 35.4300 0.1840 0.0741 0.0738 3.1540 0.3743  1.3050
24  AG  16C WK1 4  0.4451  4.8940 0.0048 0.0065 0.0566 0.3668 0.0234  0.0911
25 NOAG 16C WK2 1  3.3110  4.3920 0.0116 0.0000 0.0748 0.3584 0.0246  0.1272
26 NOAG 16C WK2 2  3.2420  3.1250 0.0295 0.0145 0.0622 0.2595 0.0246  0.1630
27 NOAG 16C WK2 3  5.1010  6.5750 0.0302 0.0148 0.0640 0.4968 0.0510  0.1650
28  AG  16C WK2 4  0.3829  2.8870 0.0119 0.0095 0.0001 0.5272 0.0120  0.1587
29 NOAG 16C WK3 1  0.8165  0.7630 0.0000 0.0014 0.0199 0.0632 0.0131  0.0125
30 NOAG 16C WK3 2  1.3900  0.5288 0.0000 0.0015 0.0000 0.0251 0.0000  0.0000
31 NOAG 16C WK3 3  1.4410  0.7187 0.0000 0.0019 0.0000 0.0372 0.0000  0.0000
32  AG  16C WK3 4  0.1992  1.3330 0.0075 0.0002 0.0259 0.0400 0.0000  0.0110
33 NOAG 16C WK4 1  0.9982  0.6328 0.0000 0.0030 0.0090 0.0496 0.0000  0.0000
34 NOAG 16C WK4 2  1.1400  0.3724 0.0000 0.0033 0.0000 0.0171 0.0000  0.0000
35 NOAG 16C WK4 3  1.2620  0.4161 0.0000 0.0017 0.0000 0.0194 0.0000  0.0000
36  AG  16C WK4 4  0.1472  0.3439 0.0108 0.0000 0.0000 0.0311 0.0000  0.0000
37 NOAG 16C WK6 1  0.6884  0.3053 0.0000 0.0016 0.0006 0.0137 0.0000  0.0081
38 NOAG 16C WK6 2  0.6797  0.2574 0.0000 0.0000 0.0000 0.0104 0.0000  0.0063
39 NOAG 16C WK6 3  0.4981  0.1997 0.0000 0.0002 0.0000 0.0069 0.0000  0.0000
40  AG  16C WK6 4  0.1090  0.5928 0.0120 0.0000 0.0000 0.0000 0.0000  0.0000
41 NOAG  4C WK1 1  9.7290 18.8100 0.0621 0.0076 0.2590 1.3210 0.1473  0.4540
42 NOAG  4C WK1 2 15.2500 32.2700 0.1068 0.0411 0.0570 2.3000 0.1557  0.7442
43 NOAG  4C WK1 3  5.9110 23.8100 0.0298 0.0210 0.0805 1.6000 0.0000  0.2948
44  AG   4C WK1 4  0.9607  2.5010 0.0044 0.0059 0.0513 0.3326 0.0392  0.0901
45 NOAG  4C WK2 1  2.5930  3.6070 0.0000 0.0076 0.0372 0.2338 0.0000  0.0441
46 NOAG  4C WK2 2  2.9080  4.1010 0.0104 0.0108 0.0349 0.2687 0.0519  0.1391
47 NOAG  4C WK2 3  1.2080  3.4630 0.0000 0.0048 0.0073 0.2025 0.0000  0.0084
48  AG   4C WK2 4  0.4244  1.8000 0.0111 0.0060 0.0040 0.2654 0.0120  0.1319
49 NOAG  4C WK3 1  0.7325  0.7520 0.0000 0.0037 0.0189 0.0443 0.0000  0.0120
50 NOAG  4C WK3 2  0.7243  0.6378 0.0000 0.0000 0.0000 0.0283 0.0000  0.0000
51 NOAG  4C WK3 3  0.5131  1.1460 0.0000 0.0010 0.0037 0.0599  0       0.0000
52  AG   4C WK3 4  0.2138  1.2720 0.0096 0.0001 0.0355 0.0200  0   0.0110
53  NOAG 4C WK4 1  0.4784  0.5589 0.0000 0.0000 0.0000 0.0271  0   0.0000
54  NOAG 4C WK4 2  0.4585  0.3805 0.0000 0.0000 0.0000 0.0169  0   0.0000
55  NOAG 4C WK4 3  0.3165  0.5265 0.0000  .0000 0.0000 0.0167  0   0.0000
56  AG   4C WK4 4  0.1330  0.6393 0.0132  .0000 0.0000 0.0110   0  0.0000
57  NOAG 4C WK6 1  0.2850  0.2409 0.0000  .0000 0.0000 0.0076   0  0.0166
58  NOAG 4C WK6 2  0.2660  0.2821 0.0000  .0000 0.0000 0.0000   0  0.0000
59  NOAG 4C WK6 3  0.1544  0.3458 0.0000  .0000 0.0000 0.0062   0  0.0000
60  AG   4C WK6 4  0.0969  0.1130 0.0307  .0001 0.0000 0.0000   0  0.0000
61 NOAG 37C WK1 1  0.3876  2.9080 0.0000  .0015 0.0000 0.4168   0  0.0699
62 NOAG 37C WK1 2  0.3875  2.1510 0.0000  .0011 0.0084 0.3499   0  0.0616
63 NOAG 37C WK1 3  0.5813  4.4350 0.0000  .0041 0.0018 0.5443   0  0.0894
64 NOAG 37C WK2 1  0.1680  0.9532 0.0097  .0013 0.0021 0.1717   0  0.0418
65 NOAG 37C WK2 2  0.1446  0.7330 0.0020  .0000 0.0070 0.1306   0  0.0000
66 NOAG 37C WK2 3  0.1189  1.2080 0.0000  .0000 0.0000 0.1529   0  0.0000
67 NOAG 37C WK3 1  0.0818  0.2151 0.0083  .0000 0.0000 0.0382   0  0.0000
68 NOAG 37C WK3 2  0.0568  0.1509 0.0057  .0000 0.0000 0.0273   0  0.0000
69 NOAG 37C WK3 3  0.0938  0.3275 0.0066  .0000 0.0050 0.0573   0  0.0000
70 NOAG 37C WK4 1  0.0386  0.0784 0.0078  .0000 0.0000 0.0059   0  0.0000
71 NOAG 37C WK4 2  0.0807  0.0656 0.0214  .0000 0.0000 0.0075   0  0.0000
72 NOAG 37C WK4 3  0.0766  0.1313 0.0134  .0000 0.0000 0.0228   0  0.0000
73 NOAG 37C WK6 1  0.0678  0.1117 0.0055  .0000 0.0000 0.0087   0  0.0040
74 NOAG 37C WK6 2  0.0770  0.0508 0.0564  .0000 0.0000 0.0166   0  0.0000
75 NOAG 37C WK6 3  0.0411  0.1560  0.0130 .0000 0.0000 0.0184   0  0.0000
;
RUN;
```

```
Proc iml;         /*To construct orthogonal polynomial coefficients */
    Opn4=orpol(1:4,2);  /*4 temperatures and linear and quadratic regression
*/
    Opn4[,1]=(1:4)`;
    Op4=opn4; print opn4;   /*This prints the coefficients */
    Create opn4 from opn4[colname={'temp'  'T1'  'T2'}];
    Append from opn4;
    Close opn4;
Run;

    Opn5=orpol(1:5,2); /*5 weeks and linear and quadratic regression */
    Opn5[,1]=(1:5)`;
Op5=opn5; print opn5;
Create opn5 from opn5[colname={'week'  'W1'  'W2'}];
Append from opn5;
Close opn5;
Run;

/*now temp is a character variable in DATA SHABNAM, but a numeric variable
in DATA opn4. We'll turn it back into a character variable. */

data new4;
input temp newtemp $;
cards;
1 4C
2 16C
3 28C
4 37C
;
run;

data opn4new;
merge opn4 new4;
by temp;
drop temp;
run;

proc print data=opn4new;
run;

/* Now week is a character variable in DATA SHABNAM but a numeric
variable in DATA opn5. We'll turn it back into a character variable in opn5*/

data new5;
input week newweek $;
cards;
1 WK1
2 WK2
3 WK3
4 WK4
5 WK6
;
RUN;

DATA opn5new;
merge opn5 new5;
```

```
by week;
drop week;
run;

Data  large;             /*Merge in polynomial coefficients */
Set Shabnam;
    idx=_n_;
Proc sort data=large;
    By newweek;
proc sort data=opn4new;
      by newtemp;
Proc sort data=opn5new;
      by newweek;

Data large;
    Merge large opn5new;
    By newweek;
Proc sort data = large;
    By newtemp;
Data large;
    Merge large opn4new;
    By newtemp;
Proc sort data=large;
    By idx;
Run;

/* The following model analyzes the data as a 3-factor factorial with the 3-
factor interaction being used as error. */

Proc glm data = large;
    Class Agtrt week temp;
    Model  Cu = Agtrt week temp Agtrt*week  Agtrt*temp  week*temp;
    Lsmeans Agtrt Agtrt*week Agtrt*temp;
Run;

/* The following model uses the linear and quadratic trends of temp and week
plus all possible interactions. */

Proc glm data = large;
    Class Agtrt  week  temp;
    Model Cu = T1 T2 W1 W2 Agtrt T1*W1 T1*W2 T2*W1 T2*W2 Agtrt*W1
    Agtrt*W2 Agtrt*T1 Agtrt*T2 Agtrt*T1*W1 Agtrt*T1*W2 Agtrt*T2*W1
    Agtrt*T2*W2;
    Lsmeans Agtrt;
Run;

/* The following model omits all parameters (terms) whose F-value did not
exceed the tabulated F-value at the 25% level.  This is the rule for
selecting parameters proposed by Bozovich et al. (1956) as and used by
Federer (1998). */

proc glm data = large;
    Class Agtrt week temp;
      Model Cu = T2 W1 W2 Agtrt T2*W1 Agtrt*W1
    Agtrt*W2 Agtrt*T2 Agtrt*T2 Agtrt*T2*W1 ;
      Lsmeans Agtrt;
Run;
```

```
/* Since the residual mean square for the above model was larger than for
either of the previous two models, this is not a good model.  The following
model uses the main effects and 2-factor interactions of the factorial model
and the four 3-factor interaction terms of treatments with the linear and
quadratic trends of temp and week. */

proc glm data = large;
    Class Agtrt week temp;
    Model  Cu = Agtrt week temp Agtrt*week  Agtrt*temp  week*temp
    Agtrt*T1*W1 Agtrt*T1*W2 Agtrt*T2*W1 Agtrt*T2*W2;
    Lsmeans Agtrt Agtrt*week ;
Run;

/* Using the selection rule described above, the parameters Agtrt*T1*W1 and
Agtrt*T1*W2 were eliminated from the model to obtain the following model. */

proc glm data = large;
    Class Agtrt week temp;
    Model  Cu = Agtrt week temp Agtrt*week  Agtrt*temp  week*temp
    Agtrt*T2*W1 Agtrt*T2*W2/solution;

/* Since not all parameters can be estimated the command /solution was put
into the Model statement.  The analyst can then see exactly which parameters
can be estimated for this model and data set.  Note that the SAS/GLM sets the
highest numbered effect equal to zero.  All other estimates given is the
effect minus the highest numbered effect.  The "standard errors" given are
standard errors of a difference between two effects or two means. */

    Lsmeans Agtrt Agtrt*week ;
Run;

/* The reason that some means are not estimable is due to the omission of the
Ag treatment at the 37C temp. */

QUIT;
/* This command may be omitted but is a good safeguard to include in order to
stop the SAS/GLM procedure from continuing to run if the program is faulty.
*/
```

The output using the above data for Cu and the above program in SAS/GLM is as follows:

The SAS System

OPN4

1	-0.67082	0.5
2	-0.223607	-0.5
3	0.2236068	-0.5
4	0.6708204	0.5

OPN5

1	-0.632456	0.5345225
2	-0.316228	-0.267261
3	1.765E-17	-0.534522
4	0.3162278	-0.267261
5	0.6324555	0.5345225

Obs	T1	T2	newtemp
1	-0.67082	0.5	4C
2	-0.22361	-0.5	16C
3	0.22361	-0.5	28C
4	0.67082	0.5	37C

The GLM Procedure

Class Level Information

Class	Levels	Values
Agtrt	2	AG NOAG
week	5	WK1 WK2 WK3 WK4 WK6
temp	4	16C 28C 37C 4C

Number of observations 75

Dependent Variable: Cu

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	26	2375.542907	91.367035	20.83	<.0001
Error	48	210.495633	4.385326		
Corrected Total	74	2586.038540			

R-Square	Coeff Var	Root MSE	Cu Mean
0.918603	70.85168	2.094117	2.955635

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Agtrt	1	132.250341	132.250341	30.16	<.0001
week	4	1109.242629	277.310657	63.24	<.0001
temp	3	306.269753	102.089918	23.28	<.0001
Agtrt*week	4	251.487246	62.871811	14.34	<.0001
Agtrt*temp	2	24.343613	12.171806	2.78	0.0723
week*temp	12	551.949325	45.995777	10.49	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Agtrt	1	225.0786763	225.0786763	51.33	<.0001
week	4	130.6709883	32.6677471	7.45	<.0001
temp	3	260.6745426	86.8915142	19.81	<.0001
Agtrt*week	4	420.7076984	105.1769246	23.98	<.0001
Agtrt*temp	2	24.3436129	12.1718065	2.78	0.0723
week*temp	12	551.9493252	45.9957771	10.49	<.0001

Least Squares Means

Agtrt	Cu LSMEAN
AG	Non-est
NOAG	3.61958833

Agtrt	week	Cu LSMEAN
AG	WK1	Non-est
AG	WK2	Non-est
AG	WK3	Non-est
AG	WK4	Non-est
AG	WK6	Non-est
NOAG	WK1	12.9730333
NOAG	WK2	2.9562917
NOAG	WK3	1.0518167
NOAG	WK4	0.7647917
NOAG	WK6	0.3520083

Agtrt	temp	Cu LSMEAN
AG	16C	0.25668000
AG	28C	0.27702000
AG	4C	0.36576000
NOAG	16C	5.94252667
NOAG	28C	5.60717333
NOAG	37C	0.16014000
NOAG	4C	2.76851333

The GLM Procedure

Dependent Variable: Cu

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	2284.568353	134.386374	25.41	<.0001
Error	57	301.470187	5.288951		
Corrected Total	74	2586.038540			

R-Square	Coeff Var	Root MSE	Cu Mean
0.883424	77.80975	2.299772	2.955635

Source	DF	Type I SS	Mean Square	F Value	Pr > F
T1	1	19.7872659	19.7872659	3.74	0.0581
T2	1	192.0717005	192.0717005	36.32	<.0001
W1	1	739.5486282	739.5486282	139.83	<.0001
W2	1	301.1461025	301.1461025	56.94	<.0001
Agtrt	1	221.0889773	221.0889773	41.80	<.0001
T1*W1	1	32.6812658	32.6812658	6.18	0.0159
T1*W2	1	20.2642212	20.2642212	3.83	0.0552
T2*W1	1	211.6513902	211.6513902	40.02	<.0001
T2*W2	1	84.5615482	84.5615482	15.99	0.0002
W1*Agtrt	1	274.4577679	274.4577679	51.89	<.0001
W2*Agtrt	1	120.0981665	120.0981665	22.71	<.0001
T1*Agtrt	1	12.4272364	12.4272364	2.35	0.1308
T2*Agtrt	1	15.5629712	15.5629712	2.94	0.0917
T1*W1*Agtrt	1	11.5243370	11.5243370	2.18	0.1454
T1*W2*Agtrt	1	3.2813685	3.2813685	0.62	0.4342
T2*W1*Agtrt	1	17.4093815	17.4093815	3.29	0.0749
T2*W2*Agtrt	1	7.0060240	7.0060240	1.32	0.2546

Source	DF	Type III SS	Mean Square	F Value	Pr > F
T1	1	1.53181490	1.53181490	0.29	0.5926
T2	1	13.80128296	13.80128296	2.61	0.1118
W1	1	52.83994643	52.83994643	9.99	0.0025
W2	1	20.97638913	20.97638913	3.97	0.0512
Agtrt	1	34.14041154	34.14041154	6.46	0.0138
T1*W1	1	2.40355443	2.40355443	0.45	0.5030
T1*W2	1	1.32602408	1.32602408	0.25	0.6185
T2*W1	1	15.10321674	15.10321674	2.86	0.0965
T2*W2	1	5.89215651	5.89215651	1.11	0.2957
W1*Agtrt	1	42.50610355	42.50610355	8.04	0.0063
W2*Agtrt	1	18.20320188	18.20320188	3.44	0.0687
T1*Agtrt	1	1.69244461	1.69244461	0.32	0.5738
T2*Agtrt	1	15.56297116	15.56297116	2.94	0.0917
T1*W1*Agtrt	1	2.44135151	2.44135151	0.46	0.4996
T1*W2*Agtrt	1	1.39805110	1.39805110	0.26	0.6091
T2*W1*Agtrt	1	17.40938150	17.40938150	3.29	0.0749
T2*W2*Agtrt	1	7.00602396	7.00602396	1.32	0.2546

Least Squares Means

Agtrt	Cu LSMEAN
AG	0.32521200
NOAG	3.84487718

Dependent Variable: Cu

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	1994.968179	221.663131	24.38	<.0001
Error	65	591.070361	9.093390		
Corrected Total	74	2586.038540			

R-Square	Coeff Var	Root MSE	Cu Mean
0.771438	102.0263	3.015525	2.955635

Source	DF	Type I SS	Mean Square	F Value	Pr > F
T2	1	178.2844344	178.2844344	19.61	<.0001
W1	1	739.5486282	739.5486282	81.33	<.0001
W2	1	301.1461025	301.1461025	33.12	<.0001
Agtrt	1	179.6810461	179.6810461	19.76	<.0001
T2*W1	1	193.5720205	193.5720205	21.29	<.0001
W1*Agtrt	1	219.0655585	219.0655585	24.09	<.0001
W2*Agtrt	1	70.6521202	70.6521202	7.77	0.0070
T2*Agtrt	1	53.0266427	53.0266427	5.83	0.0186
T2*W1*Agtrt	1	59.9916259	59.9916259	6.60	0.0125

Source	DF	Type III SS	Mean Square	F Value	Pr > F
T2	1	48.3755096	48.3755096	5.32	0.0243
W1	1	182.2768222	182.2768222	20.04	<.0001
W2	1	78.1378280	78.1378280	8.59	0.0047
Agtrt	1	119.0365176	119.0365176	13.09	0.0006
T2*W1	1	52.3145240	52.3145240	5.75	0.0193
W1*Agtrt	1	147.0736918	147.0736918	16.17	0.0002
W2*Agtrt	1	70.6521202	70.6521202	7.77	0.0070
T2*Agtrt	1	53.0266427	53.0266427	5.83	0.0186
T2*W1*Agtrt	1	59.9916259	59.9916259	6.60	0.0125

Least Squares Means

Agtrt	Cu LSMEAN
AG	0.31300800
NOAG	3.76327244

The GLM Procedure

Dependent Variable: Cu

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F

Model	30	2418.005894	80.600196	21.11	<.0001
Error	44	168.032646	3.818924		
Corrected Total	74	2586.038540			

R-Square	Coeff Var	Root MSE	Cu Mean
0.935023	66.11801	1.954207	2.955635

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Agtrt	1	132.250341	132.250341	34.63	<.0001
week	4	1109.242629	277.310657	72.61	<.0001
temp	3	306.269753	102.089918	26.73	<.0001
Agtrt*week	4	251.487246	62.871811	16.46	<.0001
Agtrt*temp	2	24.343613	12.171806	3.19	0.0510
week*temp	12	551.949325	45.995777	12.04	<.0001
T1*W1*Agtrt	1	13.331899	13.331899	3.49	0.0684
T1*W2*Agtrt	1	2.574968	2.574968	0.67	0.4160
W1*T2*Agtrt	1	14.386122	14.386122	3.77	0.0587
W2*T2*Agtrt	1	12.169997	12.169997	3.19	0.0811

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Agtrt	1	225.0786763	225.0786763	58.94	<.0001
week	4	77.5143771	19.3785943	5.07	0.0019
temp	3	260.6745426	86.8915142	22.75	<.0001
Agtrt*week	4	67.4806516	16.8701629	4.42	0.0043
Agtrt*temp	2	24.3436129	12.1718065	3.19	0.0510
week*temp	8	46.1972028	5.7746504	1.51	0.1807
T1*W1*Agtrt	1	2.1289954	2.1289954	0.56	0.4592
T1*W2*Agtrt	1	4.9232488	4.9232488	1.29	0.2623
W1*T2*Agtrt	1	14.3861224	14.3861224	3.77	0.0587
W2*T2*Agtrt	1	12.1699967	12.1699967	3.19	0.0811

Least Squares Means

Agtrt	Cu LSMEAN
AG	Non-est
NOAG	Non-est

Agtrt	week	Cu LSMEAN
AG	WK1	Non-est
AG	WK2	Non-est
AG	WK3	Non-est
AG	WK4	Non-est
AG	WK6	Non-est
NOAG	WK1	Non-est
NOAG	WK2	Non-est
NOAG	WK3	Non-est

NOAG	WK4	Non-est
NOAG	WK6	Non-est

The GLM Procedure

Class Level Information

Class	Levels	Values
Agtrt	2	AG NOAG
week	5	WK1 WK2 WK3 WK4 WK6
temp	4	16C 28C 37C 4C

Number of observations 75

Dependent Variable: Cu

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	28	2410.953649	86.105487	22.62	<.0001
Error	46	175.084891	3.806193		
Corrected Total	74	2586.038540			

R-Square	Coeff Var	Root MSE	Cu Mean
0.932296	66.00771	1.950947	2.955635

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Agtrt	1	132.250341	132.250341	34.75	<.0001
week	4	1109.242629	277.310657	72.86	<.0001
temp	3	306.269753	102.089918	26.82	<.0001
Agtrt*week	4	251.487246	62.871811	16.52	<.0001
Agtrt*temp	2	24.343613	12.171806	3.20	0.0501
week*temp	12	551.949325	45.995777	12.08	<.0001
T2*W1*Agtrt	1	25.589026	25.589026	6.72	0.0127
T2*W2*Agtrt	1	9.821716	9.821716	2.58	0.1150

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Agtrt	1	225.0786763	225.0786763	59.13	<.0001
week	4	162.4295057	40.6073764	10.67	<.0001
temp	3	260.6745426	86.8915142	22.83	<.0001
Agtrt*week	4	306.8400060	76.7100015	20.15	<.0001
Agtrt*temp	2	24.3436129	12.1718065	3.20	0.0501
week*temp	10	156.4559264	15.6455926	4.11	0.0004
T2*W1*Agtrt	1	25.5890261	25.5890261	6.72	0.0127

T2*W2*Agtrt	1	9.8217161	9.8217161	2.58	0.1150

Parameter		Estimate		Standard Error	t Value	Pr > |t|
Intercept		0.12860794	B	1.11560064	0.12	0.9087
Agtrt	AG	1.36559873	B	1.58533778	0.86	0.3935
Agtrt	NOAG	0.00000000	B	.	.	.
week	WK1	10.29474000	B	1.56616733	6.57	<.0001
week	WK2	1.83420698	B	1.56230501	1.17	0.2464
week	WK3	0.58849302	B	1.56230501	0.38	0.7081
week	WK4	0.48208698	B	1.50719369	0.32	0.7505
week	WK6	0.00000000	B	.	.	.
temp	16C	0.57513310	B	1.53499670	0.37	0.7096
temp	28C	0.38510976	B	1.53499670	0.25	0.8030
temp	37C	-0.06664127	B	1.58533778	-0.04	0.9667
temp	4C	0.00000000	B	.	.	.
Agtrt*week	AG WK1	-14.65507333	B	1.92914779	-7.60	<.0001
Agtrt*week	AG WK2	-2.62232365	B	1.92601351	-1.36	0.1800
Agtrt*week	AG WK3	-0.86577635	B	1.92601351	-0.45	0.6552
Agtrt*week	AG WK4	-0.69858698	B	1.88158548	-0.37	0.7121
Agtrt*week	AG WK6	0.00000000	B	.	.	.
Agtrt*week	NOAG WK1	0.00000000	B	.	.	.
Agtrt*week	NOAG WK2	0.00000000	B	.	.	.
Agtrt*week	NOAG WK3	0.00000000	B	.	.	.
Agtrt*week	NOAG WK4	0.00000000	B	.	.	.
Agtrt*week	NOAG WK6	0.00000000	B	.	.	.
Agtrt*temp	AG 16C	-3.28309333	B	1.42477007	-2.30	0.0258
Agtrt*temp	AG 28C	-2.92740000	B	1.42477007	-2.05	0.0456
Agtrt*temp	AG 4C	0.00000000	B	.	.	.
Agtrt*temp	NOAG 16C	0.00000000	B	.	.	.
Agtrt*temp	NOAG 28C	0.00000000	B	.	.	.
Agtrt*temp	NOAG 37C	0.00000000	B	.	.	.
Agtrt*temp	NOAG 4C	0.00000000	B	.	.	.
week*temp	WK1 16C	11.26013167	B	2.13715510	5.27	<.0001
week*temp	WK1 28C	7.94958167	B	2.13715510	3.72	0.0005
week*temp	WK1 37C	-9.90457333	B	2.23390740	-4.43	<.0001
week*temp	WK1 4C	0.00000000	B	.	.	.
week*temp	WK2 16C	1.74094786	B	2.13078503	0.82	0.4181
week*temp	WK2 28C	3.09169786	B	2.13078503	1.45	0.1536
week*temp	WK2 37C	-1.75234032	B	2.23120128	-0.79	0.4363
week*temp	WK2 4C	0.00000000	B	.	.	.
week*temp	WK3 16C	0.06912714	B	2.13078503	0.03	0.9743
week*temp	WK3 28C	0.94912714	B	2.13078503	0.45	0.6581
week*temp	WK3 37C	-0.57299302	B	2.23120128	-0.26	0.7985
week*temp	WK3 4C	0.00000000	B	.	.	.
week*temp	WK4 16C	-0.07580548	B	2.03951600	-0.04	0.9705
week*temp	WK4 28C	0.27734452	B	2.03951600	0.14	0.8924
week*temp	WK4 37C	-0.47875365	B	2.19296489	-0.22	0.8282
week*temp	WK4 4C	0.00000000	B	.	.	.
week*temp	WK6 16C	0.00000000	B	.	.	.
week*temp	WK6 28C	0.00000000	B	.	.	.
week*temp	WK6 37C	0.00000000	B	.	.	.
week*temp	WK6 4C	0.00000000	B	.	.	.
T2*W1*Agtrt	AG	-7.15388372	B	2.75905537	-2.59	0.0127
T2*W1*Agtrt	NOAG	0.00000000	B	.	.	.

```
 T2*W2*Agtrt AG            4.43209117 B     2.75905537      1.61     0.1150
 T2*W2*Agtrt NOAG          0.00000000 B      .               .        .

NOTE: The X'X matrix has been found to be singular, and a generalized
      inverse was used to solve the normal equations.  Terms whose
      estimates are followed by the letter 'B' are not uniquely estimable.

                          Least Squares Means

                          Agtrt         Cu LSMEAN

                          AG              Non-est
                          NOAG            Non-est


                      Agtrt     week         Cu LSMEAN

                      AG        WK1            Non-est
                      AG        WK2            Non-est
                      AG        WK3            Non-est
                      AG        WK4            Non-est
                      AG        WK6            Non-est
                      NOAG      WK1            Non-est
                      NOAG      WK2            Non-est
                      NOAG      WK3            Non-est
                      NOAG      WK4            Non-est
                      NOAG      WK6            Non-est
```

In the exploratory model selection phase, five different models were investigated. After reviewing the output for the first two models, the other three models were considered to be possible candidates for explaining the variation present in the experiment. It appears that the last model is an appropriate one to explain the variation in the data for Cu. An investigation should be made to determine if this model also holds for the other variables. It appears that a log transformation of the data for Cu and for Zn and perhaps for Ni would be appropriate.

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