DATA EVALUATION RECORD
CHRONIC TOXICITY TEST WITH THE HONEY BEE
Non-Guideline Chronic Feeding Study

1. **CHEMICAL:** Dicamba **PC Code:** 029801

2. **TEST MATERIAL:** Dicamba acid technical **Purity:** 98.46 ± 0.60%

3. **CITATION**

Author: Tänzler, V. and Knebel, N.

Title: Dicamba Technical: Chronic Oral Toxicity Test on the Honey Bee (*Apis mellifera* L.) in the Laboratory

Study Completion Date: February 21, 2017

Laboratory: Ibacon GmbH
Rossdorf, Germany
Sponsors: Rotam Agrochemical Europe
Lyon, France
Laboratory Report ID: 115831136
DP Barcode: 455700
MRID: 50931304

4. **REVIEWED BY:** Julie Burns, Environmental Scientist, CDM/CSS-Dynamac JV

Signature: Julie Burns **Date:** 4/1/2020

APPROVED BY: Elizabeth Krupka, Environmental Scientist, CDM/CSS-Dynamac JV

Signature: Elizabeth Krupka **Date:** 4/13/2020

5. **REVIEWED BY:** Michael Lowit, Ph.D., Senior Scientist, OCSPP/OPP/EFED/ERB6

Signature: Michael Lowit **Date:** 10/23/2020

REVIEWED BY: Michael Wagman, Senior Scientist, OCSPP/OPP/EFED/ERB2

Signature: MICHAEL WAGMAN Digitally signed by MICHAEL WAGMAN Date: 2020.10.23 12:28:07 -04'00' **Date:** 10/23/2020

6. DISCLAIMER

This Data Evaluation Record may have been altered by the Environmental Fate and Effects Division subsequent to signing by CDM/CSS-Dynamac JV personnel. The CDM/CSS-Dynamac Joint Venture role does not include establishing Agency policies.

7. STUDY PARAMETERS

Test Species: *Apis mellifera carnica* L.
Age of Test Organisms at Test Initiation: 2 days old
Exposure Duration: 10 days

8. CONCLUSIONS

The honey bee, *Apis mellifera carnica* L., was exposed to dicamba acid (PC Code 029801) technical for 10 days in a limit test feeding study at the nominal concentration/dietary dose shown in the table below. The reviewer calculated the measured concentration and measured dietary dose by applying the % recovery of the analyzed sample to the nominal concentration and nominal actual intake dietary dose. The measured concentrations/doses are reported in the table below.

Nominal Concentration (mg ai/kg diet)	Measured Concentration (mg ai/kg diet)	Nominal Dietary Dose (µg ai/bee/day)	Measured Actual Intake Dietary Dose (µg ai/bee/day)
4000.0	4200	100	64.8

Mortality was not affected in the single exposure level (no mortalities), while there was a statistically significant reduction in food consumption of 44% compared to the control.

Endpoint	Mortality	Food Consumption
Diet Concentration (mg ai/kg diet)	LC_{50} > 4200 95% CI: N/A Slope: N/A NOAEC ≥ 4200 LOAEC > 4200	IC_{50} > 4200 95% CI: N/A Slope: N/A NOAEC < 4200 LOAEC ≤ 4200
Dietary Dose (µg ai/bee/day)	LD_{50} > 64.8 95% CI: N/A Slope: N/A NOAEL ≥ 64.8 LOAEL > 64.8	ID_{50} > 64.8 95% CI: N/A Slope: N/A NOAEL < 64.8 LOAEL ≤ 64.8

9. ADEQUACY OF THE STUDY

This study is scientifically sound but is classified as **Supplemental** because a NOAEC/L was not established for food consumption. The results can be used qualitatively.

10. GUIDELINE DEVIATIONS

This study was conducted following the proposed OECD Guideline for the Testing of Chemicals: Honey bees *(Apis mellifera* L.), chronic oral toxicity test (10 day feeding test in the laboratory) and Kling and Schmitzer Proposal for a new OECD guideline for the testing of chemicals on adult honey bee (*Apis mellifera* L.) in a 10 day chronic feeding test in the laboratory and results of the recent ring test.

The reviewer evaluated the study based on the final OECD TG 245 (2017).

The following deficiencies and deviations were noted by the reviewer:

1. It was not reported if bees were kept in conditions conforming to proper cultural practices.
2. It was not reported if bees were randomly assigned to test item groups.
3. Raw data for temperature and relative humidity were not provided.
4. The reported relative humidity during the study (37-62%) fell below the OECD recommendation of 50-70%. However, the average humidity was 60% and excursions below 50% were less than 2 hours.
5. A NOAEC/L was not established for food consumption.

The lack of a NOAEC/L for food consumption impacts the acceptability of this study.

11. SUBMISSION PURPOSE

This study was conducted for the purpose of pesticide re-registration.

12. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species Honey Bee (*Apis mellifera L.*)	Honey bee (*Apis mellifera carnica* L.)

Guideline Criteria	Reported Information
Age at beginning of test Worker bees of uniform age.	2-day old emerged adult bees
Source	Colonies were bred by ibacon. Two combs from one hive were used for the test.
Were bees from diseased-free colonies?	Honey bee colonies were disease-free and queen-right.
Were bees kept in conditions conforming to proper cultural practices?	Not reported.
Acclimation conditions	After a hatching period of one day, bees were transferred to test units and acclimatized under test conditions for one further day. Before the start of the test, dead or moribund bees were excluded and replaced by new healthy bees out of reserve units.

B. Test System

Guideline Criteria	Reported Information
Test Chambers	Stainless steel chambers (8 cm x 6 cm x 4 cm) with a removable front glass sheet. The bottom of the cage was perforated with ventilation holes, and the inner walls were lined with filter paper.
Temperature during exposure	33°C, short term deviations (< 2 hours) are not reported.
Relative humidity during exposure	37-62% mean: 60%, short term deviations (< 2 hours) are not reported.
Lighting	Darkness (except during observation)

Guideline Criteria	Reported Information
Feeding	Bees were fed treated 50% sucrose solution, or untreated 50% sucrose solution (negative control). Bees were fed *ad libitum* with syringes that were replaced daily.

C. Test Design

Guideline Criteria	Reported Information
Test material	Identity: Dicamba Technical IUPAC name: 3,6-dichloro-o-anisic acid; 3,6-dichloro-2-methoxybenzoic acid CAS name: 3,6-dichloro-2-methoxybenzoic acid CAS No.: 1918-00-9 Batch No.: 20140901136 Purity: 98.46 ± 0.60% (according to supplier certificate) Storage: 20 ± 5°C, in the dark
Nominal application rates The test material should be applied at the maximum proposed label rate.	Diet concentration: 0 (negative control) and 4000.0 mg ai/kg diet Nominal dietary dose: 0 (negative control), and 100 µg ai/bee/day (nominal actual intake dose was 61.7 µg ai/bee/day)
Dose Preparation	The test item was dissolved in 50% (w/v) sucrose solution in order to receive the targeted final concentration. The feeding solution was prepared fresh daily.
Number of bees exposed	Each treatment (including untreated control and reference toxicant) had 5 replicates, with 10 bees per replicate. 50 total bees per treatment application were exposed.

Guideline Criteria	Reported Information
Application methods	The test sucrose feeding solutions were provided *ad libitum* with syringe feeders. Feeders were replaced every day with freshly prepared treatment solutions. Feeders were weighed before and after feeding. The food consumed was determined by comparing the initial and remaining weights.
Other experimental design information	Individual daily consumption was corrected for daily mortality and for estimated evaporation. Three additional test vessels containing syringes filled with negative control sucrose solution but without bees were established and the syringes were weighed daily to determine estimated evaporation.
Were bees randomly or impartially assigned to test groups?	Not reported.
Control	Negative control: 50% aqueous sucrose solution Control feeding solution was prepared at least every four days and stored tightly closed under cool conditions in the dark.
Exposure period	10 days
Positive Control	Toxic reference item: Dimethoate (1.0 mg ai/kg diet) Toxic reference feeding solution was prepared at least every four days and stored tightly closed under cool conditions in the dark.

13. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. Signed and dated Quality Assurance, Good Laboratory Practice, and No Data Confidentiality statements provided. This study was conducted in compliance with the most recent edition of the OECD Principles of Good Laboratory Practice, the German Chemicals Act (ChemG), Annex 1, Directive 2004/10/EC, and OECD Consensus Document No. 13. These requirements are accepted by regulatory authorities throughout the European Community, the US EPA and FDA, and Japan (MHLW, MAFF, and METI) on the basis of intergovernmental agreements.
Control mortality	0% mortality was observed in the negative control at test termination.
Were raw data included?	Raw mortality and food consumption data were provided. Raw data for temperature and relative humidity were not.
Were signs of toxicity described?	Bees were observed daily for mortality and any sublethal effects (e.g. apathy, moribund, affected, cramps, vomiting).

Mortality and Observations

Measured Diet Concentration, mg ai/kg diet (Measured Actual Intake Dose, µg ai/bee/day)	Number Exposed	Mortality at 10 days (%)	Behavioral Abnormalities
Negative control	50	0	None
4200 (64.8)	50	0	None

After 10 days, there was no mortality in the negative control or single treatment group. No behavioral abnormalities were present throughout the study. Mortality in the positive control (Dimethoate) was 100% at ten days. Daily food consumption averaged 27.6 mg/bee/day in the negative control and 15.4 mg/bee/day in the exposure group.

Study Author's Statistical Analysis

The NOAEC/NOAEL were determined using Fisher's Exact Test (pairwise comparison, one-sided greater, α=0.05), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. Statistical analysis was done using ToxRat Professional, Version 3.2.1. The study author's results for mortality were based on the nominal concentration and nominal actual intake daily dose:

NOAEC: 4000.0 mg ai/kg diet
NOAEL: 61.7 µg ai/bee/day

Reviewer's Statistical Verification

The reviewer analyzed mortality and mean daily food consumption using CETIS statistical software version 1.9.6.12 with database backend settings implemented by EFED on 7/25/17. The test codes were designated the MRID number, followed by the acronyms "dc" for dietary concentration and "dd" for dietary dose. The reviewer-calculated measured actual intake dietary dose and measured diet concentration were used for the analyses.

Since this was a limit test, an Equal Variance t Two-Sample Test was used to compare the single treatment group to the negative control. There was a complete absence of mortality in this experiment so no statistical analysis was necessary. However, food consumption in the treatment group was significantly reduced (44%) compared to the control.

Due to an absence of mortality and the use of a single exposure level, the reviewer empirically estimated the LC/LD_{50} to be greater than the measured concentration/dose. Based on an inhibition of 44%, the reviewer empirically estimated the food consumption IC/ID_{50} values to be greater than the measured concentration/dose.

Endpoint	Mortality	Food Consumption
Diet Concentration (mg ai/kg diet)	LC_{50} > 4200 95% CI: N/A Slope: N/A NOAEC ≥ 4200 LOAEC > 4200	IC_{50} > 4200 95% CI: N/A Slope: N/A NOAEC < 4200 LOAEC ≤ 4200
Dietary Dose (µg ai/bee/day)	LD_{50}: >64.8 95% CI: N/A Slope: N/A NOAEL ≥ 64.8 LOAEL > 64.8	ID_{50} > 64.8 95% CI: N/A Slope: N/A NOAEL < 64.8 LOAEL ≤ 64.8

14. REVIEWER'S COMMENTS

The reviewer's and study author's 10-day mortality results were in agreement when considering the differences between the measured concentrations/doses (used by the reviewer) and the nominal concentration/doses (used by the study author). The study author did not analyze food consumption, which was a significantly affected endpoint in this study. The reviewer's results are reported in the Conclusions section of this DER.

The OECD TG 245 validity requirements were met.

The in-life phase of this study was conducted from June 7 to 17, 2016.

15. REFERENCES

Kling, A. & Schmitzer, S. (2015): Proposal for a new OECD guideline for the testing of chemicals on adult honey bees (*Apis mellifera* L.) in a 10 day chronic feeding test in the laboratory and results of the recent ring test 2014. Hazards of pesticides to bees - 12th International Symposium of the ICP-PR Bee Protection Group, Ghent (Belgium), 15-17 September 2014. Julius-Kühn-Archiv, 450, pp. 69-74.

All other references are for standard guidance or methods.

CETIS Summary Report

Report Date:	14 Apr-20 18:12 (p 1 of 1)
Test Code/ID:	50931304 dc / 03-9967-2688

Special Study Honey bee Adult Chronic Oral Toxicity, 10-day Study — **ibacon GmbH**

Batch ID:	18-7037-2292	**Test Type:**	2014 Honeybee Adult Chron Oral	**Analyst:**		
Start Date:	07 Jun-16	**Protocol:**	Honeybee Adult Chronic Oral Toxicity, 10-d	**Diluent:**		
Ending Date:	17 Jun-16	**Species:**	Apis mellifera	**Brine:**		
Test Length:	10d 0h	**Taxon:**		**Source:**	IBACON GmbH	**Age:**

Sample ID:	05-9441-4743	**Code:**	50931304 dc	**Project:**	Herbicide
Sample Date:	07 Jun-16	**Material:**	Dicamba	**Source:**	Rotam Agrochemical Europe
Receipt Date:		**CAS (PC):**	1918-00-9	**Station:**	
Sample Age:	n/a	**Client:**	CDM Smith		

029801 50931304 measured concentration, record created by: J. Burns.

SIngle Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
00-7945-6998	10-Day Mortality Rate	Equal Variance t Two-Sample Test	1.0000	4200mg ai/kg d passed 10-day mortality rat	1
03-1362-5330	Food Consumption	Equal Variance t Two-Sample Test	<1.0E-37	4200mg ai/kg d failed food consumption	1

10-Day Mortality Rate Summary

Conc-mg ai/kg d	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.00%
4200		5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.00%

Food Consumption Summary

Conc-mg ai/kg d	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	5	27.58	26.57	28.59	27.1	29	0.3625	0.8106	2.94%	0.00%
4200		5	15.44	14.56	16.32	14.7	16.3	0.3187	0.7127	4.62%	44.02%

10-Day Mortality Rate Detail

Conc-mg ai/kg d	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	N	0.0000	0.0000	0.0000	0.0000	0.0000
4200		0.0000	0.0000	0.0000	0.0000	0.0000

Food Consumption Detail

Conc-mg ai/kg d	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	N	27.5	27.1	27.1	27.2	29
4200		14.7	15.1	16.3	15	16.1

10-Day Mortality Rate Binomials

Conc-mg ai/kg d	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	N	0/10	0/10	0/10	0/10	0/10
4200		0/10	0/10	0/10	0/10	0/10

CETIS Summary Report

Special Study Honey bee Adult Chronic Oral Toxicity, 10-day Study — **ibacon GmbH**

Batch ID:	07-5466-7467	**Test Type:**	2014 Honeybee Adult Chron Oral	**Analyst:**		
Start Date:	07 Jun-16	**Protocol:**	Honeybee Adult Chronic Oral Toxicity, 10-d	**Diluent:**		
Ending Date:	17 Jun-16	**Species:**	Apis mellifera	**Brine:**		
Test Length:	10d 0h	**Taxon:**		**Source:**	IBACON GmbH	**Age:**

Sample ID:	07-7669-4968	**Code:**	50931304 dd	**Project:**	Herbicide
Sample Date:	07 Jun-16	**Material:**	Dicamba	**Source:**	Rotam Agrochemical Europe
Receipt Date:	01 Apr-20 13:39	**CAS (PC):**	1918-00-9	**Station:**	
Sample Age:	n/a	**Client:**	CDM Smith		

029801 50931304 measured daily doses, record created by: J. Burns.

10-Day Mortality Rate Summary

Conc-ug/bee/day	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.00%
64.8		5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.00%

Food Consumption Summary

Conc-ug/bee/day	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	N	5	27.58	26.57	28.59	27.1	29	0.3625	0.8106	2.94%	0.00%
64.8		5	15.44	14.56	16.32	14.7	16.3	0.3187	0.7127	4.62%	44.02%

10-Day Mortality Rate Detail

Conc-ug/bee/day	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	N	0.0000	0.0000	0.0000	0.0000	0.0000
64.8		0.0000	0.0000	0.0000	0.0000	0.0000

Food Consumption Detail

Conc-ug/bee/day	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	N	27.5	27.1	27.1	27.2	29
64.8		14.7	15.1	16.3	15	16.1

10-Day Mortality Rate Binomials

Conc-ug/bee/day	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	N	0/10	0/10	0/10	0/10	0/10
64.8		0/10	0/10	0/10	0/10	0/10

CETIS Analytical Report

Report Date:	14 Apr-20 18:12 (p 1 of 2)
Test Code/ID:	50931304 dc / 03-9967-2688

Special Study Honey bee Adult Chronic Oral Toxicity, 10-day Study — **ibacon GmbH**

Analysis ID:	00-7945-6998	**Endpoint:**	10-Day Mortality Rate	**CETIS Version:**	CETISv1.9.5
Analyzed:	01 Apr-20 13:27	**Analysis:**	Parametric-Two Sample	**Status Level:**	1

Batch ID:	18-7037-2292	**Test Type:**	2014 Honeybee Adult Chron Oral	**Analyst:**			
Start Date:	07 Jun-16	**Protocol:**	Honeybee Adult Chronic Oral Toxicity, 10-d	**Diluent:**			
Ending Date:	17 Jun-16	**Species:**	Apis mellifera	**Brine:**			
Test Length:	10d 0h	**Taxon:**		**Source:**	IBACON GmbH	**Age:**	

Sample ID:	05-9441-4743	**Code:**	50931304 dc	**Project:**	Herbicide
Sample Date:	07 Jun-16	**Material:**	Dicamba	**Source:**	Rotam Agrochemical Europe
Receipt Date:		**CAS (PC):**	1918-00-9	**Station:**	
Sample Age:	n/a	**Client:**	CDM Smith		

029801 50931304 measured concentration, record created by: J. Burns.

Data Transform	Alt Hyp	Comparison Result
Untransformed	C < T	4200mg ai/kg d passed 10-day mortality rate

Equal Variance t Two-Sample Test

Control	vs	Conc-mg ai/k	Test Stat	Critical	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		4200	0	1.86	8	CDF	1.0000	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0	0	1	65540	<1.0E-37	Significant Effect
Error	0	0	8			
Total	0		9			

10-Day Mortality Rate Summary

Conc-mg ai/kg d	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.00%
4200		5	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000		0.00%

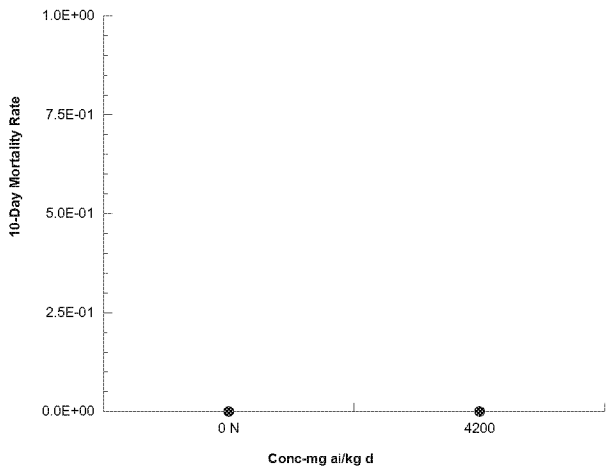
10-Day Mortality Rate Detail

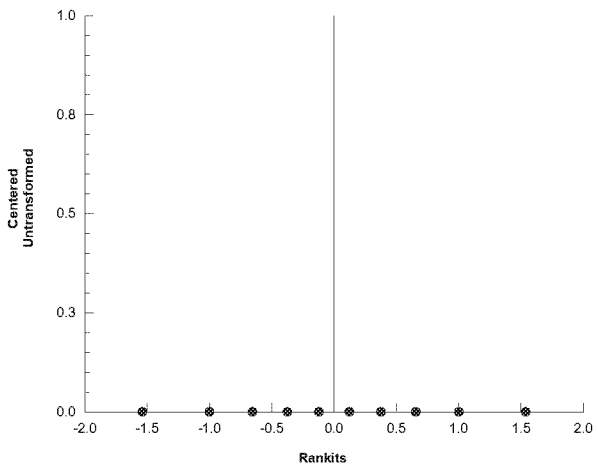
Conc-mg ai/kg d	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	N	0.0000	0.0000	0.0000	0.0000	0.0000
4200		0.0000	0.0000	0.0000	0.0000	0.0000

10-Day Mortality Rate Binomials

Conc-mg ai/kg d	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	N	0/10	0/10	0/10	0/10	0/10
4200		0/10	0/10	0/10	0/10	0/10

Graphics





Special Study Honey bee Adult Chronic Oral Toxicity, 10-day Study — **ibacon GmbH**

Analysis ID:	03-1362-5330	**Endpoint:**	Food Consumption	**CETIS Version:**	CETISv1.9.5
Analyzed:	01 Apr-20 13:28	**Analysis:**	Parametric-Two Sample	**Status Level:**	1

Batch ID:	18-7037-2292	**Test Type:**	2014 Honeybee Adult Chron Oral	**Analyst:**			
Start Date:	07 Jun-16	**Protocol:**	Honeybee Adult Chronic Oral Toxicity, 10-d	**Diluent:**			
Ending Date:	17 Jun-16	**Species:**	Apis mellifera	**Brine:**			
Test Length:	10d 0h	**Taxon:**		**Source:**	IBACON GmbH	**Age:**	

Sample ID:	05-9441-4743	**Code:**	50931304 dc	**Project:**	Herbicide
Sample Date:	07 Jun-16	**Material:**	Dicamba	**Source:**	Rotam Agrochemical Europe
Receipt Date:		**CAS (PC):**	1918-00-9	**Station:**	
Sample Age:	n/a	**Client:**	CDM Smith		

029801 50931304 measured concentration, record created by: J. Burns.

Data Transform	Alt Hyp	Comparison Result	PMSD
Untransformed	C > T	4200mg ai/kg d failed food consumption	3.25%

Equal Variance t Two-Sample Test

Control	vs	Control II	Test Stat	Critical	MSD	DF	P-Type	P-Value	Decision(α:5%)
Negative Control		4200*	25.15	1.86	0.898	8	CDF	<1.0E-37	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	368.449	368.449	1	632.5	<1.0E-37	Significant Effect
Error	4.66	0.5825	8			
Total	373.109		9			

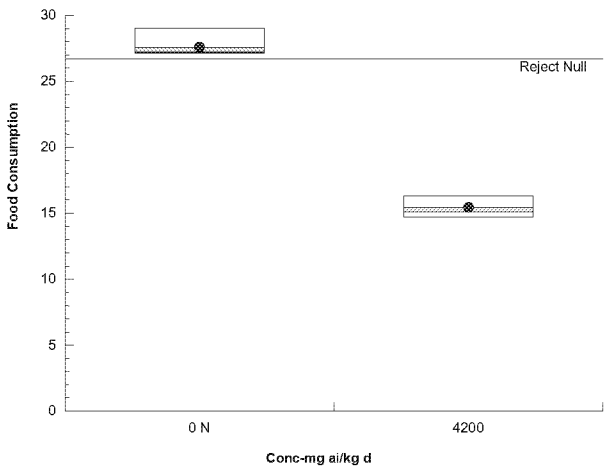
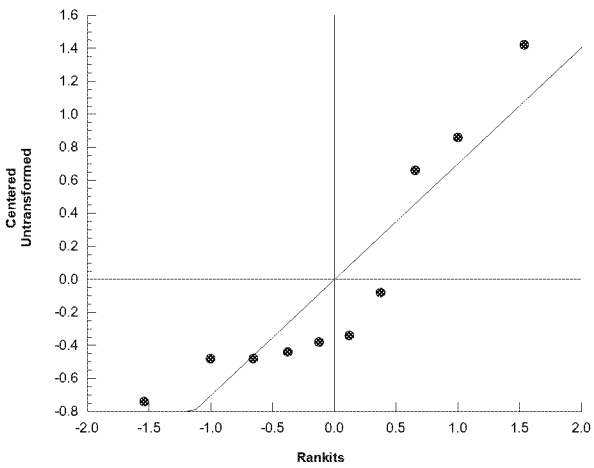
ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Variance Ratio F Test	1.293	23.15	0.8092	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.833	0.7411	0.0364	Normal Distribution

Food Consumption Summary

Conc-mg ai/kg d	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	N	5	27.58	26.57	28.59	27.2	27.1	29	0.3625	2.94%	0.00%
4200		5	15.44	14.56	16.32	15.1	14.7	16.3	0.3187	4.62%	44.02%

Food Consumption Detail

Conc-mg ai/kg d	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	N	27.5	27.1	27.1	27.2	29
4200		14.7	15.1	16.3	15	16.1

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