Model Based Statistics in Biology.
Part IV. The General Linear Model. Multiple Explanatory Variables.
Chapter 12.1 Multiple Regression. Two Explanatory Variables.

on chalk board

ReCap Part I (Chapters 1,2,3,4) Quantitative reasoning based on models.
ReCap Part II (Chapters 5,6,7)
Data Equation
Frequency distributions
Three modes of inference.
ReCap (Ch 9, 10,11) The General Linear Model with a single explanatory variable.
    Unifying concepts rather than list of statistical tests.
     GLM is more useful and flexible than a collection of special cases.

Today: Introduction to GLM, Multiple Explanatory Variable.
    Distinction among Multiple regression, Multiway ANOVA, ANCOVA
    Example: Multiple Regression

Wrap-up.

Multiple regression is a special case of the General Linear Model in which there are two or more explanatory variables on a ratio scale.

The regression coefficients estimated by most statistical packages are partial regressions. They express the rate of change in the response variable with respect to change in the explanatory variable, controlling for other variables.

The sum of squares that correspond to these partial regression coefficients are the adjusted (Type III) sum of squares. In most situations these are tested, rather than the sequential (Type I) sum of squares.

Regression coefficients express the rate of change of one variable with respect to another. Because of this relative quality, estimates can often be inferred to far larger populations than can means.

Introduction Analysis of data from Snedecor and Cochrane 1980 Table 17.2.1
Does phosphorus content of corn (ppm) from 17 Iowa soils at 20 deg C depend on inorganic and organic phosphorus in the soil?

1. Construct model

Verbal model. Plant available phosphorus depends on the amount of
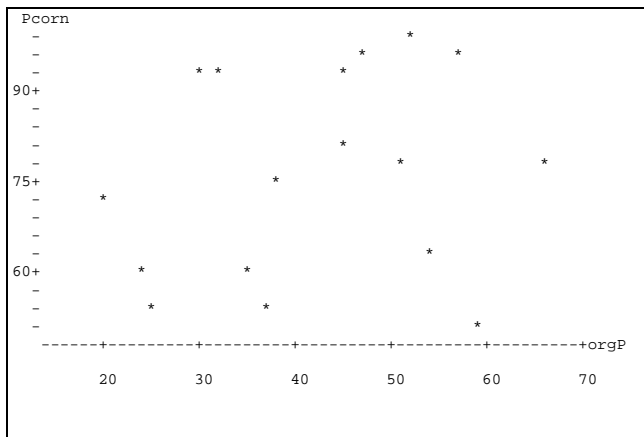both organic and inorganic soil phosphorus.
Response variable is phosphorus content of corn. *Pcorn* = ppm
Explanatory variables is organic phosphorus in soil. *oP* = ppm
Explanatory variables is inorganic phosphorus in soil. *ioP* = ppm
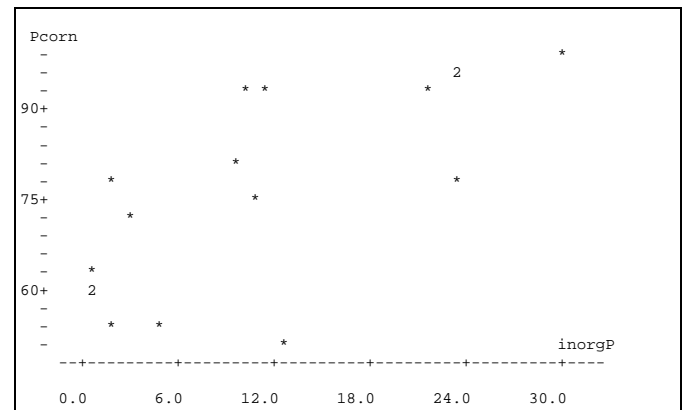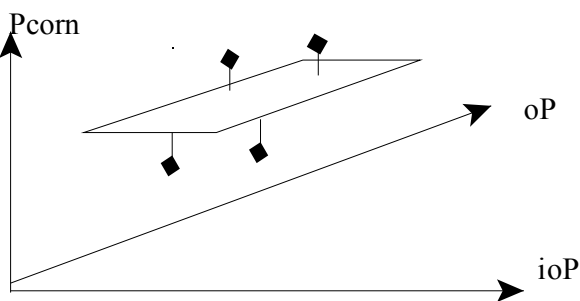All variables are on a ratio type of scale.

Graphical model.

```
Pcorn
  -
  -                        *
  -              * *    *      *
90+          * *       *
  -
  -                *
  -               *       *
75+           *          *
  -    *
  -
  -                   *
60+   *       *
  -      *      *
  -                       *
  ------+---------+---------+---------+---------+---------+orgP
        20        30        40        50        60        70
```

<---Pcorn versus *oP*

Cloud of points.
No clear trend
to describe as a line

Pcorn vs *ioP*

```
Pcorn
  -                                        *
  -                              *  *   *
  -                            * *     *
90+
  -                      *
  -               *
75+           *          *
  -        *
  -
  -     *
60+    2
  -
  -    *   *           *                    inorgP
  --+---------+---------+---------+---------+---------+----
   0.0       6.0      12.0      18.0      24.0      30.0
```

A line can be fit through the points - - >
Then fit two perpendicular lines

Formal model . We begin with one explanatory variable *ioP*

GLM: $Pcorn = \beta_o + \beta_{ioP} \cdot ioP + res$

The parameter β_{ioP} stands for rate of change in phosphorus content of corn, with respect to rate of change of inorganic phosphorus. It is represented as a line through the cloud of points in a graph of *Pcorn* versus inorganic phosphorus.

$$Pcorn = \beta_o + \beta_{oP} \cdot oP + res$$

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1. Construct model

Next, a model for the other explanatory variable, *oP*.
The parameter β_{oP} stands for rate of change in phosphorus content of corn, with respect to rate of change of organic phosphorus.

Finally, a model that includes both explanatory variables

$$Pcorn = \beta_o + \beta_{oP \cdot ioP} \cdot oP + \beta_{ioP:oP} \cdot ioP + res$$

The parameter $\beta_{ioP:oP}$ stands for rate of change in phosphorus content of corn relative to rate of change of inorganic phosphorus, adjusted for effects of organic phosphorus. It is read as 'the rate of change in available phosphorus with change in inorganic phosphorus, controlled for organic phosphorus.'

The parameter $\beta_{oP\ :ioP}$ stands for rate of change in phosphorus content of corn, relative to rate of change in organic phosphorus, adjusted for effects of inorganic phosphorus.
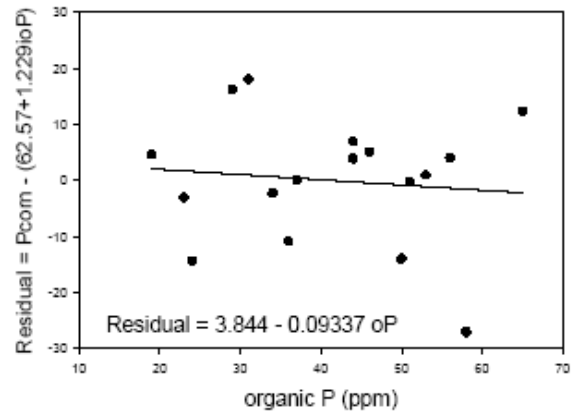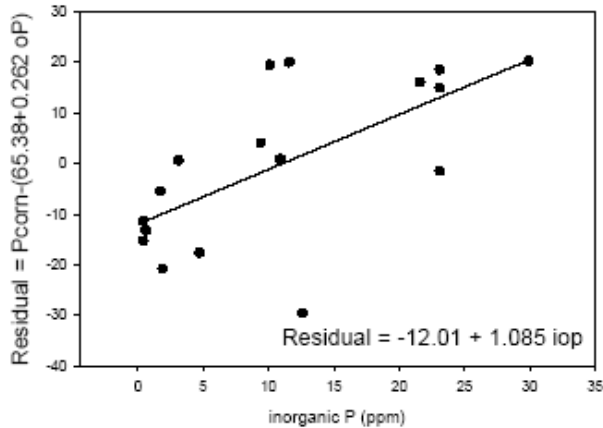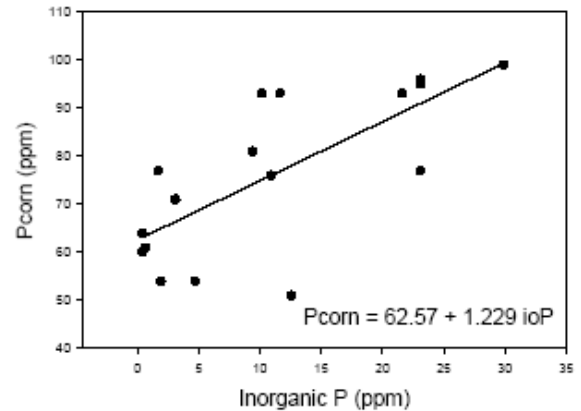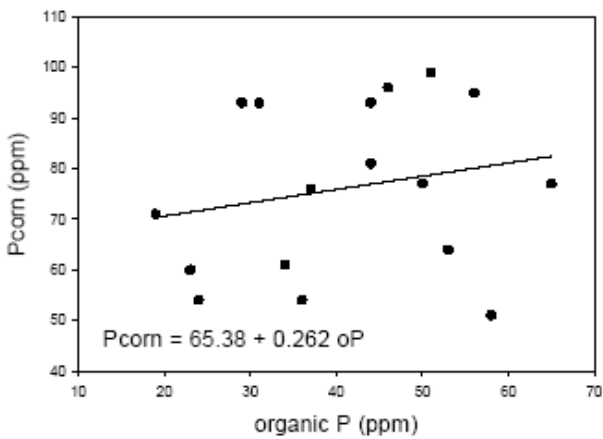
Together, these two parameters describe a plane through the data points (see Fig 1 above). These parameters are partial derivatives, for those who have had this in calculus. The next figure distinguishes the simple regression coefficients (β_{ioP} β_{oP}) from the partial coefficients ($\beta_{ioP \cdot oP}$ $\beta_{oP \cdot ioP}$)

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1.   **Construct the model** (continued)

> Partial regression is the same are regression of the residuals on the remaining variable.
>
> Regress Pcorn against ioP: Pcorn = 62.6 + 1.23 ioP
> Take residuals from this model
> Regress these against another variable, oP
>
> $$\text{Res} = 3.84 \quad - 0.09337 \quad \text{oP}$$
> $$\hat{\beta}_{oP:ioP}$$
>
> This estimate is close to the original estimate of $\hat{\beta}_{oP:ioP} = -0.111$

Because we have two explanatory variables we can investigate their interactive effects on the response variable. Does the effect of one variable on the response variable depend on the other explanatory variable? This interactive effect is described by the interaction term, $\beta_{oP*ioP} \cdot ioP \cdot oP$

$$Pcorn = \beta_o + \beta_{ioP \cdot oP} \cdot ioP + \beta_{oP \cdot ioP} \cdot oP + \beta_{oP*ioP} \cdot ioP \cdot oP + \text{res}$$

The interaction term can be visualized as the degree of curvature of the surface fitted to the data. If there is no curvature, a flat plane describes the phosphorus content of corn relative to the two measures of soil phosphorus. If there is interaction (curvature) then a flat plane will not suffice.

2. **Execute analysis.**

Place data in model format:
    Column labelled *Pcorn* with response variable phosphorus content of corn (ppm)
    Column labelled *ioP*, with explanatory variable inorganic phosphorus (ppm)
    Column labelled *oP*, with explanatory variable organic phosphorus (ppm)
Code the model statement in statistical package according to the GLM

$$Pcorn = \beta_o + \beta_{ioP:oP} \cdot ioP + \beta_{oP:ioP} \cdot oP + \beta_{oP*ioP} \cdot ioP \cdot oP + \text{res}$$

```
MTB > glm 'Pcorn' =   'ioP'        'oP'     'ioP'*'oP' ;
SUBC> covariate  'ioP'  'oP'   ;
SUBC> fits c4;
SUBC> residuals c5.
```

Fits and residuals from:
        model statement output of fitted values and residuals (as above),
        parameters reported by GLM routine,
        direct calculation of parameters.

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2. Execute analysis.

The overall mean is

      $\text{mean(Pcorn)} = \beta_o = 76.18$ ppm

The regression equation for *ioP* is

      $\text{Pcorn} = 62.6 + 1.23\ ioP$

The regression equation for *oP* is

      $\text{Pcorn} = 65.4 + 0.262\ oP$

These are the simple regression coefficients. The equations have been written in slope/intercept form, rather than in GLM form. GLM form uses the grand mean $_o$ rather than the Y-intercept. The Y-intercept is calculated from the grand mean and the slope estimate. The Y-intercept is not itself estimated because the estimate of the grand mean will be better. This is because the grand mean will, by definition, be at the centre of the cloud of data points. The Y-intercept will rarely be at the centre. In many cases the Y-intercept will be completely outside the data points, and so cannot be estimated directly.

The regression equation for both variables:

$$Pcorn = 45.92 + 0.3278\ oP + 5.304\ ioP - 0.0830\ ioP{\cdot}oP$$

These are the estimates of the partial regression coefficients. Notice that they are not the same as the estimates of the simple regression coefficients.

3.   Evaluate model.

Plot residuals versus fitted values

Structural Model. No bowls or arches are evident in a plot of residuals against fitted values, so straight line assumption acceptable.

Residuals Versus the Fitted Values
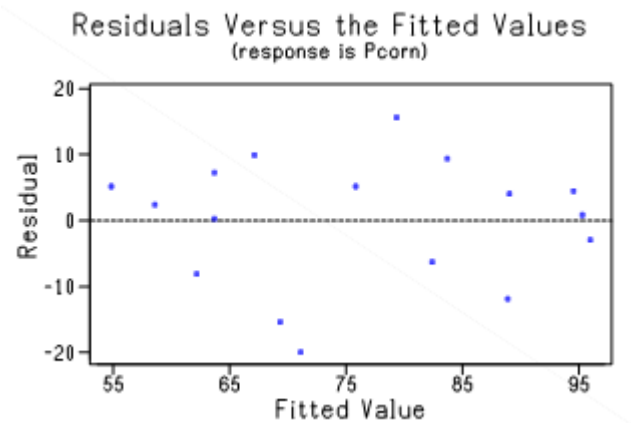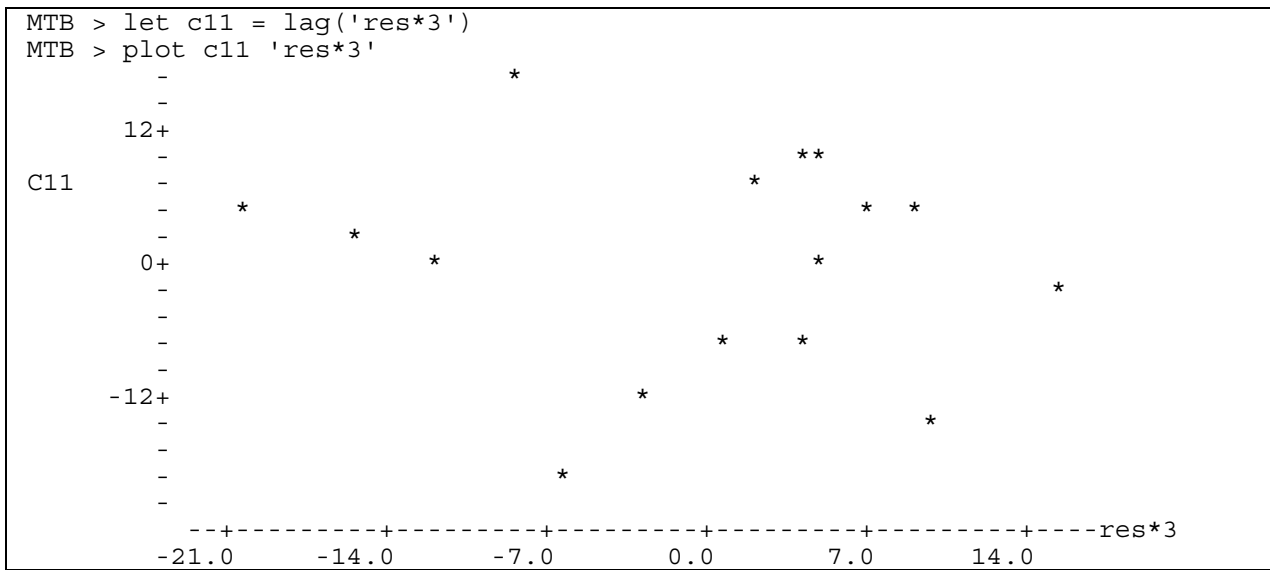(response is Pcorn)

Error model
    Residuals homogeneous ?
      No. Spindles present.
    Residuals <u>independent</u> ?
    We have no information on temporal order or on spatial arrangement. So diagnosis is limited. If we take the observations in the order presented we find no trends upward or downward.

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```
MTB > let c11 = lag('res*3')
MTB > plot c11 'res*3'
          -                              *
          -
          -
      12+
          -                                        **
C11       -                              *
          -      *                           *    *
          -           *
       0+              *                       *
          -                                              *
          -
          -                        *    *
          -
     -12+                     *
          -                                    *
          -
          -           *
          -
        --+---------+---------+---------+---------+---------+----res*3
       -21.0     -14.0      -7.0       0.0       7.0      14.0
```

The plot of residuals versus themselves (at lag 1) shows no positive or negative trends.

Normal ?

Yes. The histogram looks close to normal.

```
MTB > hist 'res';
SUBC> increment 1.
Histogram of res    N = 17
Midpoint    Count
  -2.00       1    *
  -1.00       3    ***
   0.00       9    *********
   1.00       3    ***
   2.00       1    *
```

4. Partition df and SS according to model.

$df_{tot} = 17-1$
$df_{ioP} = 1$ Because relation is expressed by regression line
$df_{oP} = 1$ Because relation is expressed by another regression line
$df_{oP*ioP} = 1$ This is the product of df_{ioP} and df_{oP}
$df_{res} = 13$ This is what is left over

Calculate SS_{total}
$$SS_{total} = \Sigma Y^2 - n^{-1}(\Sigma Y)^2 = 4426.47$$

In Minitab:

```
MTB> let k1 = stdev('Pcorn')*stdev('Pcorn')*16
MTB> print k1
   k1    4426.47
```

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4. Partition df and SS according to model

GLM:	$Pcorn - \beta_o$	$=$	$\beta_{ioP:oP} \cdot ioP$	$+$	$\beta_{oP:ioP} \cdot oP$	$+$	$\beta_{oP*ioP} \cdot oP \cdot ioP$	$+$ res
Source:	Total	$=$	ioP		oP		ioP * oP	+ res
df	17 - 1	$=$	1	$+$	1	$+$	1	+ 13
SS	4426.5	$+$	2295.2 +		29.9	$+$	626.6	+ 1474.7
	Null model							Reduced model

4. Calculate likelihood ratio for overall (omnibus) model.
$$LR = (1474.7/4426.5)^{-17/2} = 11416$$
The regression model is 11,000 times more likely than the unreduced (null) model.
The omnibus model is far more likely than the null model so we proceed to an analysis of each term in the model.

5. Choose mode of inference.
If we had prior estimates of the three model parameters, priorist inference to a revised belief (posterior probability) could be used. We have no estimates. The agronomists who made these measurements may well have had no estimates at the time. The agronomists presumably used a fixed measurement protocol, which lends itself to frequentist inference. In a research setting such as an agricultural station there is no compelling reason to control Type I error at a fixed value. An evidentialist approach (Edwards 1972, Royal 1997, Vieland and Hodge 1998) is appropriate.

Moving to a World Beyond "p < 0.05"
In 2016 the American Statistical Association published a statement on the use and abuse of p-values (Wasserstein. R and N. Lazar 2016 *The American Statistician* 70, 129–133) Of particular note was the use of $p < 0.05$ as "significant," and the tendency to treat this is yes/no conclusion as evidence. The ASA Statement stopped just short of recommending that declarations of "statistical significance" be abandoned. In 2019 that step was taken (Wasserstein et al 2019 *The American Statistician* 73- S1, 1–19: Editorial). The statement concluded, based on a review of the broader literature and on the 44 articles in the special issue, that it is time to stop using the term "statistically significant" entirely. Each of the articles in the species issued offered recommendations for change, including a wide variety of replacements. Only one article addressed teaching statistics, offering generalities. None of the articles mention the evidentialist approach. This is curious. Likelihood ratios are fundamental to both priorist and frequentist inference. They do what frequentist or priorist inference cannot do—say which model has better evidential support. They avoid the problems that attend declaring statistical significance at a fixed Type I error. They certainly have a place in Moving to a World Beyond 'p < 0.05,' the title of the 2019 ASA statement.

Moving to a World Beyond "p < 0.05"
Likelihood ratios are readily grasped by university students in the sciences. At the same time evidential inference goes unknown by most thesis supervisors, thesis examiners, and journal referees. One way of moving beyond "p<0.05" is to demonstrate an evidentialist approach relative to standard frequentist inference that stops at "statistically significant." Not surprisingly the two approaches result in similar conclusions, with the exception that Type I error and "statistical significance" are absent from evidentialist inference. Multiple regression will be presented first with a standard frequentist approach, then with an evidentialist approach for comparison.

5. Define target of inference and whether sample is representative.
The population is not enumerable (*e.g.* all corn plants in Iowa). With frequentist inference the population is the result of a data generating mechanism defined by the experimental protocol and by the procedural statements for the response and explanatory variables. The population is hypothetical, generated by repeating the experimental protocol. Thus, for the purposes of investigating the relation of phosphorus content to soil phosphorus, this sample is representative of the similar experiments on the same variety of corn plants for the range of inorganic and organic phosphorus in this experiment. We cannot infer beyond these ranges.
With an evidentialist approach inference rests on the choice of the probability model to calculate the likelihoods.
The population is represented by the model
$$Pcorn = \beta_o + \beta_{ioP:oP} \cdot ioP + \beta_{oP:ioP} \cdot oP + \beta_{oP \cdot ioP} \cdot ioP \cdot oP + \varepsilon$$
where ε represents a normally distributed error. The justification rests on two arguments. First, the residuals conform satisfactorily to a normal error distribution. Second, the process that generates a normal distribution--errors generated by large number of contributing causes-- are a reasonable supposition in this case. Inference beyond the data at hand rests on the probability model and whether it applies to measurement of phosphorus content of corn relative to the soil. The supposition rests on the propensity of corn to take up phosphorus from soil, a propensity subject to enough sources of perturbation to result in a normal distribution.

6. State statistic and sampling distribution.
For evidentialist inference we will calculate a likelihood ratio for each parameter. If we judge that the deviation from homogeneity was serious we could generate a likelihood ratio by Monte Carlo methods. (Owen, A.B. 2001. *Empirical Likelihood*. CRC Press). For frequentist inference the statistic for calculating Type I from the sum of squares is the F-ratio. Its sampling distribution is the F-distribution. If we judge that the deviation from homogeneity was serious, then we could generate the sampling distribution by Monte Carlo methods.

6. State H_A H_o pairs for parameters.

Here are the hypothesis pairs listed in the order in which they appear in the model.

The first term concerns the effect of inorganic phosphorus, controlled for organic phosphorus.

$$H_A:\ \beta_{ioP:oP} \neq 0$$
$$H_o:\ \beta_{ioP:oP} = 0$$

This is equivalent to the following hypotheses concerning parameter.

$$H_A:\ \text{var}(\beta_{ioP:oP} \cdot ioP) > 0$$
$$H_o:\ \text{var}(\beta_{ioP:oP} \cdot ioP) = 0$$

The second term concerns the effect of organic phosphorus, controlled for inorganic phosphorus.

$$H_A:\ \beta_{oP:ioP} \neq 0$$
$$H_o:\ \beta_{oP:ioP} = 0$$

This is equivalent to the following hypotheses concerning the variance.

$$H_A:\ \text{var}(\beta_{oP:ioP} \cdot oP) > 0$$
$$H_o:\ \text{var}(\beta_{oP:ioP} \cdot oP) = 0$$

The third term concerns the interactive effect of organic phosphorus and inorganic phosphorus on phosphorus content of corn.

$$H_A:\ \beta_{oP*ioP} \neq 0$$
$$H_o:\ \beta_{oP*ioP} = 0$$

This is equivalent to the following hypotheses concerning the variance.

$$H_A:\ \text{var}(\beta_{oP*ioP} \cdot oP) > 0$$
$$H_o:\ \text{var}(\beta_{oP*ioP} \cdot oP) = 0$$

7. ANOVA – Table source, df, SS.

Source	df	Seq SS.	SS_{adj}	MS_{adj}	F---->p
ioP	1	2295.2			
oP	1	29.9			
ioP·oP	1	626.6			
Error	13	1474.7			
Total	16	4426.5			

This partitioning is in the order in which the variables are listed in the model.

7. ANOVA - partition variance according to model.

If we change the order of the variables, the partitioning will change.
To take this into account, we use the adjusted Sums of Squares.
That is, we use the SS for each explanatory variable when it is entered <u>last</u> into the GLM.

Source	df	Seq SS.	adjSS	MSadj.	F---->	p
ioP	1	2295.2	1061.8			
oP	1	29.9	149.4			
ioP*oP	1	626.6	626.6			
Error	13	1474.7	1474.7			
Total	16	4426.5				

In this example, the SS for $oP*ioP$ remained the same as the previous partitioning. This is because it was the last SS in the sequential partitioning.
The SS for ioP is smaller in this partitioning, because now it is last instead of first.
This partitioning (each variable last) is called the adjusted SS.
The adjusted SS no longer add up to the total $SS_{tot} = 4426.5$
so the total SS is not shown. The SS_{error} remains the same.

Statistical packages produce both the sequential and adjusted SS. The sequential SS is the default partitioning in some packages (*e.g.* R). The adjusted SS is the default in other packages (*e.g.* Minitab). Most packages allow the user to choose the partitioning.

7. ANOVA- Frequentist. Calculate MS, F, Type I error

Source	df	Seq SS.	SS_{adj}	MS_{adj}	F---->p	
ioP	1	2295.2	1061.8	1061.8	9.36	0.009
oP	1	29.9	149.4	149.4	1.32	0.272
ioP·oP	1	626.6	626.6	626.6	5.52	0.035
Error	13	1474.7	1474.7	113.4		
Total	16	4426.5				

MS = SS/df for each term.
Calculate the correct F-ratios.
All terms in the model are regressions and so are taken as fixed. The correct F-ratio is relative to the residual MS.

$$F_{iop:oP} = 1061.8 / 113.4 = 9.36$$
$$F_{op:ioP} = 149.4 / 113.4 = 1.32$$
$$F_{op*ioP} = 626.6 / 113.4 = 5.52$$

```
MTB> cdf 5.52;
SUBC> F 1 13.
  5.52       0.965
```

Statistical routines automatically report the Type I error from the F-distribution for each F-ratio in the ANOVA table.
The probability of obtaining $F_{ioP·oP}$ this large from our sampling distribution is $p = 0.035$

8. Recompute Type I error if necessary.
Assumptions judged to be met.
Monte Carlo calculations are available if assumptions judged not met.

9. Report frequentist statistical conclusions about model terms.
H_o: $\text{var}(\beta_{ioP*oP} \cdot ioP) = 0$ $0.035 = p$

We reject H_o that there is no interactive effect of the two forms of soil phosphorus on phosphorus content of corn.

For an analysis with an interactive term it is best to report the entire table, showing Sources of variance, df, SS, MS, F and Type I error.

The SS should be clearly labelled as adjusted (Type III) SS.

Similarly, we reject the null hypothesis of no effect of inorganic phosphorus.
$F_{1,13} = 9.36$ $p = 0.009$

We cannot reject the null hypothesis of an effect of organic phosphorus.
$F_{1,13} = 1.32$ $p = 0.272$

Critique of the analysis. We rejected the null hypothesis twice. In one case the rejection was marginal, with a Type I error of 3.5%. The other rejection was at a far smaller error rate, less than a 0.1%. The reject/not reject convention leaves aside the differences. In this study there was no cost-based reason for limiting Type I error to a fixed level. An evidentialist approach is sufficient—report the likelihood ratio.

7. ANOVA- Evidentialist. Calculate SS_{full} , $SS_{reduced}$, LR

Here is an ANOVA table showing calculation of the likelihood ratio.

Source	df	SS_{adj}	$SS_{reduced}$	SS_{full}	SS_o/SS_A	LR	Evidence
ioP	1	1061.8	1474.7	2536.5	1.720	100	Good
oP	1	149.4	1474.7	1624.1	1.101	2.3	Inadequate
ioP*oP	1	626.6	1474.7	2101.3	1.425	20	Some
Residual	13	1474.7					
Total	16	4426.5					

The flow of calculation begins with the adjusted sum of squares in the previous table.
The flow proceeds from left to right as with the pervious table.
The reduced model is the same for all three terms in the model.
This will not always be so. It will vary in more complex models.
The full model is calculated as $SS_{full} = SS_{adj} + SS_{reduced}$
The full (null) relative to the reduced model is $SS_o/SS_A = SS_{full} / SS_{reduced}$
This ratio decreases to 1 as the variance SS_{adj} decreases to zero.
The likelihood ratio is $LR = (SS_o/SS_A)^{(n/2)}$.

7. ANOVA- Evidentialist.

To avoid the loss of information that accompanies yes/no decisions we sort the likelihood ratios into categories. An easily remembered ranking is

Some evidence $LR \geq 20$
Good evidence $LR \geq 100$
Strong evidence $LR \geq 1000$

The categories were chosen from the point of view of an odds ratio.
20:1 odds approximate the odds at $p = 5\%$:   Odds = $(1-5\%)/ 5\% = 0.95/0.05 = 19{:}1$
100:1 odds approximate the odds at $p = 1\%$ Odds = $(1-1\%)/ 1\% = 0.99/0.01 = 99{:}1$
1000:1 odds approximate the odds at $p = 0.1\%$ Odds = $(1-0.1\%)/ 0.1\% = 999{:}1$

These categories, while convenient, are not a recasting of $p<5\%$, $p<1\%$, *etc.*
We are no longer evaluating our results based on Type I error. We are evaluating based on a measure of relative evidence, the likelihood ratio.

8. Recompute Type I error if necessary.

Assumptions were judged to be met. If not met, we can estimate the likelihood ratio with Monte Carlo methods. In this case, inference no longer rests on the normal error model. Consequently inference applies only to the data at hand.

9. Report evidentialist statistical conclusions about model terms.

H_o / H_A: The likelihood of an interactive effect relative to no interactive effect.
   $LR = L(\beta_{ioP*oP} \neq 0) / L(\beta_{ioP*oP} = 0)$
   $LR = 20$ Some evidence for interactive effect
H_o / H_A: The likelihood of an organic phosphorus effect relative to no effect.
   $LR = L(\beta_{oP} \neq 0) / L(\beta_{oP} = 0)$
   $LR = 2.3$ Inadequate evidence for an effect of organic phosphorus
H_o / H_A: The likelihood of an in organic phosphorus effect relative to no effect.
   $LR = L(\beta_{ioP} \neq 0) / L(\beta_{ioP} = 0)$
   $LR = 100$ Good evidence for an effect of inorganic phosphorus

10. Report science conclusions.

There is some evidence for an interactive effect of soil organic and soil inorganic phosphorus on phosphorus content of corn. Our best estimate of phosphorus content of corn, given organic and inorganic phosphorus in soil is:

   $Pcorn = 45.92 + 0.3278\ oP + 5.304\ ioP - 0.0830\ ioP \cdot oP$

Most GLM routines will report standard errors or confidence limits for each parameter.

Term	Coef	SE Coef
Constant	45.92	12.24
ioP	5.304	1.734
oP	0.3278	0.2856
ioP*oP	-0.08309	0.03536

10. Report science conclusions.

Our conclusion, based on the evidence, is similar to that had we used Type I error in evaluating our model.

The failure to reject the null for organic phosphorus (and equivalently the insufficient evidence for an effect of soil organic phosphorus) suggests that we should simplify the model by dropping the *oP* term. The revised model would be:

$$Pcorn \; = \; \beta_o \; + \; \beta_{ioP:oP} \cdot ioP \; + \; \beta_{oP*ioP} \cdot ioP \cdot oP \; + \; \varepsilon$$

The *oP* term must be retained in the model in order to estimate the interaction term. Because *oP* appears in the interaction term we would still need to know the value of *oP* to predict phosphorus in corn. What if the null hypothesis for the interaction term was not rejected? Could we drop the term? From the frequentist point of view this raises the nemesis of multiple testing—the idea that we revise and re-test a model to arrive at a better model. If we do this, the limit on Type I error that we set no longer applies. We would need to set the limit lower, according to the number to tests that we do with the same data.