#### SASEG 6C - Two Way ANOVA

(Fall 2015)

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**Two-Way ANOVA with Interactions** 

In the previous section, you considered the case where you had one categorical predictor and a blocking variable. In this section, consider a case with two categorical predictors. In general, any time you have more than one categorical predictor variable and a continuous response variable, it is called *n*-way ANOVA. The *n* can be replaced with the number of categorical predictor variables.

The analysis for a randomized block design is actually a special type of *n*-way ANOVA.



Data was collected in an effort to determine whether different dose levels of a given drug have an effect on blood pressure for people with one of three types of heart disease.



 $Y_{ijk}$  the observed **BloodP** for each subject

- $\mu$  the overall base level of the response, **BloodP**
- $\alpha_i$  the effect of the *i*<sup>th</sup> **Disease**
- $\beta_j$  the effect of the *j*<sup>th</sup> **DrugDose**
- $(\alpha\beta)_{ij}$  the effect of the interaction between the *i*<sup>th</sup> **Disease** and the *j*<sup>th</sup> **DrugDose**
- $\varepsilon_{ijk}$  error term, or residual

In the model it is assumed that the:

- observations are independent
- data are normally distributed for each treatment
- variances are equal across treatments





An interaction occurs when a change in the level of one factor results in a change in the difference between levels of the other factor.

The average blood pressure change over different doses were plotted in mean plots and then connected for disease A and B.

In the left plot above, different types of disease show the same change across different levels of dose.

In the right plot, however, as the dose increases, average blood pressure **increases** in those with disease A, but **decreases** for those with disease B. This indicates an interaction between the variables **DrugDose** and **Disease**.

When you analyze an *n*-way ANOVA with interactions you should first look at any tests for interaction among factors.

If there is no interaction between the factors, the tests for the individual factor effects can be interpreted to determine their significance/non-significance.

If an interaction exists between any factors, the tests for the individual factor effects might be misleading, due to masking of these effects by the interaction. This is especially true for unbalanced data.

In the previous section, you used a blocking variable and a categorical predictor as effects in the model. It is generally assumed that blocks do not interact with other factors. In this section, there are just two separate factors. An interaction between the two might be hypothesized and tested.

# **Nonsignificant Interaction**

Analyze the main effects with the interaction in the model.

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

....or....

Delete the interaction from the model, and then analyze the main effects.

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk}$$

94

When the interaction is not statistically significant, the main effects can be analyzed with the model as originally written. This is generally the method used when analyzing designed experiments.

However, even when analyzing designed experiments, some statisticians suggest that if the interaction is nonsignificant, then the interaction effect can be deleted from the model and then the main effects are analyzed. This increases the power of the main effects tests.

Neter, Kutner, Wasserman, and Nachtsheim (1996) suggest the following guidelines for when to delete the interaction from the model:

- there are fewer than 5 degrees of freedom for the error, and
- the F Value for the interaction term is < 2.
  - When you analyze data from an observational study, it is more common to delete the non-significant interaction from the model and then analyze the main effects.

## Two-Way ANOVA with Interactions

Perform a two-way ANOVA using the DRUG data set.

The **Drug** data set contains the following variables:

DrugDose dosage level of drug (Placebo, 100 mg, 200 mg, 500 mg)

- **Disease** heart disease category
- **BloodP** change in diastolic blood pressure after 2 weeks treatment

Before conducting an analysis of variance, you should explore the data.

Presume that the initial data exploration was completed (output not shown here) and that no particular concerns were noted about unusual data values or the distribution of the data. During this exploration, you determine that the sample sizes for all treatments are not equal. The researchers recruited 240 patients (80 per heart disease category), but only 170 were randomized into the trial.

1. Open the **DRUG** data set.

DRUG -									
🐺 Filter and Sort 🕮 Query Builder   Data 👻 Describe 👻 Graph 👻 Analyze 👻   Export 👻 Send To 👻   📝									
	🔞 PatientID 🔇	DrugDose	🔌 Disease	🔞 BloodP					
1	69	2	В	13					
2	162	4	A	-47					
3	181	1	В	12					
4	209	4	A	-4					
5	308	2	A	4					
6	331	4	С	37					
7	340	4	С	-19					
8	350	1	В	-9					
9	360	2	В	-17					
10	363	4	A	-41					

Negative values for **BloodP** mean that diastolic blood pressure was reduced by that amount. Positive values mean that the **BloodP** increased.

2. Use the summary statistics task, choosing <u>BloodP</u> for the analysis variables role and <u>Disease</u> and <u>DrugDose</u> for the classification variables role.

Σ	Summary Statis	tics3 for Local:SASUSER.DRUG	×
	Data Statistics Basic Percentiles Additional Plots	Data source: Local:SASUSER.DRUG Task filter: None	
	Results Titles Properties	Variables to assign: Name PatientID DrugDose Disease BloodP BloodP Frequency classification variables Frequency classification variables	s level DrugDo: by: ormatted value order: rending
	Preview code	Select a role to view the context help for that role.	Help .::

	Analysis Variable : BloodP											
Disease	DrugDose	N Obs	Mean	Std Dev	Minimum	Maximum	Ν					
Α	1	12	1.3333333	13.5333483	-22.0000000	25.0000000	12					
	2	16	-9.6875000	18.8881577	-37.0000000	19.0000000	16					
	3	13	-26.2307692	18.1390640	-51.0000000	11.0000000	13					
	4	18	-22.5555556	21.0970369	-61.0000000	12.0000000	18					
В	1	15	- <mark>8.13333</mark> 33	16.9109714	-39.0000000	22.0000000	15					
	2	15	5.4000000	21.8886794	-45.0000000	35.0000000	15					
	3	14	24.7857143	23.7427838	-24.0000000	60.0000000	14					
	4	13	23.2307692	23.5872630	-22.0000000	55.0000000	13					
С	1	14	0.4285714	20.2929100	-38.0000000	45.0000000	14					
	2	13	-4.8461538	24.0341637	-36.0000000	50.0000000	13					
	3	14	-5.1428571	13.9827209	-26.0000000	27.0000000	14					
	4	13	1.3076923	28.7847894	-57.0000000	42.0000000	13					

The means do seem to vary from group to group. However, it is not obvious if there is a consistent trend across different levels of **DrugDose**. It seems that the trend of **BloodP** across levels of **DrugDose** may be different from disease to disease.

To test the hypothesis that effect of **DrugDose** differs across diseases, perform a 2-way ANOVA with an interaction.

- 1. Select <u>Tasks</u> (or <u>Analyze</u>)  $\Rightarrow$  <u>ANOVA</u>  $\Rightarrow$  <u>Linear Models...</u>.
- 2. Under Data, assign **Bloodp** to the dependent variable task role and **Disease** and **DrugDose** to the classification variables role.
  - DrugDose is saved in SAS as numeric and could have been assigned to the quantitative variables role. However, the scale of measurement of the variable is not continuous, but rather ordinal, so it should be added as a classification variable.
- 3. Select <u>Model</u> at the left and highlight both **Disease** and **DrugDose** in the Class and quantitative variables pane (by clicking on one and then holding down the CTRL key on the keyboard and then clicking on the other variable).

🔀 Linear Models2 fo	or Local:SASUSER.DRUG	×
Data Model Model Options Advanced Options Post Hoc Tests Least Squares Arithmetic Plots Predictions Titles Properties	Data   Variables to assign: Task roles:   Name Dependent variable (Li   PatientID DugDose   Disease Quantitative variables   BloodP DrugDose   Disease DrugDose   BloodP PatientID   PatientID DrugDose   Disease DrugDose   PatientID Prequency count (Limit)   PatienID <td></td>	
Preview code	Run 🔻 Save Cancel Help	
		.::

4. Click Factorial and see the terms, DrugDose, Disease and DrugDose\*Disease displayed in the Effects pane.

Linear Models2 for	r Local:SASUSER.DRUG	×
Data Model	Model	
Model Options Advanced Options	Class and quantitative variables: Ef	fects:
Post Hoc Tests Least Squares Arithmetic Plots Predictions Titles Properties	Image: Second	)rugDose )isease )rugDose*Disease
Provinu codo		

5. Under Model Options, deselect **Show parameter estimates**.

Ø	🗹 Linear Models2 for Local:SASUSER.DRUG						
	Data Model Model Options	Model Options					
	Advanced Options Post Hoc Tests Least Squares	Hypothesis tests Show parameter estimates Show parameter estimates Confidence limits for parameter estimater estimates					
	Arithmetic Plots Predictions	Sum of squares to show Confidence level:					

- 6. Click Preview code at the bottom of the window.
- 7. You will now see a window showing the SAS code created by the t Test task. This window is where you can directly edit the Code generated by SAS Task.
- 8. Select the Show custom code insertion points and...

Code Preview for Task

×

- 9. Scroll down in the code to just under the words **PLOTS (ONLY) =ALL**.
- 10. Click on the <insert custom code here> in the area just after PLOTS (ONLY) =ALL
- 11. Type **INTPLOT**. *This is the option to produce an interaction plot*.
- 12. Uncheck the Show custom code insertion points. The code should look like the following.



13. Close the window by clicking  $\blacksquare$  in the upper right corner.

14. Click Run

	<b>Class Level Information</b>							
	Class Levels Values							
	DrugDose	4	1234					
	Disease	3	ABC					
Nu	Number of Observations Read							
Nui	Number of Observations Used							

The next part of the output, below, shows the source table with the *F* test for the overall model. This tests the null hypothesis that none of the effects in the model are statistically different, in other words, that there are no differences among the 12 group means (one for each **DrugDose** \* **Disease** combination).

Source DF		Sum of Squ	uares	Mea	n Square	F Va	lue	Pr > F	
Model		11	36476	.8353	3	316.0759	7	.66	<.0001
Error 158		158	68366.4589			432.6991			
Corrected 1	Fotal	169	104843	.2941					
R-So		luare	Coeff Var	Root	MSE	BloodP M	/lean		
	0.34	47918	-906.7286	20.8	0142	-2.29	4118		

The descriptive statistics indicate that the average blood pressure change for all observations is -2.294118. The R<sup>2</sup> for this model is 0.347918.

The *p*-value given is <0.0001. Presuming an alpha equal to 0.05, you reject the null hypothesis and conclude that at least one treatment mean is different from one other treatment mean. Which factor(s) explain this difference?

The next part of the output shows tests of the main effects and the interaction.

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DrugDose	3	54.03137	18.01046	0.04	0.9886
Disease	2	19276.48690	9638.24345	22.27	<.0001
DrugDose*Disease	6	17146.31698	2857.71950	6.60	<.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Source DrugDose	DF 3	<b>Type III SS</b> 335.73526	Mean Square 111.91175	F Value	Pr > F 0.8551
Source DrugDose Disease	DF 3 2	<b>Type III SS</b> 335.73526 18742.62386	Mean Square 111.91175 9371.31193	F Value 0.26 21.66	Pr > F 0.8551 <.0001

The Type I SS are model-order dependent; each effect is adjusted only for the preceding effects in the model. They are known as *sequential sums of squares*. They are useful in cases where the marginal (additional) effect for adding terms in a specific order is important. An example is where X, X\*X, and X\*X\*X are in the model statement. Each term is only tested controlling for a lower order term. The TYPE I SS values are additive. They sum to the Model Sum of Squares for the overall model.

The Type III SS are commonly called *partial sums of squares*. The Type III sum of squares for a particular variable is the increase in the model sum of squares due to adding the variable to a model that already contains all the other variables in the model. Type III sums of squares, therefore, do not depend on the order in which the explanatory variables are specified in the model. The Type III SS values are not generally additive (except in a completely balanced design). The values do not necessarily sum to the Model SS.

Look at tests using the Type III SS.

You should consider the test for the interaction first, because if there is an interaction, then by definition that means that the effect of each main effect is different at each level of the other main effect. The *p*-value for **DrugDose\*Disease** is <0.0001. Presuming an alpha of 0.05, you reject the null hypothesis. You have sufficient evidence to conclude that there is an interaction between the two factors. As shown in the graph, the effect of the level of drug changes for different disease types.



You get an interaction plot, as well as a diagnostics plot.

The Quantile-Quantile plot of the residuals indicates no great departure from normality. The Residual by Predicted Value plot shows no pattern in the residuals and the variability of the Residual seems fairly similar across all values of the Predicted Value.



This plot shows the interaction. Drug A shows a pattern of lower blood pressure at higher doses. Drug B shows the opposite pattern. Drug C displays a fairly constant effect across all doses.

Because of the interaction, you do not know the significance of the **DrugDose** effect at any particular level of **Disease**. (If we were to go further, the LSMEANS tests could be used to test the effect of **DrugDose** at each level of **Disease**.)

Given all of this information, it seems that one would want to aggressively treat blood pressure in people with disease A with high doses of the drug. For those with disease B (perhaps caused by a traumatic event), treating with the drug at all would be a mistake. For those with Disease C, there seems to be no effect on blood pressure.

#### **Block Design Exercises**

- 1. Analyzing Data in a Randomized Block Design
- **a.** Test the hypothesis that the **Sales** means are equal. Include all of the variables in your MODEL statement. What can you conclude from your analysis? Was adding the blocking factor **Area** into the design and analysis detrimental to the test of **Ad**?

Perform an ANOVA on the **Ads1** data set to look for differences in sales across advertisement mode, controlling for **Area**, which is the blocking variable in the design. Check for normality of the residuals.

- 1) Open the data table **Ads1**.
- 2) Select <u>Tasks</u>  $\Rightarrow$  <u>ANOVA</u>  $\Rightarrow$  <u>Linear Models...</u>.
- 3) Under Data, assign <u>Sales</u> to the dependent variable task role and <u>Ad</u> and <u>Area</u> to the classification variable role.

4) Under Model, click Ad and Area and then click Main

Note that there is NO interaction with the Block design.

5) Under Model Options, uncheck <u>Type I</u> and <u>Show parameter estimates</u>.

6) Click Run





The QQ Plot of Residuals indicates that the normality assumption for ANOVA is met.

Class Level Information						
Class	Levels	vels Values				
Ad	4 display paper people radio					
Area	18 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17			5 16 17 18		
Number of Observations Read 144						
Number of Observations Used 144						

Source		D	F	Sum of Sq	uares	Mean	Square	F۱	alue	Pr > F	
Model		2	20	15131.38889		75	56.56944		8.43	<.0001	
Error			12	3	11037.91667		89.73916				
Corre	cted T	otal	14	3	26169.	30556					
		R-S	i <b>qu</b> a 5782	are 211	Coeff Var 14.17712	r <b>Roo</b> 2 9.4	t MSE 73076	Sales M	lean 1944		
	Sourc	e [	DF	Ту	/pe III SS	Mean	Squar	e FVa	lue	Pr > F	:
	Ad		3	586	66.083333	1955	5.36111	1 21	.79	<.0001	I
	Area		17	926	65.305556	545	5.01797	4 6	.07	<.0001	I

The p-value for Ad (<.0001) indicates that there was a difference in sales among the advertising campaign types, when controlling for Area.

The large (statistically significant) F-Value for **Area** gives evidence that area of the country was a useful factor to block on. It was definitely not detrimental.

#### **Post Hoc Pairwise Comparison**

#### 2. (optional exercise) Post Hoc Pairwise Comparisons – where is the difference?

Conduct pairwise comparisons with an experimentwise (use the Tukey adjustment) error rate of  $\alpha$ =0.05.

- Re-open the previous Linear Models task by right-clicking the icon for it and selecting Modify... from the drop-down menu.
- Select Least Squares under Post Hoc Tests and then click Add under the white space for Effects to estimate.
- A 0 line will appear and the Options for means tests area will be populated with several options. Click <u>False</u> next to Ad from the Options for means tests and then click that appears next to it. Change this value to <u>True</u> by scrolling to that value and clicking on it.
- In a similar fashion, under comparisons, change the Show p-values for differences option from None to <u>All pairwise differences</u>. The Adjustment method for comparison can be selected as <u>Tukey</u> or left at <u>Default</u> because Tukey is the default method used when <u>All pairwise differences</u> is chosen above.
- Click Add under the white space for Effects to estimate and effect 1 will appear. Choose Ad again as the Class effects to use variable. This time the choice for Show p-values for differences should be <u>Control using first level</u>. The default Adjustment method will be <u>Dunnett</u> and the default control group will be <u>display</u>.
- Click Run and do not replace the previous results.

**a.** Which types of advertising are significantly different?

Ad	Sales LSMEAN	LSMEAN Number
display	56.5555556	1
paper	73.2222222	2
people	66.6111111	3
radio	70.8888889	4

L( Pr	Least Squares Means for effect Ad Pr >  t  for H0: LSMean(i)=LSMean(j) Dependent Variable: Sales						
i/j	1	2	3	4			
1		<.0001	<.0001	<.0001			
2	<.0001		0.0190	0.7233			
3	<.0001	0.0190		0.2268			
4	<.0001	0.7233	0.2268				



The Tukey comparisons show significant differences between **display** and all other types of advertising and between paper and people (p=0.0190).

**b.** Use **display** as the control group and do a Dunnett comparison of all other advertising methods to see if those methods improved sales over just display ads in stores.

		H0:LSMean=Control
Ad	Sales LSMEAN	Pr >  t
display	56.5555556	
paper	73.2222222	<.0001
people	66.6111111	<.0001
radio	70.8888889	<.0001



All other advertising campaigns resulted in significantly better average sales (statistically significant) than **display**.

### Another Example - Two-Way ANOVA (if you want more practice)

Test the hypothesis that the means are equal, making sure to include an interaction term. What conclusions can you reach?

- Open the **Concrete** data set.
- Use the summary statistics task, choosing <u>Strength</u> for the analysis variables role and <u>Brand</u> and <u>Additive</u> for the classification variables role.
- Run the task.

Analysis Variable : Strength											
Brand	Additive	N Obs	N Obs Mean Std Dev Mini		Minimum	Maximum	Ν				
Consolidated	reinforced	5	25.8000000	2.3727621	22.7000000	29.3000000	5				
	standard	5	22.6000000	1.5313393	20.4000000	24.2000000	5				
EZ Mix	reinforced	5	27.2600000	1.9603571	25.5000000	30.2000000	5				
	standard	5	24.4000000	3.7729299	19.8000000	28.0000000	5				
Graystone	reinforced	5	30.6600000	1.3390295	29.5000000	32.6000000	5				
	standard	5	25.2800000	3.1451550	21.2000000	29.8000000	5				

The means do seem to vary from group to group. However, it is not obvious if there is a consistent difference between additives across different levels of **Brand**. The differences between **reinforced** and **standard** range from about 3 for Consolidated and EZ Mix and 5 for Graystone.

To test the hypothesis that effect of **Additive** differs across brands, perform a 2-way ANOVA with an interaction.

- Select <u>Tasks</u>  $\Rightarrow$  <u>ANOVA</u>  $\Rightarrow$  <u>Linear Models...</u>.
- Under Data, assign **Strength** to the dependent variable task role and **Brand** and **Additive** to the classification variables role.
- Select <u>Model</u> at the left and highlight both **Brand** and **Additive** in the Class and quantitative variables pane (by clicking on one and then holding down the CTRL key on the keyboard and then clicking on the other variable).
- Click Factorial and see the terms, Brand, Additive and Brand\*Additive displayed in the Effects pane.
- Under Model Options, deselect Show parameter estimates and Type I.
- Click Preview code
- You will now see a window showing the SAS code created by the t Test task. This window is where you can directly edit the Code generated by SAS Task.
- Select the Show custom code insertion points and...
- Scroll down in the code to just under the words PLOTS(ONLY)=ALL.
- Click on the <insert custom code here> in the area just after PLOTS(ONLY)=ALL

- Type INTPLOT. This is the option to produce an interaction plot.
- Uncheck the Show custom code insertion points and close the window by clicking 🗵 in the upper corner.
- Click Run

Class Level Information									
Class	Levels	Values							
Brand	3	Consolidated EZ Mix Graystone							
Additive	2	reinforced standard							
N	30								
N.	Number of Observations Used								
INC	inner or	30							

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model 5		189.9080000	37.9816000	6.04	0.0009
Error	24	150.9520000	6.2896667		
Corrected Total	29	340.8600000			

	R-Square		Coeff Var Root MSE		ot MSE	Streng		
0.55714		44	9.645849 2.507921		26.00000			
Source		DF	Type III	SS	Mean	Square	F Value	Pr > F
Brand		2	71.4980	000	35.7	490000	5.68	0.0095
Additive	)	1	109.0613	333	109.0	613333	17.34	0.0003
Brand*A	dditive	2	9.3486	667	4.6	743333	0.74	0.4862

There is no significant interaction between **Additive** and **Brand**, even though the plot shows slightly different slopes among the two additives. At this point, you may choose to remove the interaction term from the model and, if still significant, conclude that there is a difference in additive types.