DATA EVALUATION RECORD
ACUTE ORAL TOXICITY TEST WITH THE HONEY BEE
NON-GUIDELINE

1. **CHEMICAL**: Spiromesifen PC Code No.: 024875

2. **TEST MATERIAL**: BSN 2060 240 SC Purity: 24.6%

3. **CITATION**

Author: G. Sterk

Title: Toxicity of AMS 13839 240 SC on Adult Workers and Larvae of *Bombus terrestris* (L.).

Study Completion Date: May 23, 2001

Laboratory: Biobest N.V.
Ilse Velden 18
B-2260 Westerlo

Sponsor: Bayer CropScience AG
Monheim, Germany

Laboratory Report ID: BOMBTE 2000/04A

DP Barcode: D440328

MRID No.: 497173-02

4. **REVIEWED BY**: Thomas Steeger, Senior Science Advisor, ERB4, Environmental Fate and Effects Division, Office of Pesticide Programs, EPA

Signature: THOMAS STEEGER Digitally signed by THOMAS STEEGER Date: 2017.06.06 15:20:04 -04'00' **Date:** 06/06/2017

REVIEWED BY: Cameron Douglass, Biologist, ERB4, Environmental Fate and Effects Division, Office of Pesticide Programs, EPA

Signature: Cameron Douglass CAMERON DOUGLASS 2017.06.06 15:13:34 -04'00' **Date:** 06/06/2017

5. **STUDY PARAMETERS**:

Age of Test Organisms at Test Initiation: Adult [female] worker bees, male [sterile] larvae and pupae
Exposure Duration: Approximately 40 days

6. **CONCLUSIONS**: The toxicity of the spiromesifen formulated end product BSN 2060 240 SC (AMS 13939; 24.6% active ingredient) was tested with bumble bee (*Bombus terrestris*) using spike pollen (0.06%)

and sucrose solution (0.06%). The study included a negative control and two reference toxicant controls, *i.e.*, Dimethoate™ 100 EC (0.4%) and Nomolt™ (teflubenzuron) (0.10%). Treated or untreated diets were replenished every 10 days four consecutive times. In each treatment group there were 5 reps per treatment group with 10 young adult females per replicate. At the end of the study the mean (± standard deviation) number of surviving female workers was 44±1.30, 46±0.84, 0, and 48±0.55 in the negative control, spiromesifen-treated, dimethoate and tebufenzuron reference controls, respectively. There was no statistical difference between the negative control (314±14.74) and the spiromesifen-treated colonies (207±15.66) in terms of the number of hatched drones. There was a significant difference ($p<0.05$) in the total number of dead larvae in the spiromesifen-treated colonies ((total=99) and the negative control (total=203), the difference indicates that loss rates were higher in the negative control; therefore, this does not represent a treatment-related adverse effect. Colonies exposed to the dimethoate reference toxicant exhibited a 100% loss of the adult bees and as a consequence no males were produced. According the study authors analysis, exposure of colonies to tebufenzuron resulted in no larvae and no drones.

This is a non-guideline study in which exposure levels were not verified analytically; therefore, the actual exposure levels of bees is uncertain. However, given the responses to both positive reference toxicants, the data suggest that bees were exposed and that the study design was capable of detecting treatment effects.

This is a nonguideline study and given the uncertainty regarding actual exposure levels, the study is classified as supplemental. Bumble bee microcolonies exposed to nominal spiromesifen residues of 0.06% in their diet (pollen and sugar solution) did not appear to be adversely affected; therefore, the NOAEC is 0.06%

7. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: non-guideline study; exposure concentrations in sugar solutions and pollen not verified analytically; consumption rates of pollen and sugar solutions not reported.

C. Reparability: N/A

8. GUIDELINE DEVIATIONS:

This is a nonguideline study. Study was conducted with some study design elements outlined in published study by De Wael *et al.* 1995[1].

9. SUBMISSION PURPOSE: To test the toxicity of the insecticide AMS 13893 (BSN 2060 240SC) to female worker bees and newborn larva (sterile male drones) via consumption of spiked pollen and sugar solution.

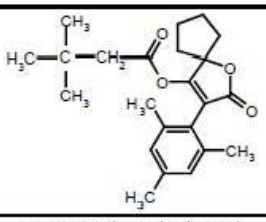
[1] De Wael, L., M. De Greef, and O. Van Laere. 1995. Toxicity of pyriproxifen and fenoxycarb to bumble bee brood using a new method for testing insect growth regulators. Journal of Apiculture Research 34(1): 3 – 8.

10. MATERIALS AND METHODS:

A. Test Material

Spiromesifen
Synonyms: BSN 2060 240 SC
IUPAC name: 3-mesityl-2-oxo-1-oxaspiro[4.4]non-3-en-4-yl 3,3-dimethylbutyrate
CAS name: 2-oxo-3-(2,4,6-trimethylphenyl)-1-oxaspiro[4.4]non-3-en-4-yl 3,3-dimethylbutanoate
CAS no.: 283594-90-1
Description: Not reported
Batch no.: Aufbau-Nr. 0220321FL-Nr06018/0028(001)
Purity: 24.6%
Storage: Not reported

Physicochemical properties of fenhexamid.

Parameter	Values	Comments
Water solubility at 25°C mg/L	0.13	USEPA 2015[2]
Vapor pressure at 25°C	7.5×10^{-8} torr	
Structure		
K_{oc}	45,296 (sandy loam) 79,472 (silt loam) 46,816 (loam) 106,000 (clay loam)	
Log K_{ow}	3.6×10^{4}	

[2] USEPA. 2015. Registration Review: Draft Problem Formulation for Environmental Fate, Ecological Risk, Endangered Species, and Human Health Drinking Water Exposure Assessments for Spiromesifen. DP Barcode D422437.

B. Test Organisms

Guideline Criteria	Reported Information
Species: Honey Bee (*Apis mellifera L.*)	Bumble bee (*Bombus terrestris*)
Age at beginning of test: Worker bees of uniform age.	Young [female] worker bees
Source:	BioBest
Were bees from diseased-free colonies?	Not reported.
Were bees kept in conditions conforming to proper cultural practices?	Not reported.

C. Test System

Guideline Criteria	Reported Information
Plot size:	N/A
Test Chambers:	Plastic hives
Temperature during exposure:	29°C
Relative humidity during exposure:	Not reported
Lighting:	Conducted in near darkness (except during 30 minutes each day for observations and water/sucrose renewals).
Feeding:	One week prior to study, colonies were fed untreated sucrose and pollen. During study, colonies were fed treated or untreated diet

D. Test Design

Guideline Criteria	Reported Information
Maximum proposed label rate:	Not reported.

Test application rates: The test material should be applied at the maximum proposed label rate.	Nominal: 0.06% formulation in sugar (Biogluc[3]); 0.06% formulation in pollen Measured: not reported
Dose Preparation:	Test solutions were prepared by mixing the test compound with sugar water until the required dose rate (0.06%). Spiked pollen prepared by spraying pollen with 20 – 25 mL of 0.06% spiked sugar solution; pollen was allowed to dry then "small balls were made of the spiked pollen and given to the hives)
Crop species:	N/A
Number of bees exposed:	Five (5) replicates with ten (10) young female worker bees of the same age per replicate allowed to produce brood for one week. Bees provided pollen and sugar solution during this week.
Residue aging intervals:	N/A
Application methods:	350 mL treated solution provided to bees; bees reported to consume approximately 50 mL/week; spike sugar solution refreshed every 10 days for four (4) consecutive times.
Other experimental design information:	Assessments conducted "normally almost twice a week." Assessments consisted of counting the number of dead worker bees, dead larvae, and newly hatched young males (drones). Dead larvae/drones were removed from hive. Behavioral changes were also recorded.
Were bees randomly or impartially assigned to test groups?	Not recorded
Control(s):	Sugar solution alone and water-treated pollen.
Exposure period:	40 days

[3] Biogluc Yellow reported to consist of saccharose (34%), dextrose (33%), fructose (33%), canary yellow (E102), natrium mentylparahydroxybenzoate (E219) and sodium mentyl parahydroxide benzoate.

Positive Control, (if any):	Dimethoate™ 100 EC (0.4%) used as a toxic standard for the adults; Nomolt™ (teflubenzuron) (0.10%) used as a toxic standard to larvae

11. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Were there no observed adverse effects on bees at the greatest aging interval?	NA
Control mortality:	There was 12% female worker bee mortality in the negative control; 100% mortality in the dimethoate reference control; and, 4% mortality in the teflubenzuron reference control
Were raw data included?	Yes
Were signs of toxicity (if any) described?	No behavioral abnormalities were reported.
Were residues measured? (optional)	No

Mortality and Observations:

The study authors reported that mean (± standard deviation) number of female worker bees at the end of the study was 44±1.30, 46±0.84, 0, and 48±0.55 in the negative control, spiromesifen-treated, dimethoate and tebufenzuron reference controls, respectively (**Table 1**). There was a statistically significant difference ($p<0.05$) in survival between the negative control and the dimethoate-treated colonies.

According to the study authors, there was a significant difference ($p<0.05$) between the negative control (314±14.74) and the spiromesifen-treated colonies (207±15.66) in terms of the number of hatched drones. No drones were produced in either the dimethoate treated colonies (where all of the female worker bees died) or in the tebufenzuron-treated colonies.

The study authors concluded that AMS 13839 had no toxic effect on adult female worker bees and "very little effect" on young larvae and only a "slight negative effect on number of newborn drones: They noted that the number of dead larvae was higher in the negative controls than in the spiromesifen-treated colonies. According to the study authors, there were no changes in behavior of bees in colonies treated with spiromesifen.

Table 1. Summary of bumble bee (*Bombus terrestris*) survival and number of hatched drones following 40-day exposure to formulated spiromesifen-spiked (0.06%) pollen and sugar solution.

Experimental Group (Nominal, µg ai/bee)	Number Exposed	Mortality		Hatched Drones
		Workers	Larvae	
Negative Control	50	6	203	314
Dimethoate Control	50	50	26	0
Teflubenzuron Control	50	2	96	0
Spiromesifen	50	4	99	207

12. REVIEWER'S COMMENTS:

Exposure concentrations in the pollen and sugar solutions were not verified analytically. Although the study authors noted that roughly 50 mL of sugar solution was consumed per week, that actual volume of sugar solution and quantity of pollen consumed is not reported; therefore, actual exposure levels have not been documented.

Other than noting that the colonies were made of plastic, no additional information is provided on the colonies.

The reviewer assumed that the pollen had been treated with the spiked Biogluc solution. If this is the case, then the negative control should have included pollen spiked with untreated Biogluc solution rather than water alone.

The conditions under which the colonies were maintained are not specified; therefore, there is uncertainty regarding the extent to which the treatment and control colonies were kept separate and whether alternative sources of pollen/sugar solution may have been available. The source of the pollen is not specified and there is uncertainty regarding the extent to which pesticides residues may have been in the pollen.

The study does not indicate why/how the spiromesifen exposure level (0.06%) was selected.

Data were analyzed using SAS® (SAS Institute, Cary, NC; release 9.0) using both parametric (Proc ANOVA with Bonferroni and t- multiple means tests) and nonparametric (Proc NPAR1WAY) Wilcoxon procedures. Statistical output in **Appendix 1**.

Reviewer calculated total numbers of dead adult worker bees, number of males produced, and number of dead larvae were consistent with those reported by the study author (**Table 1**). By the end of the study, there were no statistical differences between the negative and spiromesifen-treated colonies in the number of dead female worker bees. Although there were fewer males produced in spiromesifen-treated colonies (total=207) than the negative control (total=314), the difference was not statistically significant. Based on parametric tests, there is a marginally significant (p=0.0507) difference between spiromesifen-treated colonies (total=99) and the negative control (total=203) in the number of larvae killed; based on nonparametric tests, there is a significant difference between the two (p=0.0278); however, since fewer larvae died in the spiromesifen-treated colonies, the effect is not considered adverse.

Based on the results of this study, exposure of bumble bee microcolonies to formulated spiromesifien at nominal levels of 0.06% in their diet over a period of roughly 40 days did not appear to significantly impact the number of adult female worker bees, number of larvae produced. The number of males produced was significantly higher in spiromesifen-treated colonies; however, this effect is not considered adverse. Colonies exposed to the dimethoate reference toxicant exhibited a 100% loss of the adult bees and as a consequence no males were produced. According the study authors analysis, exposure of colonies to tebufenzuron resulted in no larvae and no drones.

This is a non-guideline study in which exposure levels were not verified analytically; therefore, the actual exposure levels of bees is uncertain. However, given the responses to both positive reference toxicants, the data suggest that bees were exposed and that the study design was capable of detecting treatment effects. Bumble bee microcolonies exposed to nominal spiromesifen residues of 0.06% in their diet (pollen and sugar solution) did not appear to be adversely affected; therefore, the NOAEC is 0.06%.

Appendix 1 SAS Output

COMPARISON OF NUMBER OF DEAD FEMALE ADULTS IN CONTROL VERSUS SPIROMESIFEN-TREATED COLONIES

The ANOVA Procedure

Class Level Information

Class	Levels	Values
TREAT	2	c s

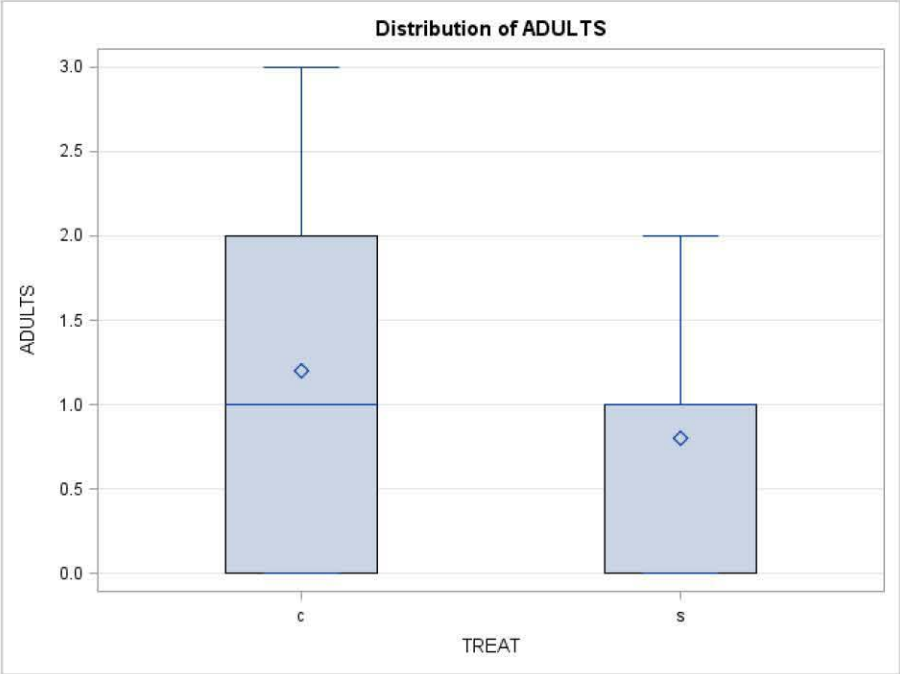
Number of Observations Read 10

Dependent Variable: ADULTS

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.40000000	0.40000000	0.33	0.5796
Error	8	9.60000000	1.20000000		
Corrected Total	9	10.00000000			

R-Square	Coeff Var	Root MSE	ADULTS Mean
0.040000	109.5445	1.095445	1.000000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	1	0.40000000	0.40000000	0.33	0.5796



COMPARISON OF NUMBER OF DEAD FEMALE ADULTS IN CONTROL VERSUS SPIROMESIFEN-TREATED COLONIES

The ANOVA Procedure

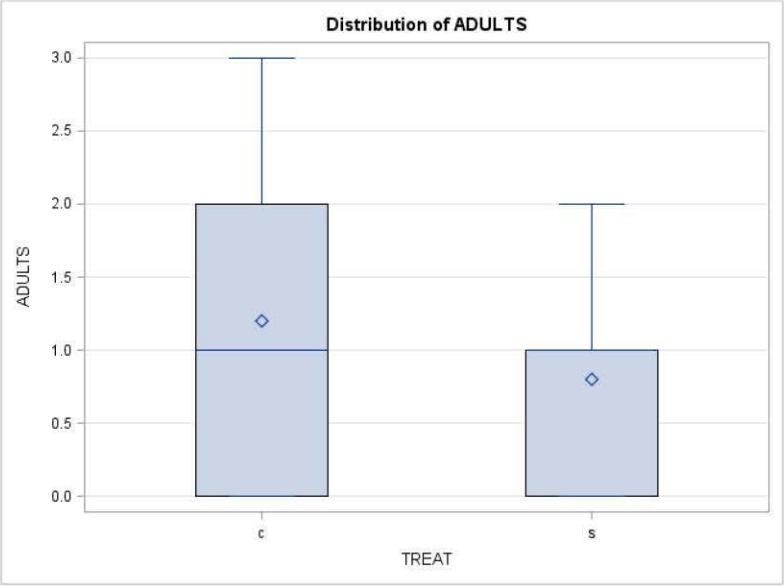
Bonferroni (Dunn) t Tests for ADULTS

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

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	1.2
Critical Value of t	2.30600
Minimum Significant Difference	1.5976

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	TREAT
A	1.2000	5	c
A			
A	0.8000	5	s



COMPARISON OF NUMBER OF DEAD FEMALE ADULTS IN CONTROL VERSUS SPIROMESIFEN-TREATED COLONIES

The ANOVA Procedure

t Tests (LSD) for ADULTS

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	1.2
Critical Value of t	2.30600
Least Significant Difference	1.5976

Means with the same letter are not significantly different.

t Grouping	Mean	N	TREAT
A	1.2000	5	c
A			
A	0.8000	5	s

NONPARAMETRIC COMPARISON OF NUMBER OF FEMALE ADULTS IN CONTROL VS SPIROMESIFEN-TREATED COLONIES

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable ADULTS Classified by Variable TREAT

TREAT	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
c	5	29.50	27.50	4.564355	5.90
s	5	25.50	27.50	4.564355	5.10

Average scores were used for ties.

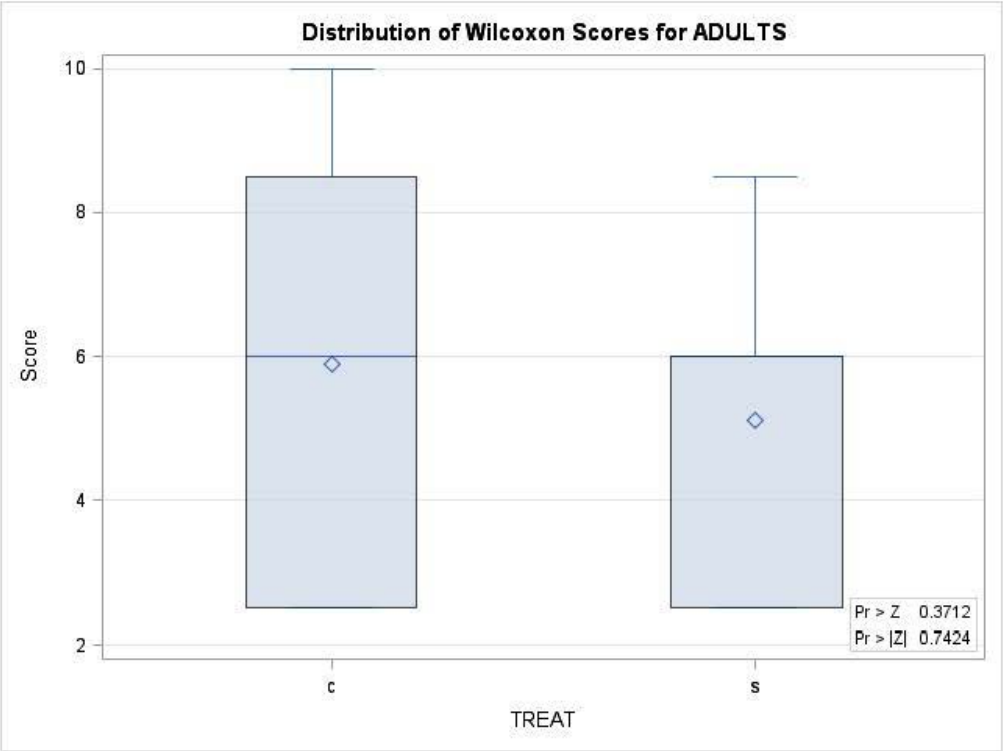
Wilcoxon Two-Sample Test

Statistic	29.5000
Normal Approximation	
Z	0.3286
One-Sided Pr > Z	0.3712
Two-Sided Pr > |Z|	0.7424
t Approximation	
One-Sided Pr > Z	0.3750
Two-Sided Pr > |Z|	0.7500

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	0.1920
DF	1
Pr > Chi-Square	0.6613



COMPARISON OF NUMBER OF MALES IN CONTROL VERSUS SPIROMESIFEN-TREATED COLONIES

The ANOVA Procedure

Class Level Information

Class	Levels	Values
TREAT	2	c s

Number of Observations Read 10

The ANOVA Procedure

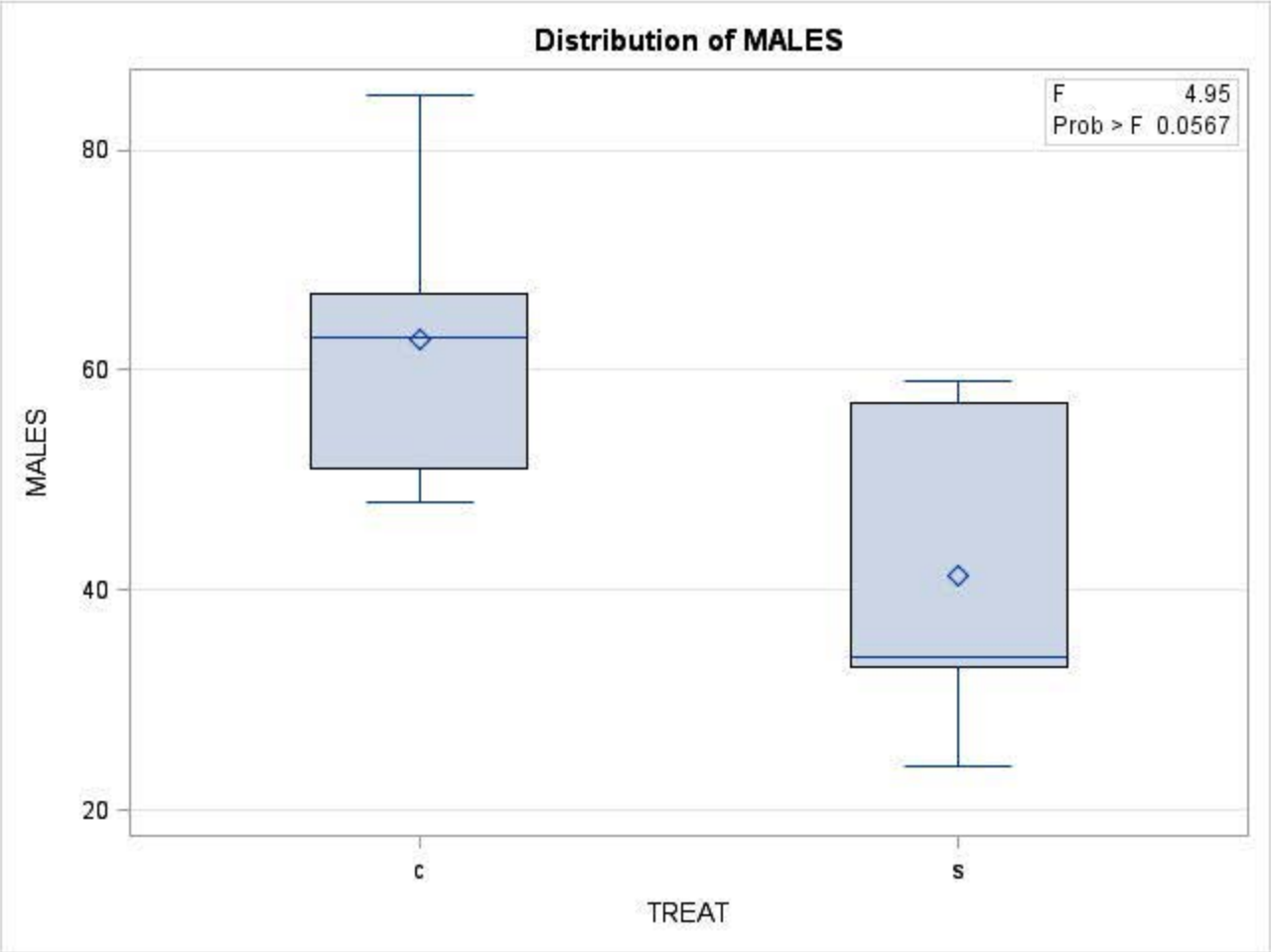
Dependent Variable: MALES

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1144.900000	1144.900000	4.95	0.0567
Error	8	1850.000000	231.250000		
Corrected Total	9	2994.900000			

R-Square	Coeff Var	Root MSE	MALES Mean

R-Square	Coeff Var	Root MSE	MALES Mean
0.382283	29.18792	15.20691	52.10000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	1	1144.900000	1144.900000	4.95	0.0567



COMPARISON OF NUMBER OF MALES IN CONTROL VERSUS SPIROMESIFEN-TREATED COLONIES

The ANOVA Procedure

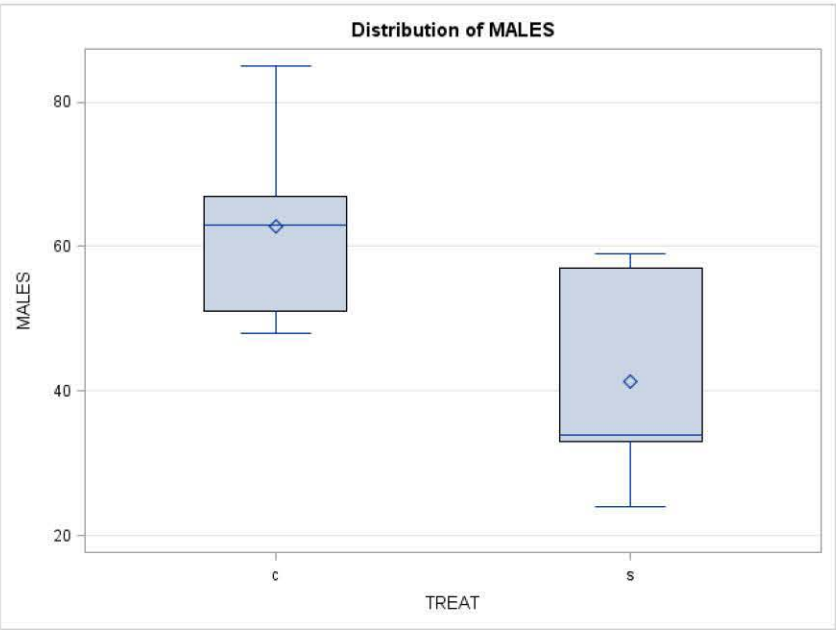
Bonferroni (Dunn) t Tests for MALES

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

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	231.25
Critical Value of t	2.30600
Minimum Significant Difference	22.178

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	TREAT
A	62.800	5	c
A			
A	41.400	5	s



COMPARISON OF NUMBER OF MALES IN CONTROL VERSUS SPIROMESIFEN-TREATED COLONIES

The ANOVA Procedure

t Tests (LSD) for MALES

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	231.25
Critical Value of t	2.30600
Least Significant Difference	22.178

Means with the same letter are not significantly different.

t Grouping	Mean	N	TREAT
A	62.800	5	c
A			
A	41.400	5	s

NONPARAMETRIC COMPARISON OF NUMBER OF MALES IN CONTROL VS SPIROMESIFEN-TREATED COLONIES

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable MALES Classified by Variable TREAT

TREAT	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
c	5	36.0	27.50	4.787136	7.20
s	5	19.0	27.50	4.787136	3.80

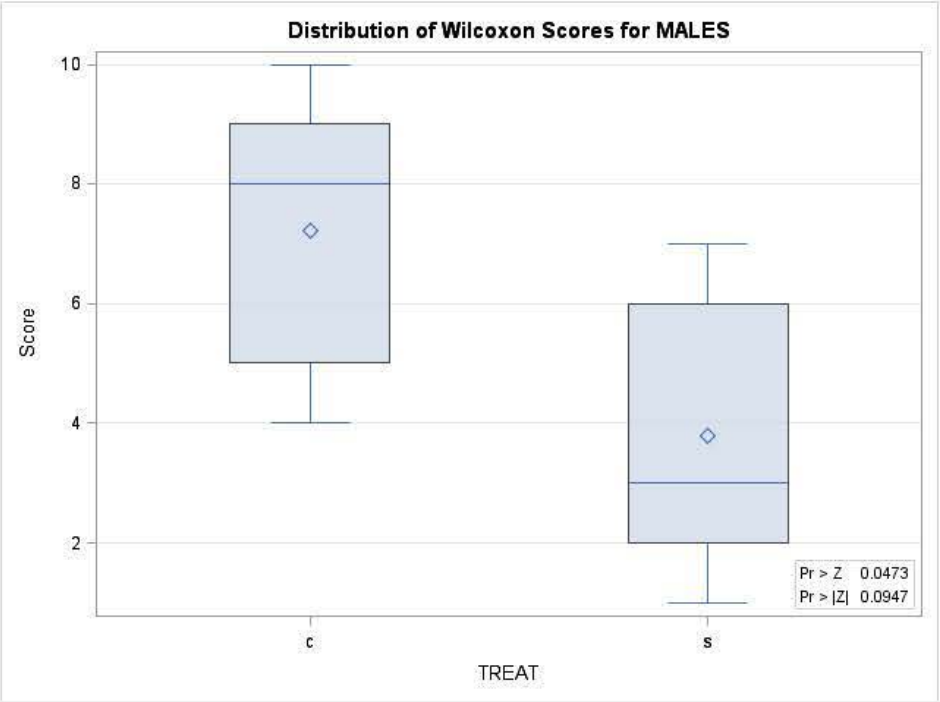
Wilcoxon Two-Sample Test

Statistic	36.0000
Normal Approximation	
Z	1.6711
One-Sided Pr > Z	0.0473
Two-Sided Pr > |Z|	0.0947
t Approximation	
One-Sided Pr > Z	0.0645
Two-Sided Pr > |Z|	0.1290

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	3.1527
DF	1
Pr > Chi-Square	0.0758


Distribution of Wilcoxon Scores for MALES
Score
10
8
6
4
2
c
s
TREAT
Pr > Z 0.0473
Pr > |Z| 0.0947

COMPARISON OF NUMBER OF LARVAE IN CONTROL VERSUS SPIROMESIFEN-TREATED COLONIES

The ANOVA Procedure

Class Level Information

Class	Levels	Values
TREAT	2	c s

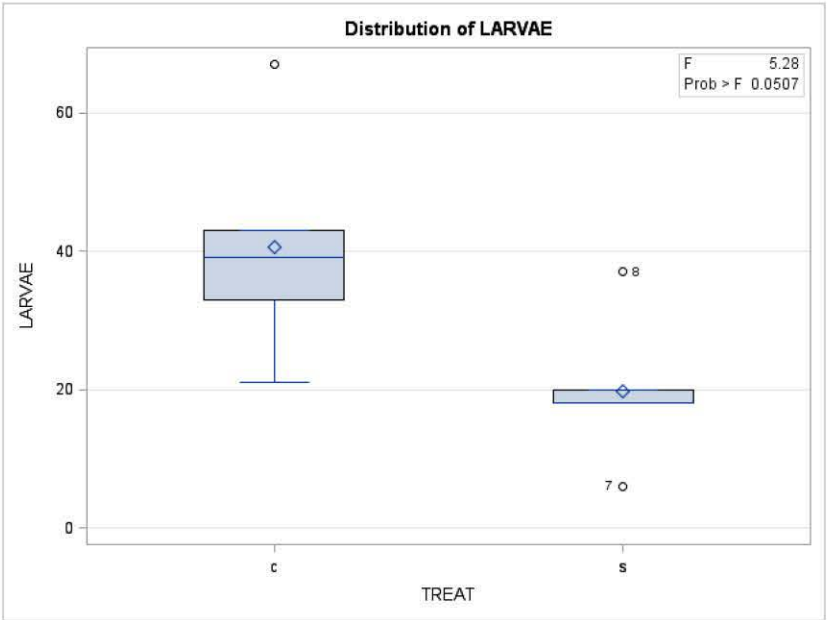
Number of Observations Read 10

The ANOVA Procedure
Dependent Variable: LARVAE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	1081.600000	1081.600000	5.28	0.0507
Error	8	1640.000000	205.000000		
Corrected Total	9	2721.600000			

R-Square	Coeff Var	Root MSE	LARVAE Mean
0.397413	47.41000	14.31782	30.20000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TREAT	1	1081.600000	1081.600000	5.28	0.0507



COMPARISON OF NUMBER OF LARVAE IN CONTROL VERSUS SPIROMESIFEN-TREATED COLONIES

The ANOVA Procedure

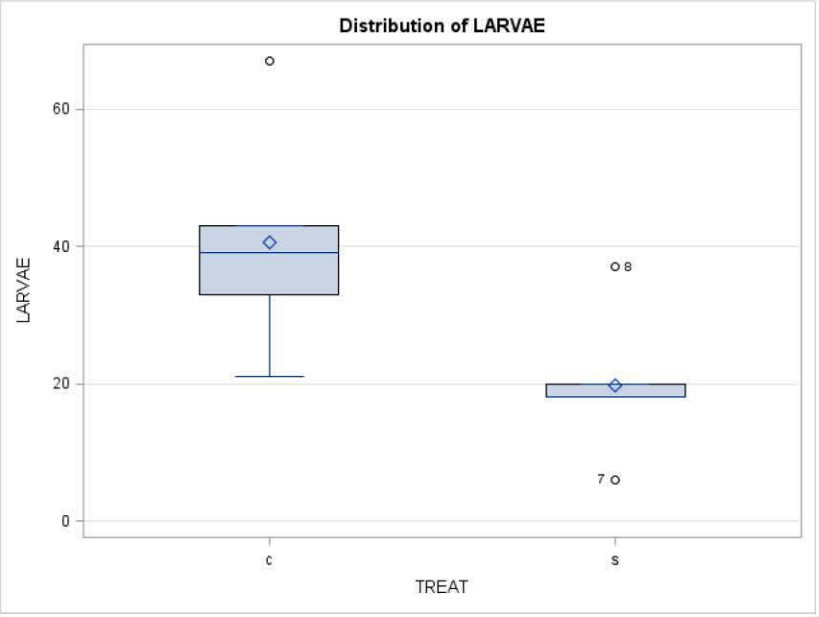
Bonferroni (Dunn) t Tests for LARVAE

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

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	205
Critical Value of t	2.30600
Minimum Significant Difference	20.882

Means with the same letter are not significantly different.

Bon Grouping	Mean	N	TREAT
A	40.600	5	c
A			
A	19.800	5	s



COMPARISON OF NUMBER OF LARVAE IN CONTROL VERSUS SPIROMESIFEN-TREATED COLONIES

The ANOVA Procedure

t Tests (LSD) for LARVAE

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	205
Critical Value of t	2.30600
Least Significant Difference	20.882

Means with the same letter are not significantly different.

t Grouping	Mean	N	TREAT
A	40.600	5	c
A			
A	19.800	5	s

NONPARAMETRIC COMPARISON OF NUMBER OF FEMALE LARVAE IN CONTROL VS SPIROMESIFEN-TREATED COLONIES

The NPAR1WAY Procedure

Wilcoxon Scores (Rank Sums) for Variable LARVAE Classified by Variable TREAT

TREAT	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
c	5	38.0	27.50	4.772607	7.60
s	5	17.0	27.50	4.772607	3.40

Average scores were used for ties.

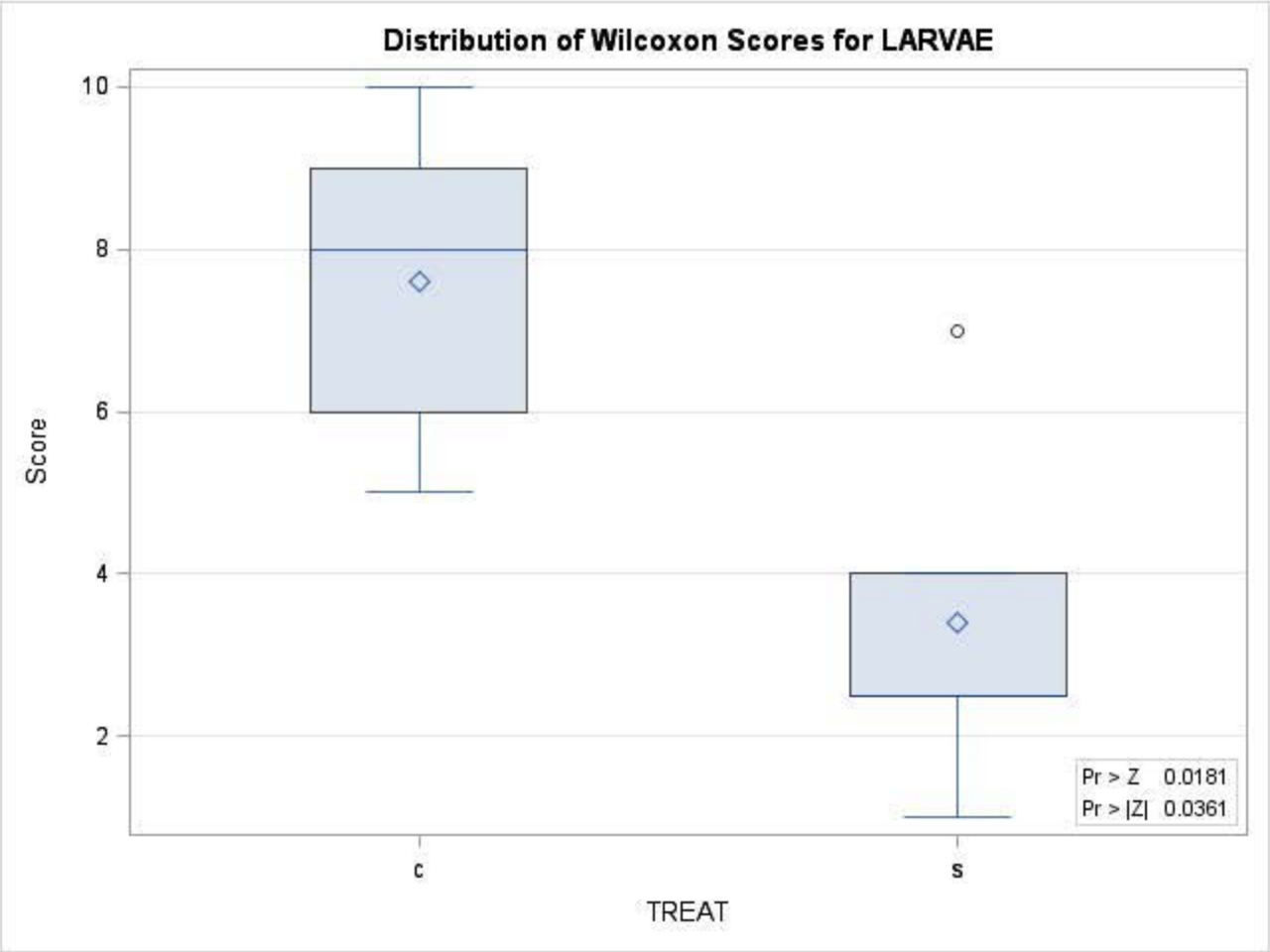
Wilcoxon Two-Sample Test

Statistic	38.0000
Normal Approximation	
Z	2.0953
One-Sided Pr > Z	0.0181
Two-Sided Pr > |Z|	0.0361
t Approximation	
One-Sided Pr > Z	0.0328
Two-Sided Pr > |Z|	0.0656

Z includes a continuity correction of 0.5.

Kruskal-Wallis Test

Chi-Square	4.8402
DF	1
Pr > Chi-Square	0.0278



NONPARAMETRIC COMPARISON OF NUMBER OF FEMALE LARVAE IN CONTROL VS SPIROMESIFEN-TREATED COLONIES

Obs	TREAT	_TYPE_	_FREQ_	ADULTS	MALES	LARVAE
1	c	0	60	6	314	203
2	s	0	60	4	207	99