Two-way ANOVA without interaction

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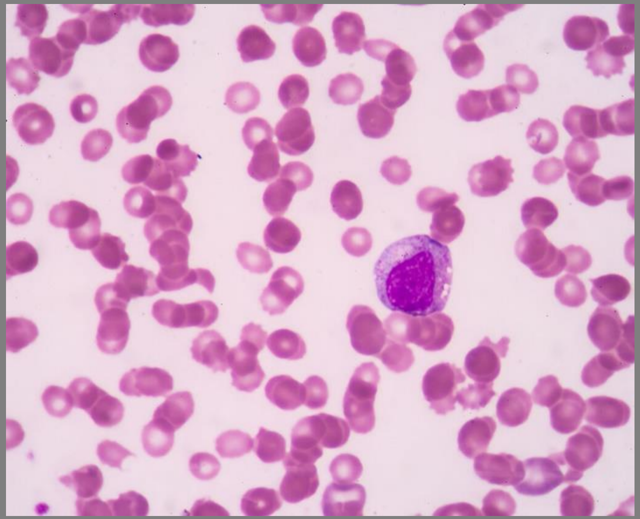
Effect of drugs and cages on lymphocyte counts



Effect of drugs and cages on lymphocyte counts

In an experiment to compare the effect of three drugs on lymphocyte counts in mice, a design with three mice from four different cages was used

	Cages			
Drugs	1 (1)	2 (2)	3 (3)	4 (4)
a (1)	7.1	6.1	6.9	5.6
b (2)	6.7	5.0	5.9	5.1
c (3)	6.6	5.4	5.8	5.2

Entering data and defining variables

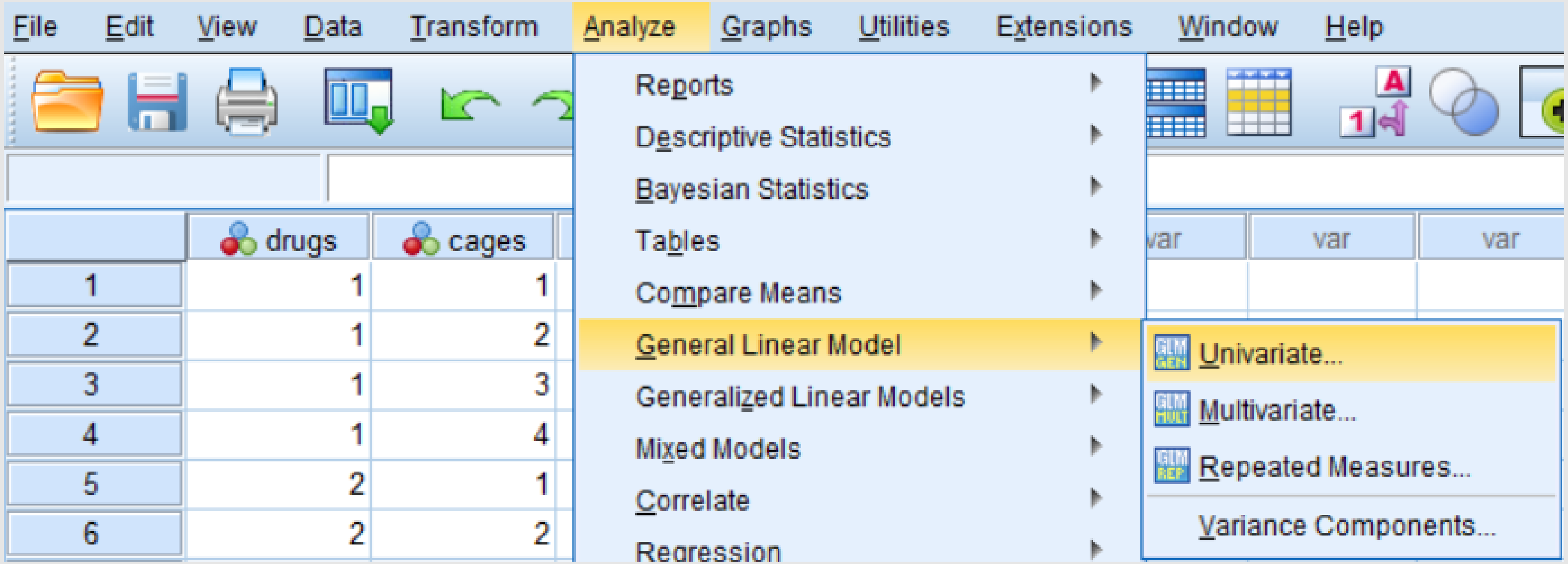
- Enter the data in the **Data View.**
- One variable represents the **cages**, another represents the **drugs**, and the third represents the corresponding **number of lymphocytes**
- Then, name the variables accordingly in the **Variable View**

	drugs	cages	cells
1	1	1	7.10
2	1	2	6.10
3	1	3	6.90
4	1	4	5.60
5	2	1	6.70
6	2	2	5.00
7	2	3	5.90
8	2	4	5.10
9	3	1	6.60
10	3	2	5.40
11	3	3	5.80
12	3	4	5.20

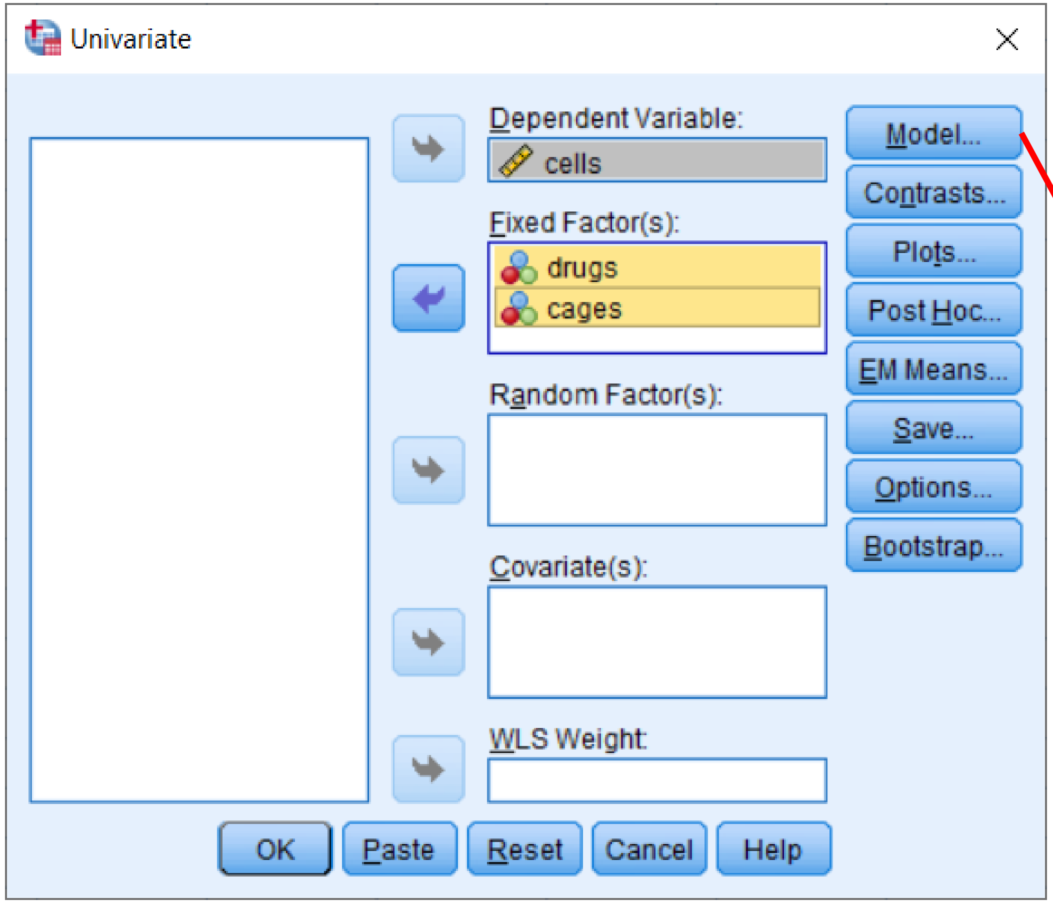
	Name	Type	Width	Decimals	Label	Values	Missing	Columns	Align	Measure	Role
1	drugs	Numeric	8	0		None	None	8	Right	Nominal	Input
2	cages	Numeric	8	0		None	None	8	Right	Nominal	Input
3	cells	Numeric	8	2		None	None	8	Right	Scale	Input

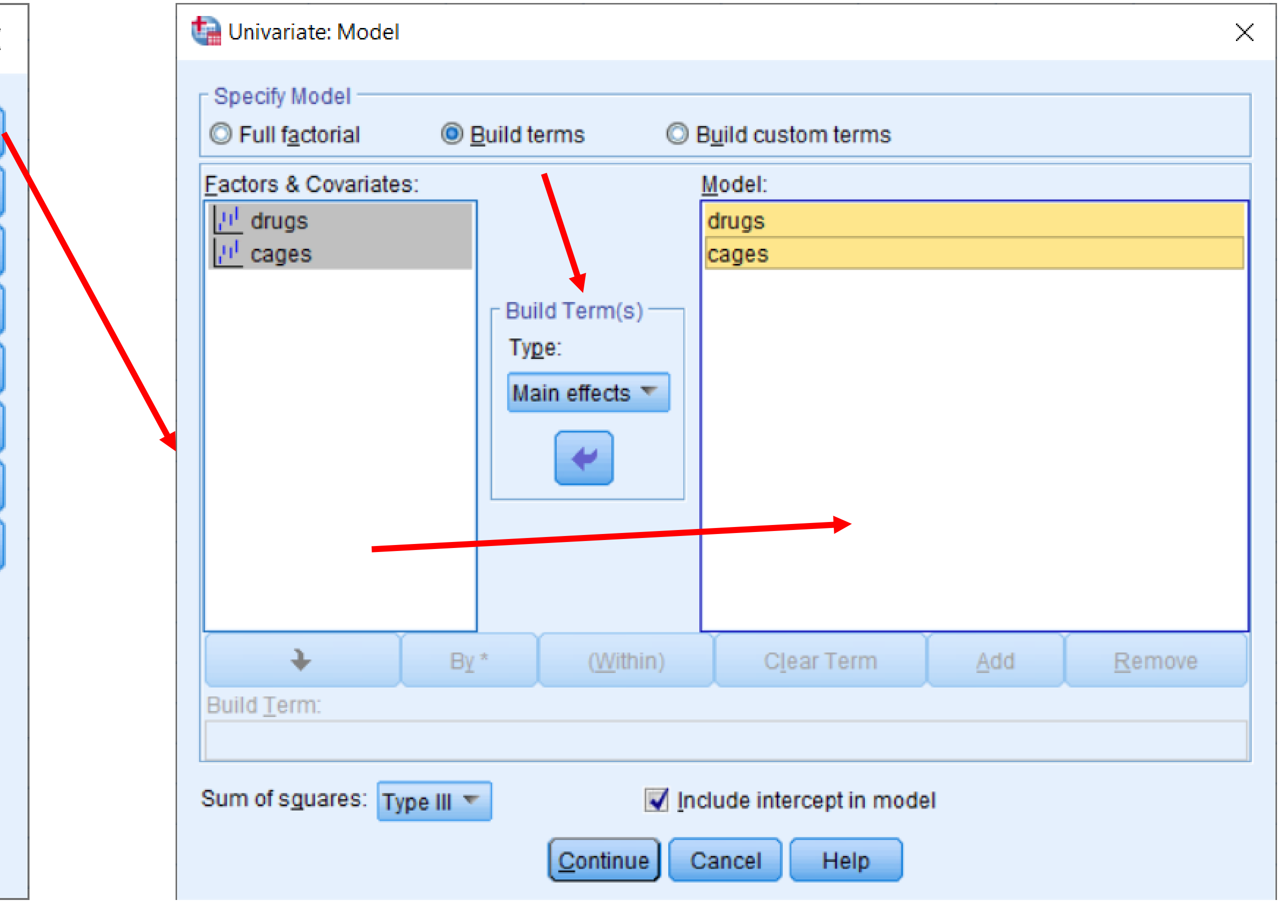
Running the two-way ANOVA test

To analyze the data, select **Analyze -> General Linear Model -> Univariate** from the menu



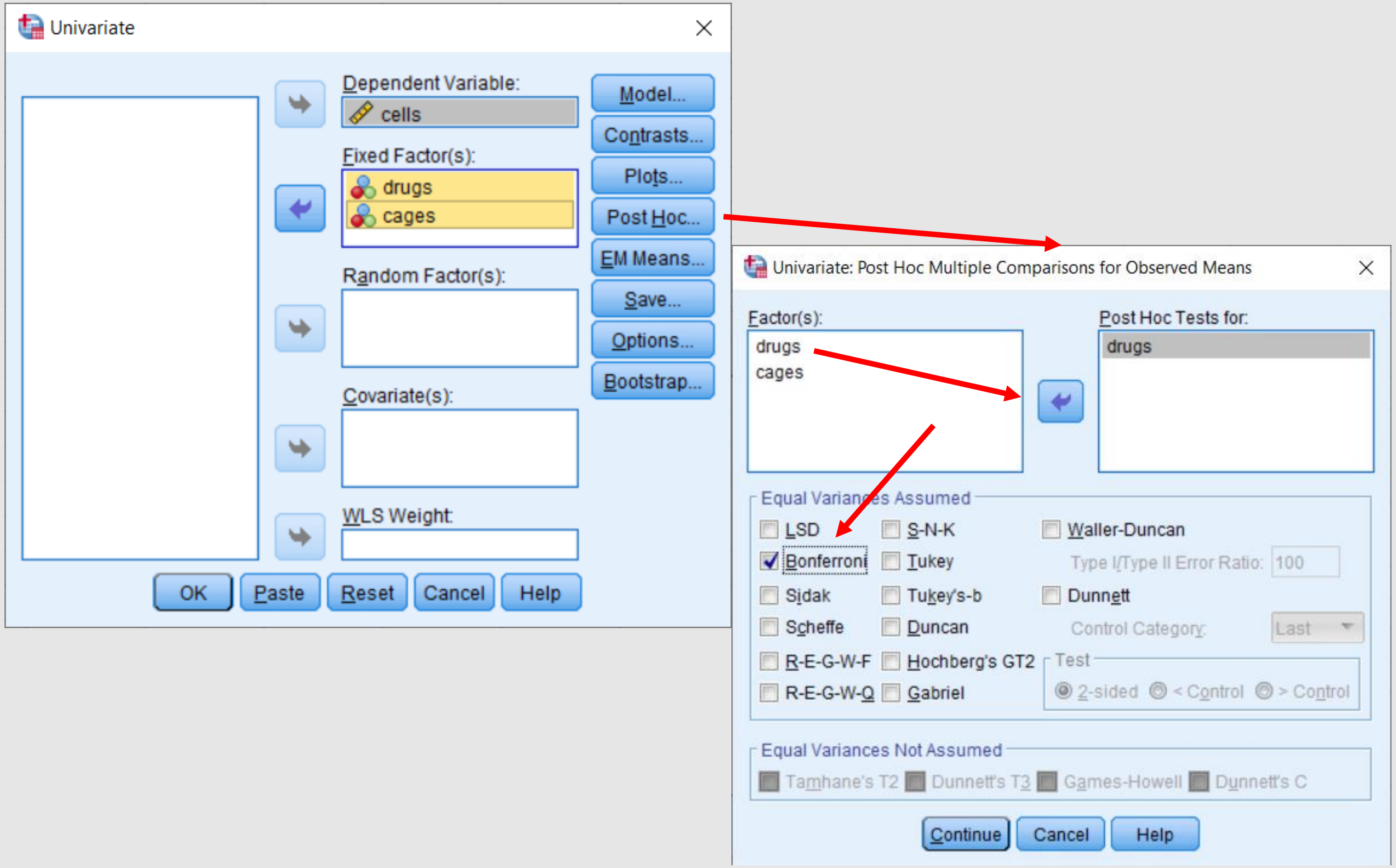
- In the **Univariate** window, drag the variable **cells** from the left box into the **Dependent Variable** field, and drag the variables **drugs** and **cages** from the left box into the **Fixed Factor(s):** box
- Select **Model**. In the **Univariate: Model** window, under the **Build terms** field, choose **Main effects**, and drag the variables **cages** and **drugs** from the **Factors & Covariates** box into the **Model** box. Click **Continue**





Post Hoc - Bonferroni

- Choose **Post Hoc**. In the window that appears, drag the variable drugs from the **Factor(s): box** into the **Post Hoc Test for:** box
- Then, select the **Bonferroni** option
- Press **Continue** and then **OK** to display the results of the analysis



Results and interpretation

- The actual results of the two-way anova, indicating whether the two independent variables (drugs and cages) are statistically significant, are shown in the **Tests of Between-Subjects Effects** table, as shown on the right
- We are interested in the **drugs** row
- This row informs us whether our independent variable (drug) has a statistically significant effect on the dependent variable, cells
- We see from the significance value (Sig.) that there was **a statistically significant difference in mean lymphocyte counts** between the drugs a, b, and c ($p<0.05$)

Tests of Between-Subjects Effects

Dependent Variable: cells

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	5.595[a]	5	1.119	24.415	.001
Intercept	424.830	1	424.830	9269.018	.000
drugs	1.365	2	.683	14.891	.005
cages	4.230	3	1.410	30.764	.000
Error	.275	6	.046		
Total	430.700	12			
Corrected Total	5.870	11			

a. R Squared = .953 (Adjusted R Squared = .914)

Post Hoc Tests

- The **Multiple Comparisons** table shows the individual comparisons between the drugs with **Bonferroni correction**
- From the results, we can see that there is a statistically significant difference between the drugs **1** and **2**, as the significance value (Sig.) is less than 0.05. The 95% confidence interval (CI) for the difference ranges from 0.2523 to 1.2477, and 0 is not included
- A significant difference was also found between drugs 1 and 3

Post Hoc Tests

Drugs

Multiple Comparisons

Dependent Variable: Cells

Bonferroni

(I) Drugs	(J) Drugs	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
1	2	.7500*	.15138	.008	.2523	1.2477
	3	.6750*	.15138	.013	.1773	1.1727
2	1	-.7500*	.15138	.008	-1.2477	-.2523
	3	-.0750	.15138	1.000	-.5727	.4227
3	1	-.6750*	.15138	.013	-1.1727	-.1773
	2	.0750	.15138	1.000	-.4227	.5727

Based on observed means.
The error term is Mean Square(Error) = .046.

*. The mean difference is significant at the 0.05 level.

Practical exercise

To study the effects of smoking and the type of exercise on an individual's physical condition, the time (in minutes) until the body reaches maximum oxygen consumption during exercise was measured. The following design was used for the study:

	Bike	*Running*	*Walking*
Non-smokers	12.4	22.7	16.7
Moderate smokers	10.8	20.2	15.7
Smokers	8.4	16.4	14.6

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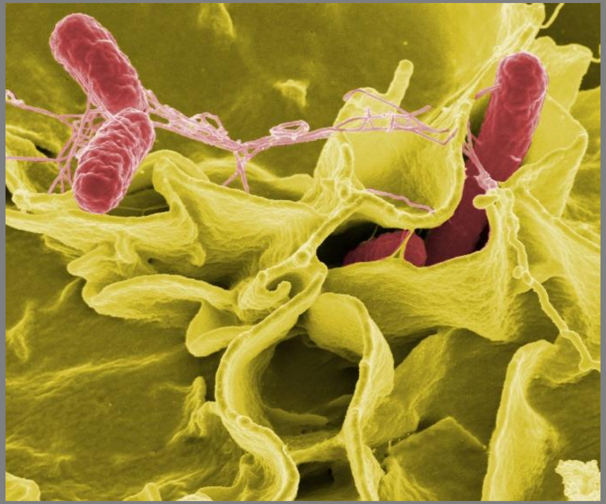
Effects of sorbic acid (sa) and water pH on Salmonella survival



Effects of sorbic acid (sa) and water pH on salmonella survival

To investigate the effects of sorbic acid (sa) and water pH on **salmonella** survival, we used three pH levels (5.0, 5.5, 6.0) and two levels of sorbic acid (0, 100 p.p.m.).

For each sa and pH combination, there were three observations.

One week later, the number of surviving **salmonella** was measured (log(density/ml)).

	ph		
sa	6.0 (1)	5.5 (2)	5.0 (3)
0 (1)	8.2 8.4 8.3	5.9 6.0 6.1	4.3 4.3 4.2
100 (2)	7.6 7.8 7.6	5.0 5.3 5.8	4.1 4.4 4.2

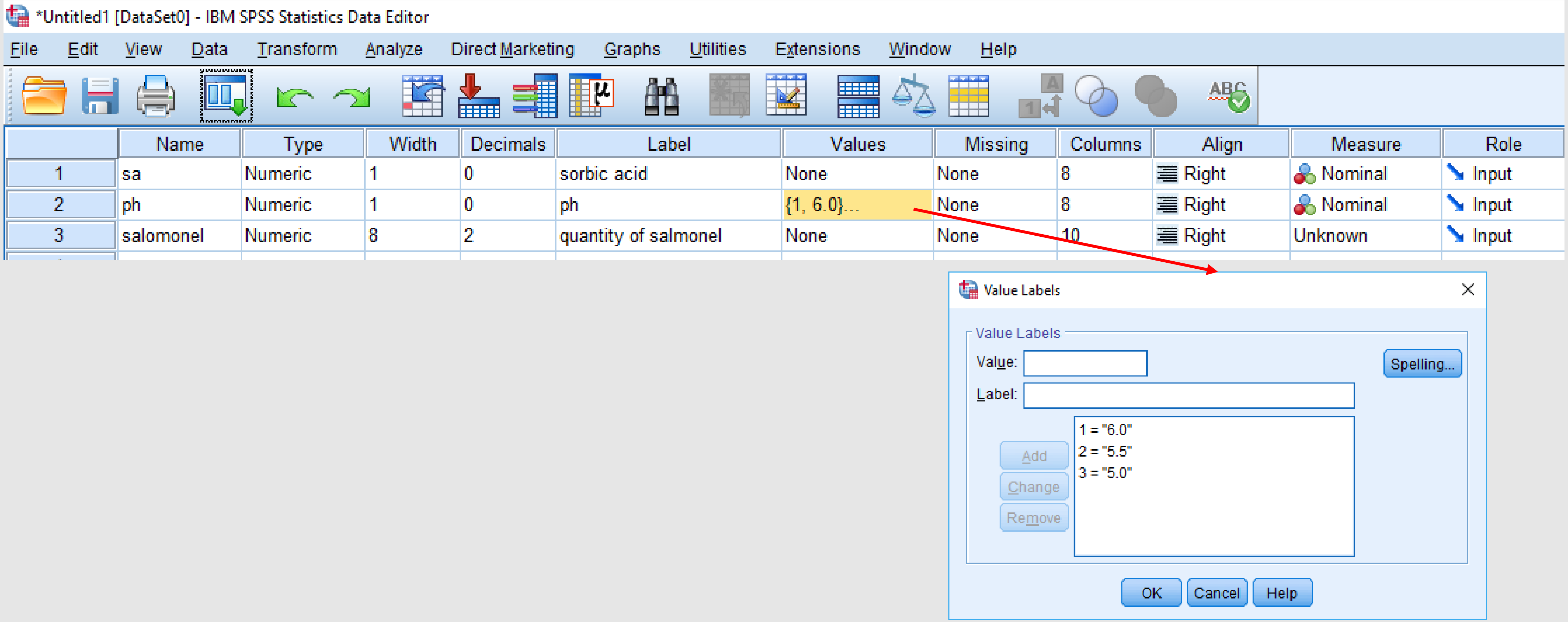
Entering data

Enter the data in the **Data View** and …

	sa	ph	salmonel
1	1	1	8.20
2	1	1	8.40
3	1	1	8.30
4	1	2	5.90
5	1	2	6.00
6	1	2	6.10
7	1	3	4.30
8	1	3	4.30
9	1	3	4.20
10	2	1	7.60
11	2	1	7.80
12	2	1	7.60
13	2	2	5.00
14	2	2	5.30
15	2	2	5.80
16	2	3	4.10
17	2	3	4.40
18	2	3	4.20

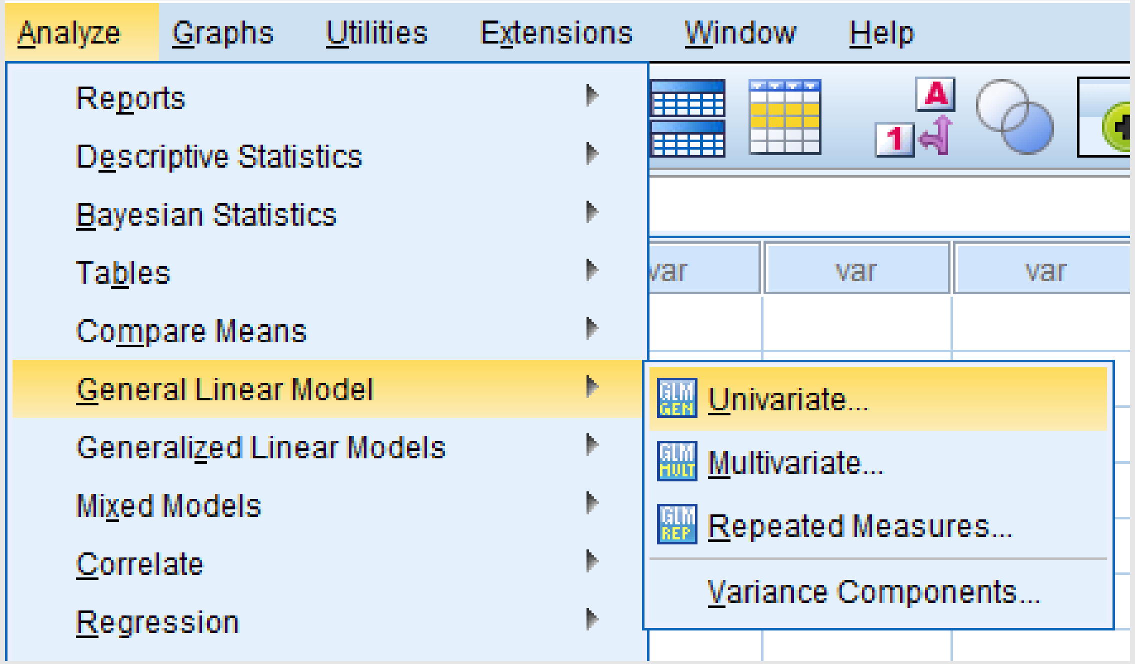
Defining variables

… define the variables in the **Variable View**

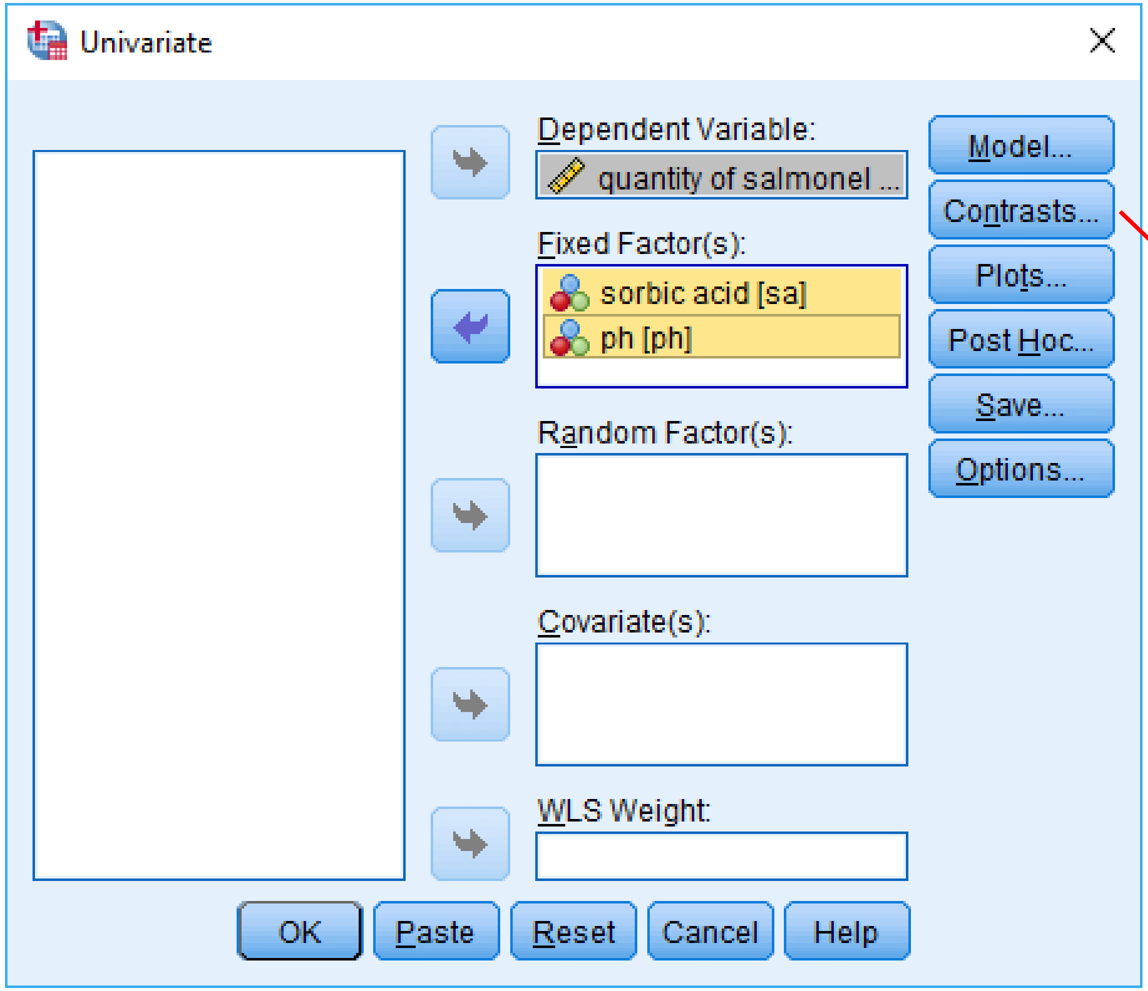


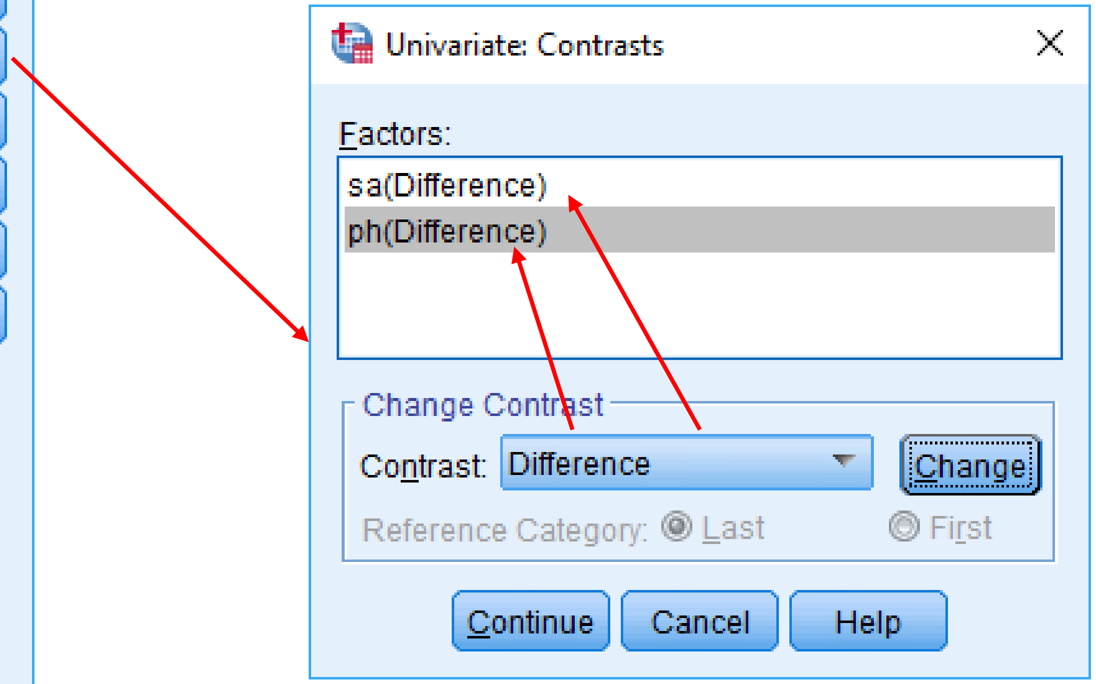
Running the two-way ANOVA test

To analyze the data, select **Analyze -> General Linear Model -> Univariate** from the menu

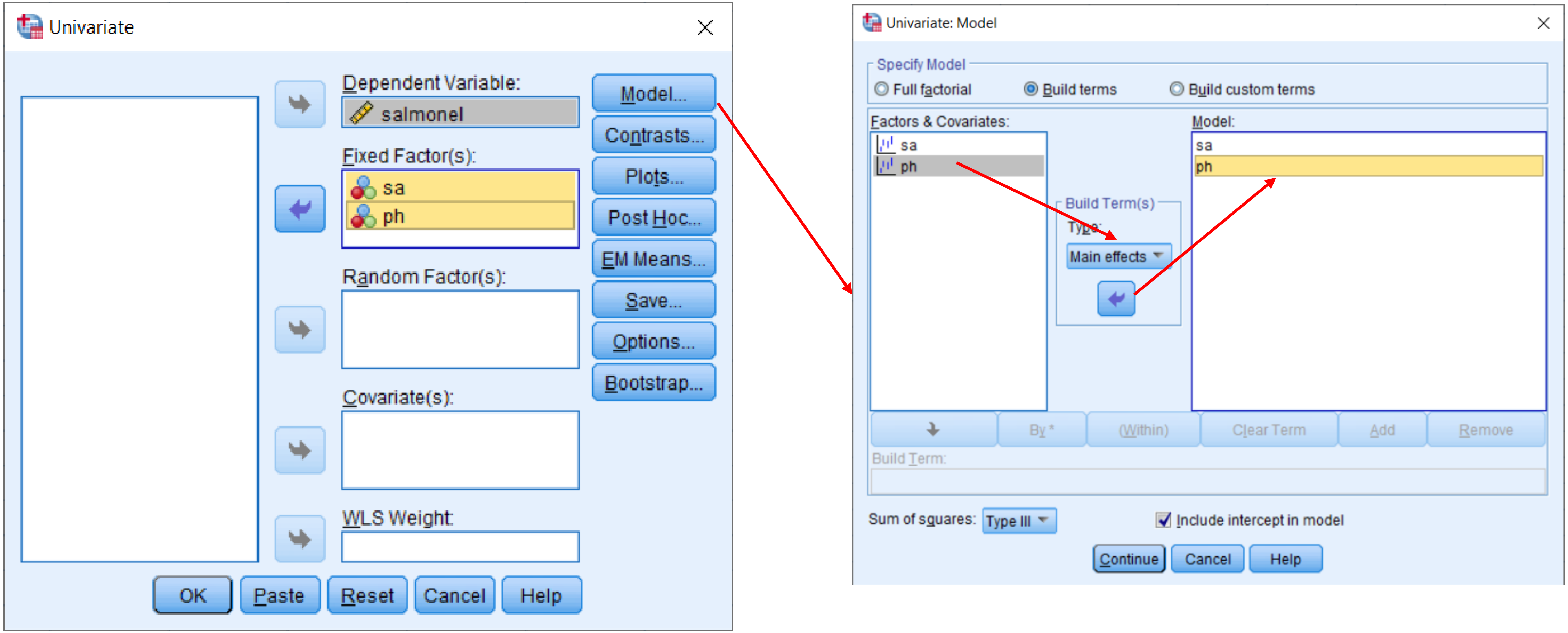


- In the **Univariate** window, drag the variable **salmonel** from the left box into the **Dependent Variable** field, and drag the variables **sa** and **ph** from the left box into the **Fixed Factor(s):** box
- Select **Contrasts….** In the **Univariate: Constrasts** window, under the **Factors:** box, select each variable one by one, choose **Difference** from the **Contrast** field, and click **Change**. Then, click **Continue**

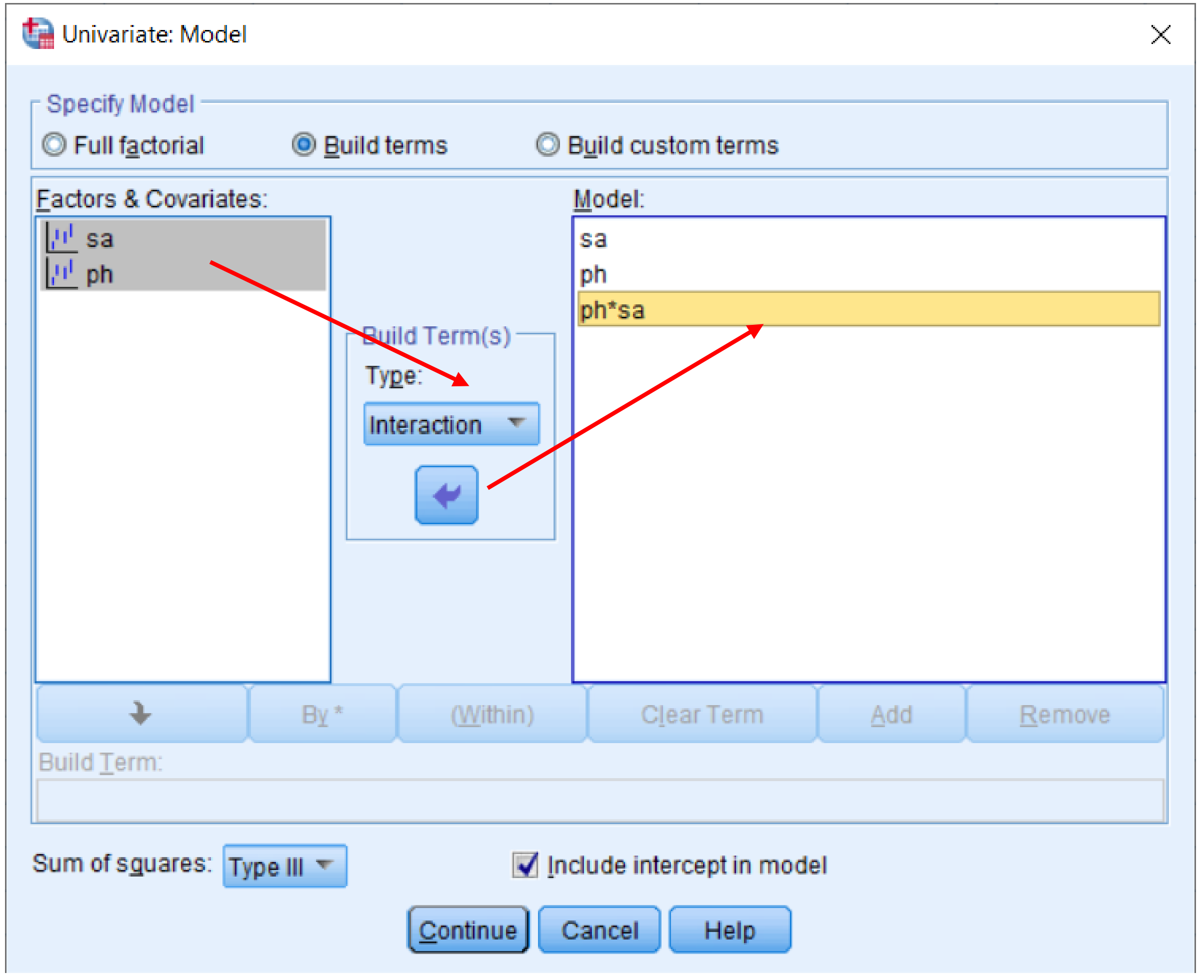




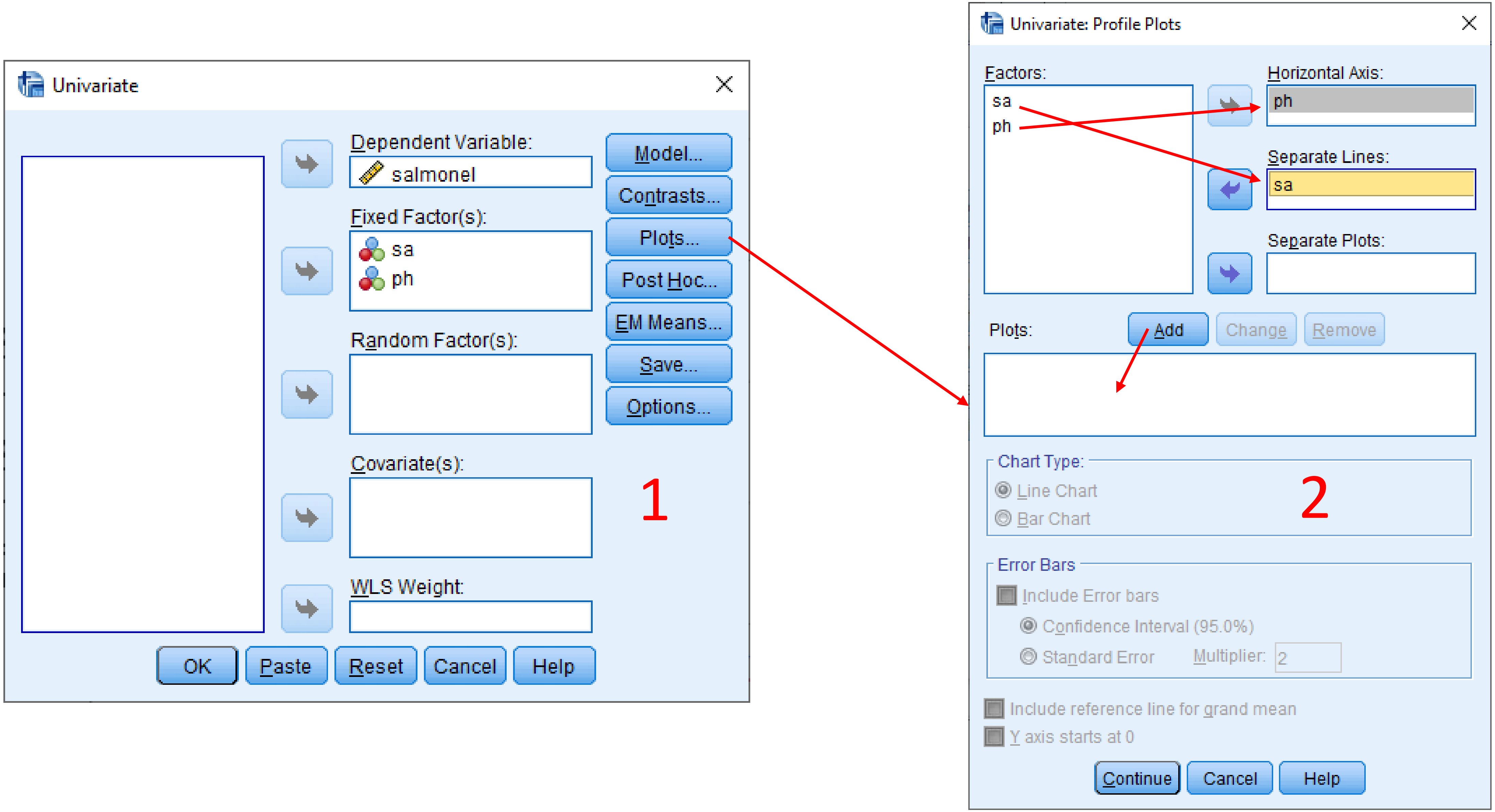
Select **Model**. In the **Univariate: Model** window, under the **Build terms** field, choose **Main effects**, and drag the variables **sa** and **ph** from the **Factors & Covariates** box into the **Model** box.

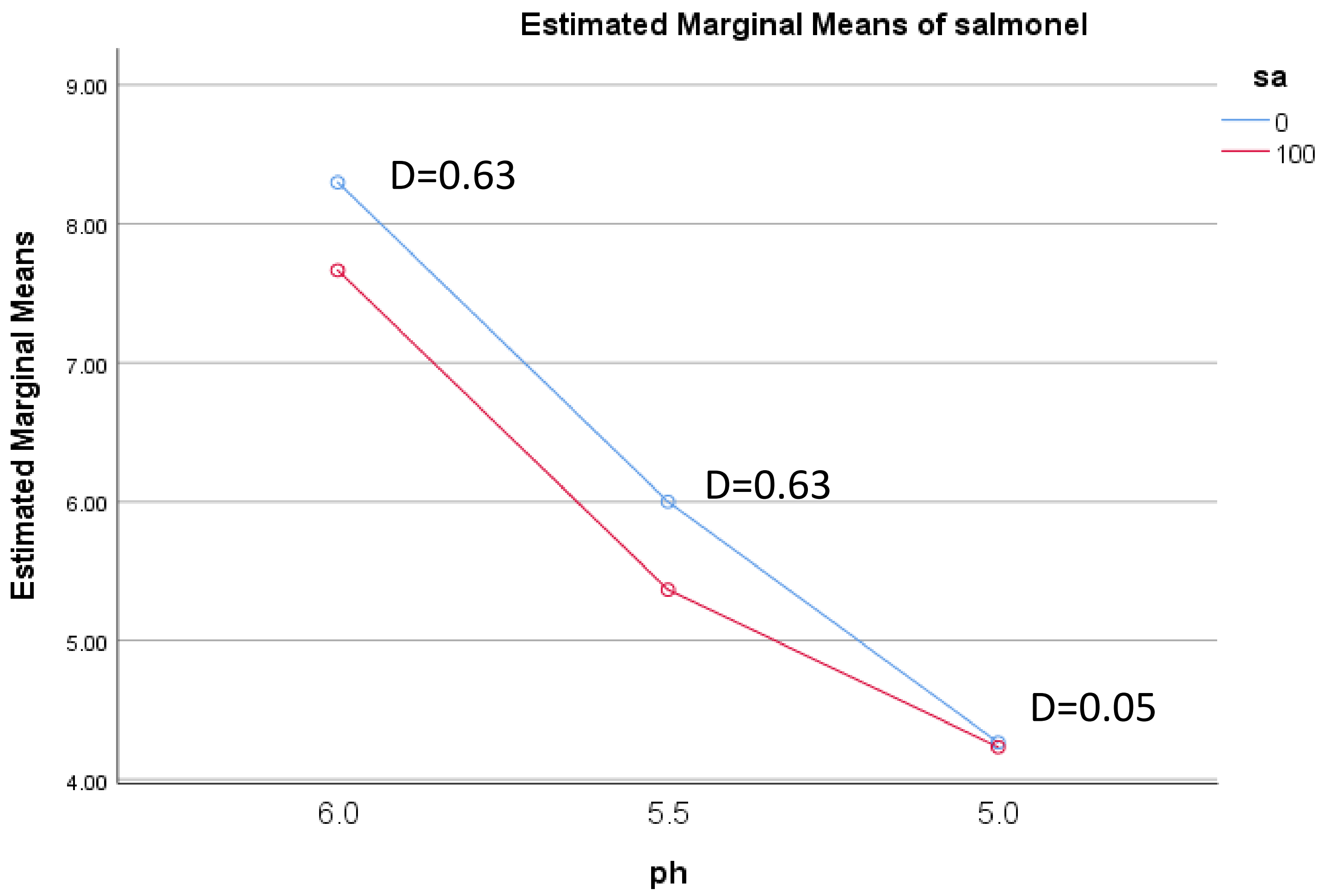


Under the **Build terms** field, choose **Interaction**, and drag both variables **sa** and **ph** from the **Factors & Covariates** box into the **Model** box. Then, click **Continue**.



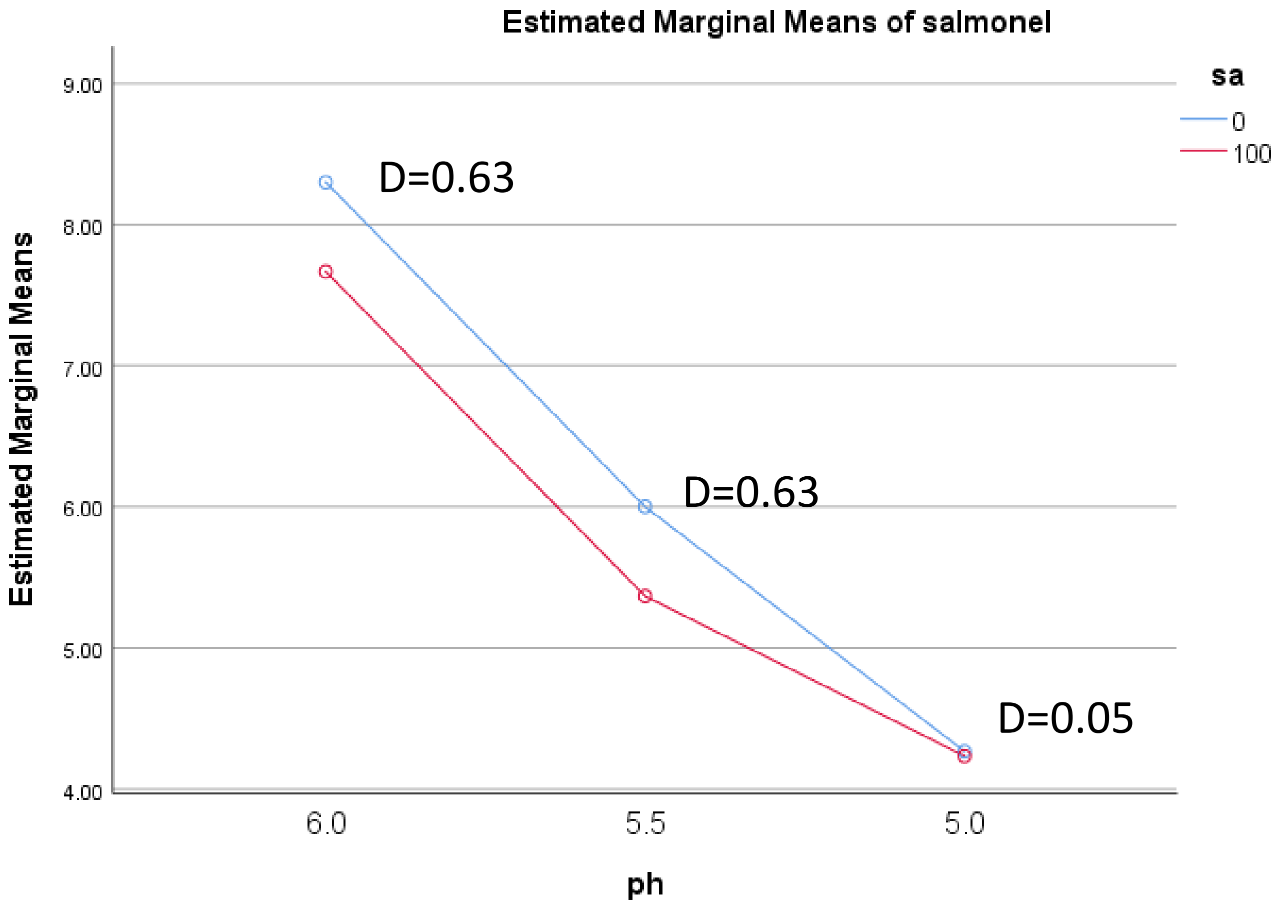
Select **Plots** (1) and adjust the settings as shown in the Image 2.





- For the pH levels 5.5 and 6.0, we observe a higher number of surviving **salmonella** at the 0 level of sa
- However, at the pH 5.0 level, we observed no difference in the number of surviving **salmonella** between the two levels of sa

Do the two levels of sa consistently increase **salmonella** survival across all pH levels?



The answer is: NO

This kind of situation is called an **interaction**

The presence of the **interaction** means that the difference (D) sa0−sa100 is not constant

In a **marginal means graph**, a general rule is to examine whether the lines converge or tend to converge, as this indicates a **statistically significant interaction**.

Results and interpretation

- **Interaction Effect (sa * pH)**
  - There was a statistically significant interaction between the effects of **sa** and **ph** on the number of salmonella survival, F (2, 12) = 4.836, p = 0.029.
- **Main Effect of Sorbic Acid**
  - The result shows that the effect of **sa** on the number of surviving **Salmonella** is statistically significant (p<0.001)
- **Main Effect of pH:**
  - The result shows that the effect of **ph** on the number of surviving **Salmonella** is statistically significant (p<0.001)

Tests of Between-Subjects Effects

Dependent Variable: quantity of salmonel

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	43.769[a]	5	8.754	235.179	.000
Intercept	642.014	1	642.014	17248.134	.000
sa	.845	1	.845	22.701	.000
ph	42.564	2	21.282	571.761	.000
sa * ph	.360	2	.180	4.836	.029
Error	.447	12	.037		
Total	686.230	18			
Corrected Total	44.216	17			

a. R Squared = .990 (Adjusted R Squared = .986)

Note: When there is a **statistically significant interaction**, reporting the **main effects** can be misleading. Therefore, you will need to report the **simple main effects** (advance course).

Practical exercise

The expiratory flow (PEmax) of patients with cystic fibrosis is as follows:

PEmax	BMP	sex
95	high	M
85	low	F
100	low	M
85	low	F
95	high	M
80	low	F
110	high	M
85	low	F
105	high	M
90	high	F
100	low	M

Is there difference in PEmax between the two genders? Is there interaction between BMP and sex in terms of PEmax?