DATA EVALUATION RECORD
HONEYBEES FIELD TEST
Apis mellifera
Non-Guideline Semi-Field and Residue Study

1. **CHEMICAL:** Sulfoxaflor   PC Code No.: 005210

2. **TEST MATERIAL: Closer SC (ai: Sulfoxaflor)**   Purity: 22.7% (252 g/L)

3. **CITATION**

Author:	Louque, J.
Title:	GF-2032 (Sulfoxaflor): Assessment of Effects on Development of the Brood and Adult Workers of the Honey Bee *(Apis mellifera)* in a Semi-Field Tunnel Study After One Application on Buckwheat (F. *esculentum)*
Study Completion Date:	December 18, 2017
Laboratory:	Smithers Viscient, Carolina Research Center (CRC) 2900 Quackenbush Road, Snow Camp, NC 27349
Sponsor:	Dow AgroSciences 9330 Zionsville Road Indianapolis, IN 46268
Laboratory Report ID:	14050.4114
DP Barcode:	445512
MRID No.:	50494501

4. **REVIEWED BY:** Adrian Graff, Environmental Scientist, CDM/CSS-Dynamac JV
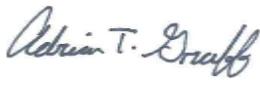
Signature: **Date:** 4/12/2018

APPROVED BY: Moncie V. Wright, Environmental Scientist, CDM/CSS-Dynamac JV

Signature: *Moncie V Wright*   **Date:** 6/1/2018

5. **APPROVED BY:** Meghann Niesen, Ecologist, OPP/EFED/ERB5

   Signature:

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: Honeybees (*Apis mellifera* L.)
   Age of Test Organism at Test Initiation: Healthy colonies ideally contained one queen with 6000 to 8000 bees (all ages of honeybees included) although the study author notes that some colonies did not meet the specified size criteria as indicated in Deviation 4 (p. 73); no visible disease, parasites, or pests were present
   Test Duration: 10-day exposure with additional 30 days of monitoring plus overwintering/spring assessments

8. **CONCLUSIONS:** **Closer SC (ai: Sulfoxaflor)** was applied at nominal rates of 0.023, 0.071, and 0.090 lb ai/acre to flowering buckwheat (*Fagopyrum esculentum*) near Belvidere, North Carolina along with a water control and three reference controls. The honey bee (*Apis mellifera*) colonies were exposed for 10 days using six replicate tunnel tents per treatment level plus an additional two replicates per treatment for sampling. Following the 10-day test exposure, the hives were monitored for an additional 30 days at another site near Snow Camp, North Carolina. The 0.023, 0.071, and 0.090 lb ai/acre treatment groups are referred to as T1, T2, and T3 by the reviewer in select portions of this DER and in the Conclusions section.

The study author concluded, based on study results, that 10-day honey bee exposure to an application of **Closer SC (ai: Sulfoxaflor)** to a flowering crop may have mild, short-term effects on a honey bee colony's foraging intensity and adult bee mortality. The study author concluded that exposed colonies would recover quickly after 10 days of exposure. However, Fall and Winter colony fatality rates in treatments T2 and T3 exceeded the fatality rate of the control.

The reviewer's general assessment of the study results has resulted in the following conclusions regarding toxicity of the test material:

1.) The study author did not take the appropriate steps to ensure that all colonies were similar before installation in the tunnels.

2.) Total mortality was affected on the day after exposure, decreasing over time until the controls and treatment groups were similar.

3.) Flight intensity was likely unaffected and was highly variable within and between groups over time.

4.) The lowest number of adult bees and lowest estimates of colony strength were recorded in the spring after overwintering in T1 and T3.

5.) The lowest number of capped brood cells was observed in the spring after overwintering in T1, T2, and T3.

6.) Bee brood development was similar between the control and treatment groups for both cohorts 1 and 2.

7.) Many colonies were lost during overwintering due to temperature swings, making interpretations of endpoints after overwintering challenging.

8.) Before exposure, pollen estimates were different in T2 and T3 as compared to the control. After 10 days, pollen estimates were increased further in T2 and T3.

9.) In general, residues were greatest in whole plant samples, followed by larval tissue, nectar, and bee bread. Sulfoxaflor accounted for the majority of the total sulfoxaflor residue (TSR) in bee bread, nectar, and larval tissues, whereas the X11719474 metabolite accounted for the majority of TSR in whole plant samples.

Further, it is important to recognize the inherent strengths and limitations of this study as results are interpreted and potentially considered in risk assessment. In the context of available field studies involving honey bees, this study contains some strengths including:

- Inclusion of multiple colony-level endpoints reflecting hive condition, brood development, and nectar/pollen availability.
- Quantification of exposure to sulfoxaflor and metabolites in hive matrices (uncapped nectar, honey, bee bread)
- Sulfoxaflor was quantified in the solutions used to treat the crops for the exposure.

A number of limitations were noted, including:

- Sulfoxaflor was detected in matrices of several control groups.
- Residues were only reported from nectar and not pollen.
- Not all colonies had enough eggs for brood analysis in the first cycle (across controls and treatment hives) which led to a weaker analysis. Brood cycle two was conducted and

analyzed.
- Pupal samples were inadvertently collected and analyzed instead of larval samples for residue analysis at two timepoints.
- Information on initial colony condition was not reported and some hives did not meet the population criteria listed in the protocol. At the first CCA there were indications that some of the colonies did not meet the specified criteria. Additionally, hives were 6 weeks old at the time of study initiation.
- Overwintering survival was very poor in control hives which excludes use of that endpoint in analysis.

9. ADEQUACY OF THE STUDY: This study **is scientifically sound** and is classified as **supplemental (quantitative)**.

10. GUIDELINE DEVIATIONS: This semi-field study was conducted following the OECD guidance document No. 75 (2007), OEEP/EPPO Guideline No. 170 (2010), and OCSPP 850.3040: Field Testing for Pollinators (2012). No deviations were noted.

11. SUBMISSION PURPOSE: This study was conducted to investigate the potential effects of sulfoxaflor exposure to honey bee (*Apis mellifera*) adult mortality, foraging activity, behavior, colony strength, and brood development after foliar application to buckwheat (*Fagopyrum esculentum*) under semi-field conditions. This study was submitted under the Pesticide Registration Improvement Act (PRIA) for the purpose of chemical registration/re-registration.

12. MATERIALS AND METHODS

Test Material:

Identity:	Closer SC (Sulfoxaflor)
IUPAC name (ai):	[1-(6-trifluoromethylpyridin-3-yl)ethyl](methyl)oxido-λ6-sulfanylidenecyanamide
CAS name (ai):	
CAS No.:	946578-00-3

Lot No.:   D074F1M200
Description: White/off-white powder
Purity: 22.7% (252 g/L)
        Storage: Ambient; 5-30°C.

Test Organisms/Hives: The honeybee (*Apis mellifera* L.) colonies used in the test were purchased as packages from a commercial apiary and supplied with sister queens. Colonies were free of symptoms of nosemosis, varroosis, foulbrood and other bee diseases. At the start of the test, the colonies were apparently healthy, with laying queens and no visible disease, parasites, or pests present.

Test Design: The semi-field test location was near Belvidere, North Carolina (36°19'56.29"N, 76°28'57.28"W) at a test site with planted buckwheat (*F. esculentum*). For biological assessments, three sulfoxaflor treatment groups and one control group had six replicate tunnels plus two additional replicates for residue sampling. This, combined with three reference groups consisting of three replicates and an additional two replicates for sampling totaled 47 tunnels for the study.

Each tunnel enclosed an area of 121 m² and measured 20 m long x 5 m wide x 3.5 m high in the center. The two applied crop areas in each tunnel measured 82.72 m². The minimum distance between the tunnels of the treatment, control, or reference material was at least 3.0 m, while a minimum distance of 12.0 m was maintained between tunnels belonging to the treatment, control, and reference groups. The tunnel frames were constructed of PVC pipe and were covered with light plastic mesh with a 2 x 7 mm mesh size. During tunnel exposure period, the bees were supplied with water that was covered with floatable material to prevent the honey bees from drowning. Prior to study start, some plants were removed, paths were created in each tunnel, and the paths were covered with linen sheets for the dead honey bee assessments.

Once 20-30% of flowers were open (BBCH 62-63), one small commercial honey bee colony was introduced to each tunnel in the evening after daily honey bee flight activity (2DBE). The 10-day exposure phase was conducted in the tunnel tents and the hives were relocated to a monitoring site on 12DAA (DAE=Days After Exposure) until 60DAE. The monitoring site was located 213 miles from the field testing site at the CRC main building apiary. Untreated wildflowers, pumpkin, and buckwheat were available for foraging.

Nominal application rates/volumes: The nominal sulfoxaflor application rates were 0 (negative tap water control), 0.023, 0.071, and 0.090 lb ai/acre. The reference items had targeted application rates of 0.0778 lb ai/acre for novaluron, and 0.1 L/ha and 1 L/ha for dimethoate.

Application procedure: The test item solution was prepared one hour before the application and applied using a calibrated portable boom sprayer equipped with a Quick TeeJet nozzle, simulating a commercial application. Treatment applications (June 12, 2016) were performed during daily bee flight activity on flowering *F. esculentum* (BBCH 60-63) in the replicate treatment tunnels. During application, wind speed was estimated at 0 mph, temperatures ranged from 27.7 to 31.2°C, humidity was 69%, and there was no cloud cover. The crops were in BBCH growth stage 62-64, ground cover was 80-100%, and the crops were reported to be in fair/good health.

During the applications, honey bee colonies in the tunnels were covered with plastic sheets and the water supply was moved out of the tunnels until the end of application to

avoid direct contamination. The amount of control, test item, or reference item solution actually applied was determined by measuring the initial and the remaining spray solution.

Observations: Temperature, humidity, and rainfall were recorded from the on-site weather station daily during exposure. Additively, a temperature data recorder was placed underneath each hive in every "a" tunnel to provide microclimate information during exposure. Cloud cover at the field site was estimated during the exposure and post-exposure periods during daily assessments. Air temperature, relative air humidity, and wind speed (GLP) at the field site was recorded during applications. At the monitoring site (10DAA to 63DAA), meteorological data (non-GLP) were provided by the EAS weather station located 8.7 km from the monitoring site.

Sampling: Adult bee mortality was determined daily by counting dead bees in drop-zone dead bee traps and on linen strips. Dead bees were differentiated between adult worker bees, males, freshly emerged bees, pupae, and larvae during each assessment. For statistical analysis, dead bee counters were expressed as a percentage of adult colony strength recorded from the previous CCA. On the day of application, mortality was assessed three times, at 0930, 1130, and 1330. Dead bees were counted only in the dead bee traps during the post-exposure period (DAE11-13 to DAE31).

Foraging activity was recorded within areas of 1 m^3 at three different locations in each tunnel. At each assessment interval, the number of bees foraging on flowering buckwheat were counted for approximately 15 seconds at each location. Simultaneously, behavior of bees around the hives and in the crop was being observed. Potential behaviors included: aggressiveness, clustering at hive entrance, and intoxication symptoms. On the day of application, behavior and foraging activity were assessed three times, at 0930, 1130, and 1330 after the morning application. During the exposure period, foraging was assessed once a day during daily bee flight activity.

Colony condition assessments were conducted once before exposure, once during exposure, and three times post-exposure. Colony strength (no. of adult bees) and comb area containing capped pupae were quantified. Additively, colonies were examined for any bee diseases at each assessment according to standard beekeeping practices.

Bee brood developmental status in individual marked comb cells was captured at specified intervals with digital photography and quantified using image processing software Honeybee Complete©. Hive frames were assessed at the first digital photography session. A brood frame, with sufficient egg coverage, was marked at 4 corners to identify the area of eggs to be monitored at each hive. Approximately 300 eggs were marked per colony. On each brood fixing day (BFD) assessment, the same area was photographed, and software was used to determine success. A termination of brood could

be assigned if several criteria were met. Observed cells for brood determination rate were classified as successful development or bee brood termination. Termination rates were determined for each colony separately and the mean value per treatment group was calculated. Brood index was calculated for each assessment day and colony, starting from brood fixing day 0 (BFD0) to BFD21. Brood compensation index was also calculated for each assessment day and colony. Evaluation codes for further calculations of these indices were provided in Table 6 (page 49).

Residue samplings on various honey bee and plant matrices were conducted during the study using two replicates for T1, T2, T3, and C for sampling. Whole buckwheat plants and bee bread samples were collected once before exposure, once on the day of exposure, and seven times after exposure. All plant samples were macerated under dry ice to obtain a homogenous sample (as described in Appendix). Bee bread samples were collected as available, once before exposure, once during exposure, and seven times after exposure. No bee bread samples were available during the Fall CCA. Nectar and larvae were collected once before exposure, once on the day of exposure, and eight times after exposure, notably in Fall 2016 and Spring 2017.

Larval samples were not available at 4DAE and 7DAE and pupae were sampled instead. Samplings occurred All samples were collected according to Smithers Viscient protocol 14050.4114 (further described in Appendix). Honey (capped nectar) was collected at the final assessment only, to determine remaining residues from the exposure phase. All samples were frozen on blue or dry ice as soon as possible and shipped to the Smithers Viscient Wareham facility for analytics. Samples were stored in temperature-monitored freezers (-10 to 25°C), except when removed for analysis, during the study.

Residue Analysis Method: Residues of sulfoxaflor and its metabolites were extracted from bee bread, nectar, and whole plant samples by extraction with 80:20 acetonitrile:purified reagent water (v:v) using a Geno Grinder; concentration under nitrogen followed by enzymatic deconjugation with 10 mg/mL ß-glucosidase; clean-up with solid phase extraction (SPE), and eluted with 80:20 acetonitrile:purified reagent water (v:v) with 0.1% formic acid. Nectar recovery samples were concentrated to near dryness and reconstituted with 20:80 acetonitrile:purified reagent water (v:v) with 0.1% formic acid. Larval bee tissue recovery samples were extracted with 80:20 acetonitrile:purified reagent water (v:v) using a Geno Grinder. Dosing solution (50% sucrose solution) recovery samples were diluted with 50:50 acetonitrile:purified reagent water (v:v) (for sulfoxaflor) or 50:50:0.1 acetonitrile:purified reagent water:formic acid (v:v:v) (for metabolites). All recovery samples were further diluted into the calibration range with an internal standard diluent. All samples were analyzed using liquid chromatography with tandem mass spectrometry detection (LC-MS/MS).

Separate method validations were performed to determine recoveries and precision of the

methods used for the quantification of sulfoxaflor and its metabolites in bee bread, nectar, larval bee tissue, whole plant, and dosing solutions.

The limits of detection (LOD) and quantitation (LOQ) were determined during the method validation study. The LOD was determined to be 0.003 mg/kg for bee bread (both sulfoxaflor and metabolites), 0.0003 and 0.003 mg/kg for nectar (sulfoxaflor and metabolites, respectively), 0.000257 mg/kg (sulfoxaflor), 0.00257 mg/kg (metabolites) and 0.00234 mg/kg (novaluron, and dimethoate) for larval bee tissue, 0.003 mg/kg for whole plants (both sulfoxaflor and metabolites), and 1.30 and 0.00244 mg/kg for dosing solutions (sulfoxaflor and metabolites, respectively). The LOQ was determined to be 0.010 mg/kg for bee bread (both sulfoxaflor and metabolites), 0.0010 and 0.010 mg/kg for nectar (sulfoxaflor and metabolites, respectively), 0.0010 (sulfoxaflor) and 0.010 mg/kg for larval bee tissue (metabolites, novaluron, and dimethoate), 0.010 mg/kg for whole plants (both sulfoxaflor and metabolites), and 4.07 and 0.00813 mg/kg for dosing solutions (sulfoxaflor and metabolites, respectively). These values are in alignment with empirically-derived LOD and LOQ values determined within the method validation study.

Statistical Analysis: The data for adult bee mortality, flight intensity inside the tunnels, colony condition, development of bee brood, detailed brood assessment (brood termination rate, brood-index, compensation-index) were tested for normality using a Ryan-Joiner test (similar to Shapiro-Wilk test) with alpha set at 0.01. Equality of variance was tested using Levene's test (Alpha = 0.05). If data for a variable were both normally distributed and of equal variance, an analysis of variance (ANOVA) was conducted followed by a Tukey Simultaneous Test for paired comparisons. If data were not normal or variances unequal, a non-parametric Kruskal-Wallis test was conducted and, if significant differences were indicated, a Dunn's test was run to provide paired comparisons. Fall and overwinter survival data for the colonies were analyzed by comparing each treatment and reference group to the control group via a Fisher's Exact test.

Summary of Study Dates:
(DAE=Days after exposure)

DAE	Date	Action
-12	Tuesday, May 10, 2016	Protocol Signed
-11	Wednesday, June 01, 2016	Amendment 1 Signed
-6	Monday, June 06, 2016	Tunnel Construction begins
-5	Tuesday, June 07, 2016	Hives moved from Dowry to Belvidere
-4	Wednesday, June 08, 2016	CCA 1
-3	Thursday, June 09, 2016	CCA 1
-2	Friday, June 10, 2016	Tunnel Construction Ends
-1	Saturday, June 11, 2016	DAE -1 Samples, Brood B1
0	Sunday, June 12, 2016	Application, DAE 0 Samples
1	Monday, June 13, 2016	DAE 1 Samples

3	Wednesday, June 15, 2016	Brood B2, DAE 4 Samples
5	Friday, June 17, 2016	DAE 4 Samples cont'd
7	Sunday, June 19, 2016	DAE 7 Samples
8	Monday, June 20, 2016	CCA 2
9	Tuesday, June 21, 2016	CCA 2, Brood B3
10	Wednesday, June 22, 2016	Move out of tunnels, DAE 10 Samples
12	Friday, June 24, 2016	Hives moved from Belvidere to CRC
17	Wednesday, June 29, 2016	Brood Bb1
18	Thursday, June 30, 2016	Brood B4
23	Tuesday, July 05, 2016	Brood B5
24	Wednesday, July 06, 2016	Brood Bb2, DAE 21 Samples
25	Thursday, July 07, 2016	CCA 3
29	Monday, July 11, 2016	Brood Bb3
33	Friday, July 15, 2016	Brood Bb4
36	Monday, July 18, 2016	DAE 35 Samples
37	Tuesday, July 19, 2016	All current samples sent to Analytical Chemistry
38	Wednesday, July 20, 2016	Brood Bb5, samples received by Analytical Chemistry
60	Thursday, Aug. 11, 2016	Hives moved to CRC from Dowry
64	Monday, Aug. 15, 2016	Fall CCA
66	Wednesday, Aug. 17, 2016	Fall CCA, Fall Samples
95	Thursday, Sept. 15, 2016	Varroa Treatment #1
107	Tuesday, Sept. 27, 2016	Don Hopkins apiary inspection (sugar shakes)
109	Thursday, Sept. 29, 2016	Bottom Boards switched to Solid, Varroa Tr. #2
149	Tuesday, Nov. 08, 2016	First Frost, Mortality Check
152	Friday, Nov. 11, 2016	AP-23 Winter Patties fed to colonies (1/2 lb)
157	Wednesday, Nov. 16, 2016	All current samples sent to Analytical Chemistry
158	Thursday, Nov. 17, 2016	Samples received by Analytical Chemistry
192	Wednesday, Dec. 21, 2016	AP-23 Winter Patties fed to colonies (1 lb)
219	Tuesday, Jan. 17, 2017	Entrance reducers added to colony on large opening
254	Tuesday, Feb. 21, 2017	AP-23 Winter Patties fed to colonies (1/2 lb)
288	Monday, March 27, 2017	Spring 2017 sampling, Spring CCA
295	Monday, April 03, 2017	All current samples sent to Analytical Chemistry
296	Tuesday, April 04, 2017	Samples received by Analytical Chemistry

13. REVIEWERS RESULTS

Statistics. For the reviewer calculated results statistics were run using R programing (R Core Team 2013). As the data permitted comparisons were run between control and treatment groups as outlined in Appendix A. Where appropriate Dunnets test or Wilcoxen tests were used for these comparisons.

Adult Mortality. Adult foraging bees exposed to GF-2032 at rates of 0.090, 0.071, and 0.023 lb a.i./A (during flight) exhibited a statistically-significant increases in mortality of up to 8X the rate observed in controls on the day of application. This increase in mean daily worker bee mortality was short lived, however, having returned to not significantly different from controls by 1DAA (for the 0.071, and 0.023 lb a.i./A treatments) and 3DAA (for the 0.090 lb

a.i./A treatment). No statistically significant increases in daily mortality rates were detected after 4DAA.
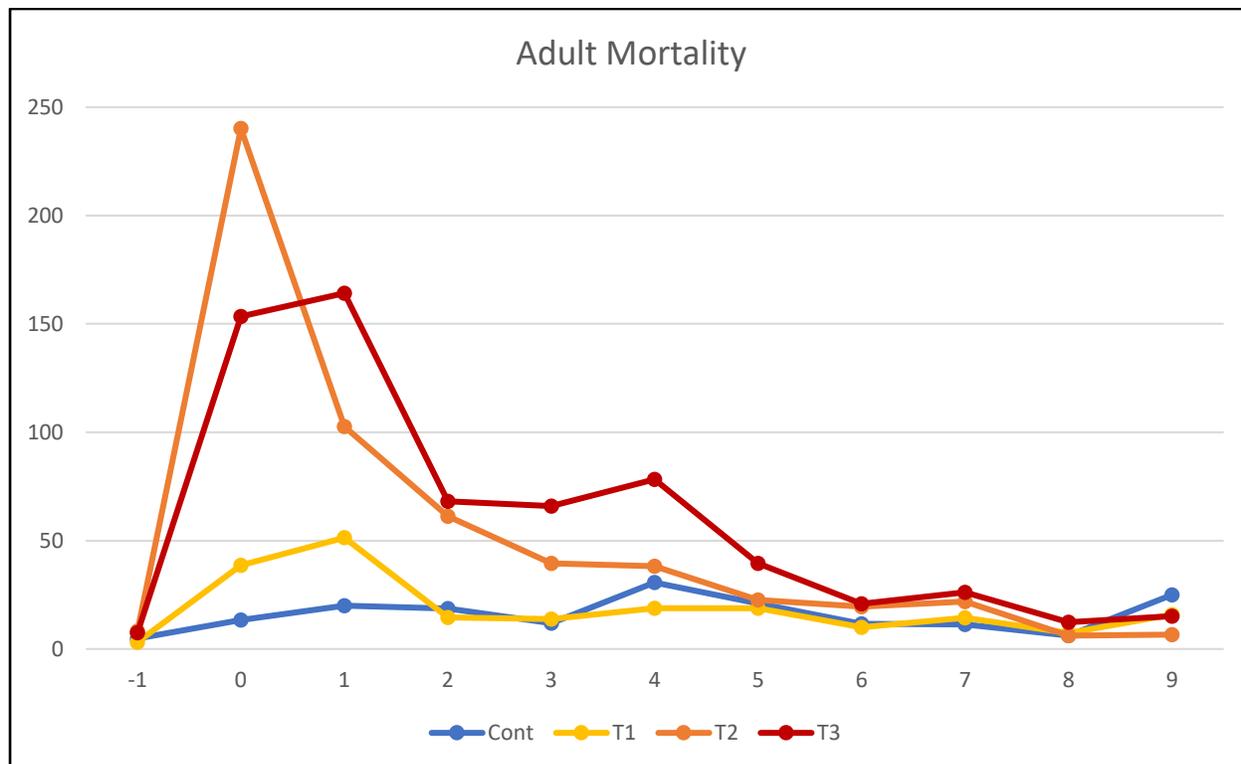
Figure 1. Mean mortality of adult bees per day.

Foraging Activity. There were slight decreases in flight intensity in the treatment groups as compared to the control during the exposure period, but the largest decreases in any treatment group was a 2-fold decrease as compared to the control. This endpoint was highly variable within the same group over time, fluctuating up and down in a manner likely attributable to chance alone and not due to treatment.

Colony Strength. The effect of sulfoxaflor on colony strength is difficult to interpret due to large variation between hives. There were no sustained effects to colony strength at any timepoint. There were no obvious dose-dependent trends in colony strength apparent among hives. Pollen stores were significantly different from control at 8DAA and 66DAA. These differences were not sustained in between these timepoints.

Brood Condition. There were not enough eggs in all colonies to perform a 300-egg assessment for the 1st cohort. As indicated by the brood termination rate, most eggs did not move forward in development past the first stages. It is known that poor brood performance

is a common issue with tunnel tests and work is being done to optimize the test design by (ICPPR). Cohort 2 was marked later and all but one colony, had recovered from the tunnels effects enough to have sufficient eggs for marking. Overall, the control and all treatments were similar across endpoints. Control variation was wide and limited the ability to pick up any statistical differences between the control and treatments.

Residues. Residues of sulfoxaflor up to 0.03 mg/kg were detected in hive nectar in the 0.071 and 0.09 lb a.i./A treatment groups and showed decline over time after the peak at 10DAA. Residues for in-hive bee bread were only detected at 0.09 lb a.i./A at 7DAA at 0.24 mg/kg.
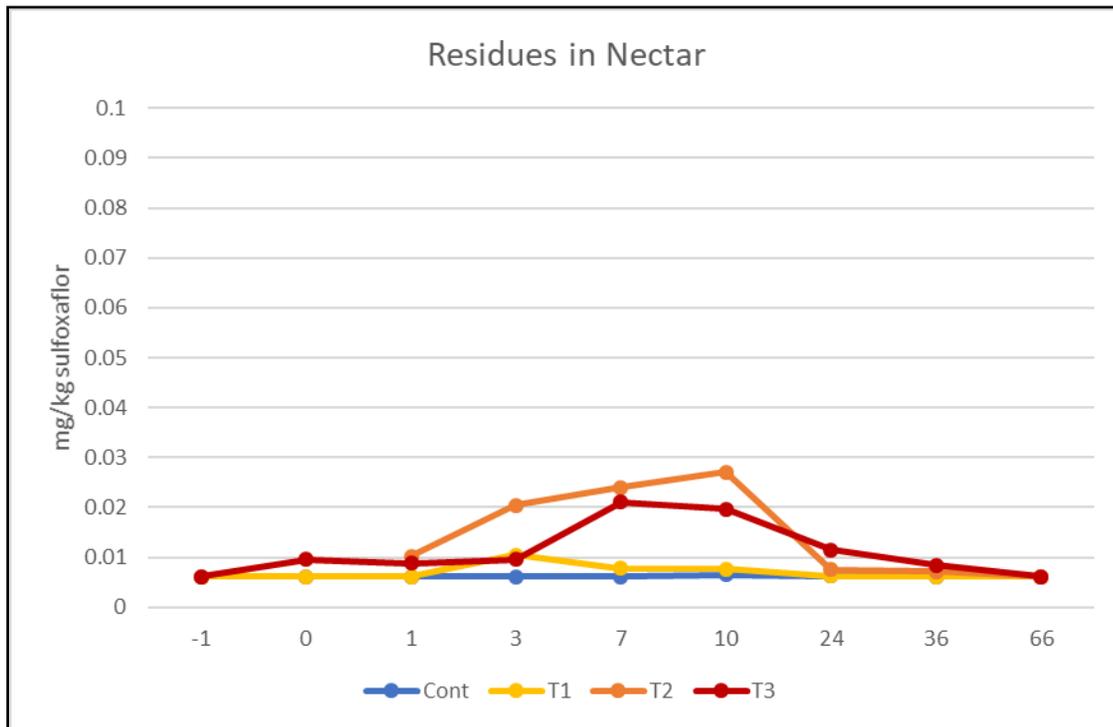

Figure 2. Sulfoxaflor residues from in hive nectar per day.
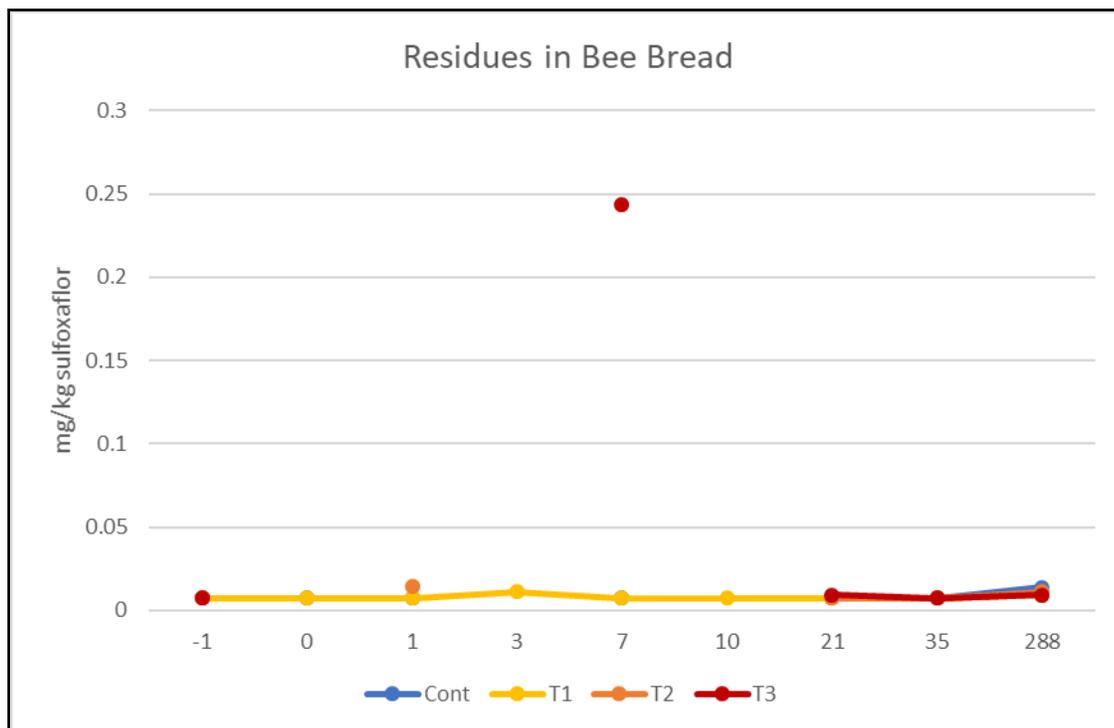
Figure 3. Sulfoxaflor residues from in hive bee bread per day.

Overwintering. The majority of colonies were lost in the late winter, which was attributed to temperature swings. With 50% mortality in the controls; 17% mortality in T1; and 83% mortality in both T2 and T3. High control mortality confounds the interpretation of impact of sulfoxaflor treatment on overwintering success.

14. STUDY AUTHOR REPORTED RESULTS

Mortality: Before exposure, mean mortality (adult workers, males, freshly emerged, pupae, and larvae) was similar between the control and all treatment groups. On the day after exposure, mortality was increased ~2-fold in treatment T1, 5-fold in T2, and 8-fold in T3 as compared to the control. Over the next 8 days, mortality slowly decreased in the treatment groups until the 9th day of exposure whereupon the treatments became similar to the control. By the end of the exposure period, control mortality was slightly higher than mortality in each of the three treatment groups.

Table 1. Mortality at pre-exposure and during the 10-day exposure (adult workers, males, freshly emerged, pupae, and larvae).

Date	CONTROL Mean	CONTROL Stdev		TREATMENT 1 Mean	TREATMENT 1 Stdev	TREATMENT 2 Mean	TREATMENT 2 Stdev	TREATMENT 3 Mean	TREATMENT 3 Stdev
6/11/2016									
Pre-exposure	5	5		4	2	9	10	8	4
6/12/2016									
Pre-exposure	31	22		18	17	21	18	24	11
Post-exposure	4	4		11	9	80	108	51	53
6/13/2016	20	18		50	55	101	81	164	53
6/14/2016	19	7		14	9	61	75	68	40
6/15/2016	12	6		14	20	40	30	66	40
6/16/2016	31	9		16	6	38	26	78	55
6/17/2016	21	10		18	10	23	16	40	36
6/18/2016	11	5		10	8	20	15	21	18
6/19/2016	11	7		14	4	22	28	26	28
6/20/2016	6	4		7	4	6	5	12	7
6/21/2016	25	15		16	9	7	2	15	9

Flight Intensity: Before exposure, mortality was similar across the control and treatment groups. There were slight decreases in flight intensity in the treatment groups as compared to the control during the exposure period, but the largest decreases in any treatment group was a 2-fold decrease as compared to the control. This endpoint was highly variable within the same group over time, fluctuating up and down in a manner likely attributable to chance alone and not due to treatment.

Table 2. Flight intensity at pre-exposure and during the 10-day exposure.

Date	C	T1	T2	T3	R1	R2	R3
11-Jun-2016	0.2	0.2	0.6	0.6	0.9	0.1	1.3
12-Jun-16 pre	1.5	0.1	1.1	0.4	4.1	0.3	0.9
12-Jun-16 post 1	1.2	0.8	0.6	0.8	2.2	0.1	1.2
12-Jun-16 post 2	0.4	0.5	0.2	0.3	0.9	0.0	1.7
13-Jun-2016	2.9	1.4	1.1	1.3	2.8	0.0	0.9
14-Jun-2016	2.9	1.1	1.2	0.9	2.2	0.1	0.3
15-Jun-2016	4.0	1.3	2.4	1.3	3.8	0.1	3.1
16-Jun-2016	4.5	1.2	2.9	0.9	1.0	0.0	3.4
17-Jun-2016	2.2	0.5	1.6	0.6	0.2	0.2	0.8
18-Jun-2016	3.1	0.5	2.9	1.6	1.6	0.4	0.7
19-Jun-2016	3.4	1.0	1.7	1.1	0.7	0.7	0.9
20-Jun-2016	4.4	1.4	3.8	1.9	1.9	1.1	1.9
21-Jun-2016	5.4	1.2	5.7	2.8	4.7	1.0	2.1

Behavior: Behavior was considered normal prior to exposure. There was no abnormal behavior of bees was observed in any treatment during exposure.

Colony Condition:

No. of adult bees:
Before exposure (CCA1), the number of adult bees was similar across the control and treatment groups. After the 10-day exposure period (CCA2), the control and treatment T3 were comparable, and the number of adult bees was slightly lower in T2 and T3 as compared to the control. At CCA3, the number of adult bees in T2 and T3 were similar to the control, and T1 had a decreased number of bees as compared to the control. In the last assessment before overwintering, similar conditions observed at CCA3 prevailed. In the very last assessment in the spring after overwintering, the control and T2 were similar, while T1 and T3 were decreased by ~2 and 3-fold, respectively, as compared to the control.

Table 3. Mean number of adults at each CCA sampling event.

Adult Average Estimates								
Date	CCA	C Average	R1 Average	R2 Average	R3 Average	T1 Average	T2 Average	T3 Average
8-Jun-16	CCA1	5366	7090	3935	7804	4326	4406	5616
21-Jun-16	CCA2	6246	5914	4377	6044	5700	5837	6639
7-Jul-16	CCA3	7274	6188	4576	5992	5284	6231	6069
17-Aug-16	Fall 2016	7173	5537	6261	8379	5160	7880	8883
27-Mar-17	Spring 2017	7220	0	10031	0	3197	7691	2134
Fall Results:								
Percent Change:		134%	78%	159%	107%	119%	179%	158%
Percent Difference:		34%	-22%	59%	7%	19%	79%	58%
Fall to Spring Results:								
Percent Change:		101%	Dead	160%	Dead	62%	98%	24%
Percent Difference:		1%		60%		-38%	-2%	-76%
Overall Change:								
Percent Change:		135%	Dead	255%	Dead	74%	175%	38%
Percent Difference:		35%		155%		-26%	75%	-62%

Capped Brood Cell Counts:
Before exposure (CCA1), the number of capped brood cells was similar across the control and treatment groups. After the 10-day exposure period (CCA2), the number of cells was lower in T1 and T2 as compared to the control, while the number of cells was increased in T3. At CCA3, the number of capped brood cells in T2 and T3 were similar to the control, and T1 had a slightly decreased number of cells as compared to the control. In the last assessment before overwintering, there were slight decreases in T1, T2 was similar to the control, and there were increases in T3 as compared to the control. In the very last assessment in the spring after overwintering, treatments T1, T2, and T3 were decreased by ~2, 1.5, and 3-fold, respectively, as compared to the control.

Table 4. Mean number of capped brood cells at each CCA sampling event.

Capped Average Estimates								
Date	CCA	C Average	R1 Average	R2 Average	R3 Average	T1 Average	T2 Average	T3 Average
8-Jun-16	CCA1	9544	6176	9818	9925	9363	8629	8562
21-Jun-16	CCA2	1848	1948	704	2006	1159	1147	2317
7-Jul-16	CCA3	8486	5576	5865	3251	7570	8373	8646
17-Aug-16	Fall 2016	5429	5785	5371	3509	4376	5817	7369
27-Mar-17	Spring 2017	6786	0	11315	0	3282	4391	2134
Fall Results:								
Percent Change:		57%	94%	55%	35%	47%	67%	86%
Percent Difference:		-43%	-6%	-45%	-65%	-53%	-33%	-14%
Fall to Spring Results:								
Percent Change:		125%	Dead	211%	Dead	75%	75%	29%
Percent Difference:		25%		111%		-25%	-25%	-71%
Overall Change:								
Percent Change:		71%	Dead	115%	Dead	35%	51%	25%
Percent Difference:		-29%		15%		-65%	-49%	-75%

Colony Strength:
Before exposure (CCA1), colony strength was similar across the control and treatment groups. After the 10-day exposure period (CCA2), the control and treatment T3 were comparable, and colony strength was slightly lower in T2 and T3 as compared to the control. At CCA3, colony strength in T2 and T3 were similar to the control, and T1 had decreased colony strength as compared to the control. In the last assessment for overwintering, similar conditions observed at CCA3 prevailed, with the exception that colony strength was higher in T3 as compared to the control. In the very last assessment in the spring after overwintering, the control and T2 were similar, while T1 and T3 were decreased by ~2- and 3-fold, respectively, as compared to the control.

Table 5. Mean colony strength at each CCA sampling event.

Colony Strength Average Estimates								
Date	CCA	C Average	R1 Average	R2 Average	R3 Average	T1 Average	T2 Average	T3 Average
8-Jun-16	CCA1	14909	13265	13753	17729	13689	13034	14178
21-Jun-16	CCA2	8093	7862	5081	8050	6859	6984	8956
7-Jul-16	CCA3	15760	11764	10441	9243	12854	14604	14715
17-Aug-16	Fall 2016	12602	11322	11632	11888	9536	13697	16252
27-Mar-17	Spring 2017	14006	0	21346	0	6479	12082	4268
Fall Results:								
Percent Change:		85%	85%	85%	67%	70%	105%	115%
Percent Difference:		-15%	-15%	-15%	-33%	-30%	5%	15%
Fall to Spring Results:								
Percent Change:		111%	Dead	184%	Dead	68%	88%	26%
Percent Difference:		11%		84%		-32%	-12%	-74%
Overall Change:								
Percent Change:		94%	Dead	155%	Dead	47%	93%	30%
Percent Difference:		-6%		55%		-53%	-7%	-70%

Pollen Estimates:
Before exposure (CCA1), pollen estimates were similar between the control and treatment group T1. However, estimates were notably higher in T2 and T3 by 2 and 3-fold, respectively. After the 10-day exposure period (CCA2), estimates were lower in T1 as compared to the control, and were increased 9 and 7-fold in T2 and T3, respectively.

At CCA3, pollen estimates for T1 were similar to the control, while the estimates were increased 1.6 and 2-fold in T2 and T3, respectively. In the last assessment before overwintering, there were notable increases in pollen estimates in T1, T2, and T3 as compared to the control, with increases that were 5.3, 5.6, and 6.8-fold, respectively, of the estimates in the control. In the very last assessment in the spring after overwintering, treatments T1, T2, and T3 were decreased by 2.6, 2, and 4-fold, respectively, as compared to the control.

Table 6. Mean pollen estimates at each CCA sampling event.

Pollen Average Estimates								
Date	CCA	C Average	R1 Average	R2 Average	R3 Average	T1 Average	T2 Average	T3 Average
8-Jun-16	CCA1	241	815	620	1120	272	484	668
21-Jun-16	CCA2	9	254	5	89	5	80	65
7-Jul-16	CCA3	1364	1734	1879	3615	1341	2240	2885
17-Aug-16	Fall 2016	156	600	500	376	824	879	1056
27-Mar-17	Spring 2017	4721	0	6236	0	1841	2361	1213
Fall Results:								
Percent Change:		65%	74%	81%	34%	303%	181%	158%
Percent Difference:		-35%	-26%	-19%	-66%	203%	81%	58%
Fall to Spring Results:								
Percent Change:		3023%	Dead	1247%	Dead	223%	269%	115%
Percent Difference:		2923%		1147%		123%	169%	15%
Overall Change:								
Percent Change:		1956%	Dead	1005%	Dead	677%	488%	182%
Percent Difference:		1856%		905%		577%	388%	82%

Bee Brood Development: There were not enough eggs in all colonies to perform a 300-egg assessment for the 1st cohort. As indicated by the brood termination rate, most eggs did not move forward in development past the first stages. However, the study author notes that the compensation index for cohort 1 does indicate that there was some recovery from the tunnel stress and hive relocation in attempt to repopulate the colony. Overall, the control and all treatments were similar across endpoints.

Cohort 2 was marked as BFD1 on June 29th. All but one colony, Ce, had recovered from the tunnels effects enough to have sufficient eggs for marking. The study author noted that Cd was the largest hive in the study and appeared to have insufficient pollen resources for its population and therefore, it should be removed from the brood evaluation data as it is an incomparable data point. Overall, the control and all treatments were similar across endpoints. There was a slight increase in the cells with terminated/unexpected development and the brood termination rate in treatments T2 and T as compared to the control.

Table 7. Bee brood development of cohort 1 at each CCA sampling event.

DEVELOPMENT OF EGGS:	C	T1	T2	T3	R1	R2	R3
No. of cells:	224	234	287	268	300	239	198
Cells with normal development:	0	0	0	15	62	0	23
Cells with terminated/unexpected devel.:	224	234	287	254	238	239	175
Brood termination rate [%]:	100.0	100.0	100.0	95.0	79.2	100.0	92.3
Brood index:	0.0	0.0	0.0	0.3	1.0	0.0	0.4
Compensation index:	1.31	1.19	0.94	1.87	1.72	0.94	0.68

Table 8. Bee brood development of cohort 2 at each CCA sampling event.

DEVELOPMENT OF EGGS:	C	T1	T2	T3	R1	R2	R3
No. of cells:	281.8	300.0	300.0	300.0	300.0	300.0	300.0
Cells with normal development:	258.5	275.0	267.8	262.3	162.7	213.7	241.3
Cells with terminated/unexpected devel.:	23.3	25.0	32.2	37.7	137.3	86.3	58.7
Brood termination rate [%]:	8.0	8.3	10.7	12.6	45.8	28.8	19.6
Brood index:	4.6	4.6	4.5	4.4	2.7	3.6	4.0
Compensation index:	4.7	4.7	4.6	4.6	3.0	4.5	4.4

Winter Mortality:

The majority of colonies were lost in the late winter, which was attributable to temperature swings.

Table 9. Mortality in the Fall and Spring CCAs.

Mortality							
	C	T1	T2	T3	R1	R2	R3
Fall CCA	0	0	0	0	1	0	1
Spring CCA	3	1	5	5	3	0	3
Fall % Mortality	0%	0%	0%	0%	33%	0%	33%
Spring % Mortality	50%	17%	83%	83%	100%	0%	100%

Residues: Residue data were based on the plots used for residue sampling (Cs, TS1, TS2, and TS3). Values less than the LOQ but greater than the LOD were set as ½ the LOQ for mean calculations, whereas values <LOD were set as ½ the LOD. The LOQs and LODs are showing in Table 10. In general, residues were greatest in whole plant samples, followed by larval tissue, nectar, and bee bread (Tables 11-14).

For bee bread, only TS3a had any detectable sulfoxaflor residues at 0.0903 mg/kg (7DAE). No other sulfoxaflor or metabolite residues were detected in bee bread. By Spring 2017, a concentration of 0.0109 mg/kg was detected in one control group plot; no

other residues were detected in bee bread in Spring 2017.

Sulfoxaflor was detected in one control larval tissue sample above the LOQ (0.0013 mg/kg, 24 DAE); no other sulfoxaflor or metabolite residues were detected in control larval tissue at any sampling point. Concentrations in larval tissues peaked in all treatment levels 1 DAE (0.0746, 0.0836, and 0.070 mg/kg in T1, T2, and T3, respectively), and declined to <LOQ by Fall 2016 (66 DAE). The X11721061 metabolite was detected in T1 1DAE (0.0109 to 0.0111 mg/kg); no other metabolites were detected above the LOQ in the control or treatment larval tissue samples.

No sulfoxaflor or metabolite residues were detected above the LOD in control nectar. Sulfoxaflor residues peaked between 3 and 7 DAE in all three treatment groups, with maximum means of 0.00447, 0.0163, and 0.116 mg/kg in T1, T2, and T3, respectively; residues declined to <LOD in all treatments by Fall 2016 (66 DAE). The X11519540 metabolite was detected 10 DAE in T2 (0.0152 mg/kg); no other metabolites were detected above the LOQ.

No sulfoxaflor or metabolite residues were detected above the LOD in any control whole plant samples. Maximum sulfoxaflor residues were detected on the day of application in T1 (0.526 mg/kg) and T3 (3.31 mg/kg) and on 1DAE in T2 (0.541 mg/kg). Sulfoxaflor residues declined steadily in all treatment groups through 10 DAE, and remained above the LOQ. However, the X11719474 metabolite accounted for the majority of the total sulfoxaflor residues (TSR), with maximum concentrations occurring on the day of application (1.20, 3.82, and 6.24 mg/kg in T1, T2, and T3, respectively). The other metabolites were detected, though accounted for a much smaller portion of the TSR levels; metabolite residue levels increased with increasing nominal sulfoxaflor application rate.

Table 10. LOQs (mg/kg) and LODs (mg/kg) for sulfoxaflor and metabolites in each matrix.

Analyte	Whole Plant	Nectar	Bee Bread	Larval Tissue
Sulfoxaflor	LOQ: 0.010 LOD: 0.0030	LOQ: 0.0010 LOD: 0.00030	LOQ: 0.010 LOD: 0.0030	LOQ: 0.0010 LOD: 0.000257
Metabolites	LOQ: 0.010 LOD: 0.0030	LOQ: 0.010 LOD: 0.0030	LOQ: 0.010 LOD: 0.0030	LOQ: 0.010 LOD: 0.00257

Table 11. Mean (min, max) sulfoxaflor and metabolite residues in bee bread across all sampling dates.

DAE	Sulfoxaflor (mg/kg)	X11579457 (mg/kg)	X11719474 (mg/kg)	X11519540 (mg/kg)	X11721061 (mg/kg)	TSR (mg/kg)
Control						
-1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
21	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
35	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
T1 (0.023 lb ai/A)						
-1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
3	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
7	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
10	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
21	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
35	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
T2 (0.071 lb ai/A)						
-1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
1	<LOQ	<LOD	<LOQ	<LOD	<LOD	<LOQ
21	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
35	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
T3 (0.090 lb ai/A)						
-1	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
7	0.0903	<LOD	0.123	0.0152	0.0138	0.244
21	<LOQ	<LOD	<LOD	<LOD	<LOD	<LOQ
35	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
288	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

LOQ- 0.010 mg/kg for sulfoxaflor and metabolites
LOD- 0.0030 mg/kg for sulfoxaflor and metabolites

Table 12. Mean (min, max) sulfoxaflor and metabolite residues in larval tissue across all sampling dates.

DAE	Sulfoxaflor (mg/kg)	X11579457 (mg/kg)	X11719474 (mg/kg)	X11519540 (mg/kg)	X11721061 (mg/kg)	TSR (mg/kg)
Control						
-1	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
0	<LOQ (<LOD, <LOQ)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOQ (<LOD, <LOQ)
1	0.00130 (<LOD, 0.00248)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.0064 (<LOD, 0.00762)
3	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
7	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
10	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
24	<LOQ (<LOD, 0.00130)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOQ (<LOD, 0.00644)
66	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
T1 (0.023 lb ai/A)						
-1	0.00157 (<LOD, 0.00301)	<LOQ (<LOD, <LOQ)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.00857 (<LOD, 0.0119)
0	0.00438 (0.00366, 0.00509)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.00952 (0.00880, 0.0102)
1	0.0600 (0.0453, 0.0746)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.011 (0.0109, 0.0111)	0.0748 (0.0603, 0.0894)
3	<LOQ (<LOD, <LOQ)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOQ (<LOD, <LOQ)
7	<LOQ (<LOD, <LOQ)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOQ (<LOD, <LOQ)
10	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
24	0.00221 (<LOD, 0.0043)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.00735 (<LOD, 0.00944)
66	<LOQ (<LOD, <LOQ)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOQ (<LOD, <LOQ)
T2 (0.071 lb ai/A)						
-1	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
1	0.0524 (0.0212, 0.0836)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOQ (<LOQ, 0.0137)	0.0656 (0.0301, 0.102)
3	0.00776 (<LOD, 0.0154)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.0129 (<LOD, 0.0205)
7	<LOQ (<LOD, <LOQ)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOQ (<LOD, <LOQ)
10	0.00133 (<LOD, 0.00253)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.00647 (<LOD, 0.00767)
24	0.00179	<LOD	<LOD	<LOD	<LOD	0.00693

	(0.00104, 0.00254)	(<LOD, <LOD)	(<LOD, <LOD)	(<LOD, <LOD)	(<LOD, <LOD)	(0.00618, 0.00768)
36	<LOQ (<LOQ, <LOQ)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOQ (<LOQ, <LOQ)
T3 (0.090 lb ai/A)						
-1	<LOD (LOD, <LOD)	<LOD (LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (LOD, <LOD)	<LOD (<LOD, <LOD)
0	0.00165	<LOD	<LOD	<LOD	<LOD	0.00679
1	0.070	<LOD	<LOD	<LOD	0.0238	0.0977
3	0.0296 (<LOD, 0.0590)	<LOQ (<LOD, <LOQ)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.0366 (<LOD, 0.00679)
7	0.00925 (<LOQ, 0.018)	<LOD (<LOD, <LOD)	<LOQ (<LOD, 0.0151)	<LOQ (<LOD, <LOQ)	<LOQ (<LOD, <LOQ)	0.0250 (<LOQ, 0.0444)
10	0.00191 (<LOD, 0.00369)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.00705 (<LOD, 0.00883)
24	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
36	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
66	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
288	<LOQ (<LOQ, <LOQ)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)

LOQ- 0.0010 mg/kg for sulfoxaflor and 0.010 mg/kg for metabolites
LOD- 0.000257 mg/kg for sulfoxaflor and 0.00257 mg/kg for metabolites

Table 13. Mean (min, max) sulfoxaflor and metabolite residues in nectar across all sampling dates.

DAE	Sulfoxaflor (mg/kg)	X11579457 (mg/kg)	X11719474 (mg/kg)	X11519540 (mg/kg)	X11721061 (mg/kg)	TSR (mg/kg)
Control						
-1	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
0	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
1	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
3	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
7	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
10	<LOQ (<LOQ, <LOQ)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
24	<LOQ (<LOD, <LOQ)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
36	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
66	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
T1 (0.023 lb ai/A)						
-1	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
0	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

	(<LOD, <LOD)	(<LOD, <LOD)	(<LOD, <LOD)	(<LOD, <LOD)	(<LOD, <LOD)	(<LOD, <LOD)
1	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
3	0.00447 (<LOD, 0.00879)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.0105 (<LOD, 0.0148)
7	0.00179 (0.00133, 0.00225)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.0779 (0.0065, 0.00881)
10	0.00167 (<LOQ, 0.00281)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.00633 (<LOQ, 0.00650
24	<LOQ (<LOQ, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOQ (<LOD, <LOQ)
36	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
66	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
T2 (0.071 lb ai/A)						
-1	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
1	0.00420 (0.00241, 0.00599)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.0102 (0.00841, 0.0120)
3	0.0144 (0.00695, 0.0219)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.0204 (0.0130, 0.0279)
7	0.0163 (0.0121, 0.0205)	<LOD (<LOD, <LOD)	<LOQ (<LOD, <LOQ)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.0241 (0.0181, 0.0300)
10	0.0124 (0.00637, 0.0185)	<LOD (<LOD, <LOD)	<LOQ (<LOD, <LOQ)	0.00835 (<LOD, 0.0152)	<LOD (<LOD, <LOD)	0.0270 (0.0124, 0.0417)
24	0.00154 (0.00127, 0.00180)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.00754 (0.00727, 0.00780)
36	0.00123 (<LOQ, 0.00195)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.00723 (<LOQ, 0.00795)
66	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
T3 (0.090 lb ai/A)						
-1	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
0	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOQ (<LOQ, <LOQ)	<LOD (<LOD, <LOD)
1	0.00289 (0.00176, 0.00401)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.00889 (0.00776, 0.0100)
3	0.00364 (0.00272, 0.00455)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.00964 (0.00872, 0.0106)
7	0.0116 (0.0114, 0.0118)	<LOD (<LOD, <LOD)	<LOQ (<LOQ, <LOQ)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.0211 (0.0209, 0.0213)
10	0.0102 (0.00851, 0.0119)	<LOD (<LOD, <LOD)	<LOQ (<LOQ, <LOQ)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	0.0197 (0.0180, 0.0214)

24	0.00201 (0.00140, 0.00261)	<LOD (<LOD, <LOD)	<LOQ (<LOD, <LOQ)	<LOD (<LOD, <LOD)	<LOQ (<LOD, <LOQ)	0.0115 (0.00861, 0.0144)
36	<LOQ (<LOQ, 0.00101)	<LOD (<LOD, <LOD)	<LOQ (<LOD, <LOQ)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOQ (<LOQ, 0.0105)
66	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)

LOQ- 0.0010 mg/kg for sulfoxaflor and 0.010 mg/kg for metabolites
LOD- 0.00030 mg/kg for sulfoxaflor and 0.0030 mg/kg for metabolites

Table 14. Mean (min, max) sulfoxaflor and metabolite residues in whole plants across all sampling dates.

DAE	Sulfoxaflor (mg/kg)	X11579457 (mg/kg)	X11719474 (mg/kg)	X11519540 (mg/kg)	X11721061 (mg/kg)	TSR (mg/kg)
Control						
-1	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
0	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
1	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
4	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
6	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
7	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
8	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
9	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
10	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
T1 (0.023 lb ai/A)						
-1	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
0	0.393 (0.260, 0.526)	<LOQ (<LOQ, <LOQ)	1.17 (1.14, 1.20)	<LOQ (<LOQ, <LOQ)	0.0146 (0.0132, 0.0160)	1.59 (1.42, 1.75)
1	0.175 (0.0777, 0.273)	<LOD (<LOD, <LOD)	0.206 (0.149, 0.263)	<LOQ (<LOQ, <LOQ)	0.0152 (0.0102, 0.0202)	0.403 (0.253, 0.553)
4	0.243 (0.191, 0.294)	<LOD (<LOD, <LOD)	0.151 (0.148, 0.153)	<LOQ (<LOQ, <LOQ)	0.0131 (0.0121, 0.0140)	0.413 (0.360, 0.466)
6	0.0634	<LOD	0.0820	<LOD	0.0113	0.160
7	0.0160 (0.0158, 0.0162)	<LOD (<LOD, <LOD)	0.159 (0.124, 0.193)	<LOD (<LOD, <LOD)	0.0156 (0.0138, 0.0173)	0.193 (0.160, 0.226)
8	0.0471 (0.0302, 0.0639)	<LOD (<LOD, <LOD)	0.0792 (0.0504, 0.108)	<LOD (<LOD, <LOD)	0.00940 (<LOQ, 0.0138)	0.139 (0.131, 0.146)
9	0.0642 (0.0595, 0.0689)	<LOQ (<LOD, <LOQ)	0.0923 (0.0456, 0.139)	<LOQ (<LOQ, <LOQ)	0.0121 (0.0102, 0.0140)	0.175 (0.118, 0.232)
10	0.0135 (0.0128, 0.0141)	<LOQ (<LOD, <LOQ)	0.173 (0.125, 0.220)	<LOQ (<LOQ, <LOQ)	0.0119 (0.0100, 0.0137)	0.204 (0.156, 0.253)

T2 (0.071 lb ai/A)					
-1	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
0	0.436 (0.414, 0.457)	0.00965 (<LOQ, 0.0143)	3.17 (2.51, 3.82)	0.0228 (0.0219, 0.0236)	0.0457 (0.0446, 0.0468)	3.68 (3.00, 4.36)
1	0.468 (0.394, 0.541)	<LOQ (<LOQ, <LOQ)	1.20 (1.17, 1.23)	0.0301 (0.0264, 0.0338)	0.0910 (0.0833, 0.0987)	1.79 (1.68, 1.91)
4	0.247 (0.148, 0.346)	<LOQ (<LOD, <LOQ)	0.502 (0.406, 0.598)	0.0148 (0.0124, 0.0172)	0.0635 (0.0583, 0.0687)	0.831 (0.829, 0.832)
6	0.0433 (0.0300, 0.0566)	<LOD (<LOD, <LOD)	0.357 (0.351, 0.362)	<LOQ (<LOQ, <LOQ)	0.0740 (0.0645, 0.0835)	0.480 (0.463, 0.498)
7	0.0640 (0.0517, 0.762)	<LOQ (<LOQ, <LOQ)	0.541 (0.483, 0.599)	<LOQ (<LOQ, 0.0107)	0.0775 (0.0642, 0.0907)	0.695 (0.660, 0.731)
8	0.0397 (0.0253, 0.0541)	<LOQ (<LOD, <LOQ)	0.311 (0.297, 0.325)	<LOQ (<LOQ, <LOQ)	0.0432 (0.0387, 0.0476)	0.402 (0.368, 0.437)
9	0.162 (0.158, 0.166)	<LOQ (<LOD, <LOQ)	0.242 (0.226, 0.257)	<LOQ (<LOQ, <LOQ)	0.0458 (0.0387, 0.0529)	0.458 (0.443, 0.472)
10	0.0282 (0.0250, 0.0314)	<LOQ (<LOD, <LOQ)	0.310 (0.260, 0.360)	<LOQ (<LOQ, <LOQ)	0.0343 (0.0276, 0.0410)	0.381 (0.333, 0.429)
T3 (0.090 lb ai/A)					
-1	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)	<LOD (<LOD, <LOD)
0	2.58 (1.84, 3.31)	0.0123 (0.0118, 0.0128)	6.21 (6.18, 6.24)	0.0521 (0.0451, 0.0590)	0.104 (0.0887, 0.119)	8.95 (8.26, 9.65)
1	0.639 (0.442, 0.835)	<LOQ (<LOQ, <LOQ)	0.975 (0.710, 1.24)	0.0260 (0.0229, 0.0290)	0.0599 (0.0590, 0.0607)	1.70 (1.63, 1.78)
4	0.511 (0.143, 0.878)	<LOQ (<LOQ, <LOQ)	0.826 (0.680, 0.971)	0.0192 (0.0171, 0.0213)	0.0667 (0.0586, 0.0747)	1.43 (1.19, 1.66)
6	0.222 (0.0826, 0.362)	<LOQ (<LOQ, <LOQ)	0.455 (0.374, 0.535)	<LOQ (<LOQ, <LOQ)	0.0671 (0.0482, 0.0859)	0.754 (0.676, 0.832)
7	0.0633 (0.0369, 0.0896)	<LOQ (<LOQ, <LOQ)	0.531 (0.515, 0.547)	<LOQ (<LOQ, <LOQ)	0.0554 (0.0502, 0.0605)	0.660 (0.612, 0.707)
8	0.0768 (0.0567, 0.0969)	<LOQ (<LOQ, <LOQ)	0.474 (0.430, 0.518)	<LOQ (<LOQ, <LOQ)	0.0612 (0.0481, 0.0743)	0.622 (0.545, 0.699)
9	0.0753 (0.0653, 0.0852)	<LOQ (<LOQ, <LOQ)	0.513 (0.401, 0.624)	<LOQ (<LOQ, <LOQ)	0.0713 (0.0608, 0.0817)	0.669 (0.537, 0.801)
10	0.0498 (0.0400, 0.0595)	<LOQ (<LOQ, <LOQ)	0.638 (0.586, 0.690)	0.0111 (0.0106, 0.0115)	0.0529 (0.0457, 0.0601)	0.757 (0.721, 0.792)

LOQ- 0.010 mg/kg for sulfoxaflor and metabolites
LOD- 0.0030 mg/kg for sulfoxaflor and metabolites

15. REVIEWER'S CONSIDERATION OF STUDY STRENGTHS, LIMITATIONS, AND INTERPRETATION

It is important to recognize the inherent strengths and limitations of this study as results are interpreted and potentially considered in risk assessment.

In the context of available field studies involving honey bees, this study contains some strengths including:

- Inclusion of multiple colony-level endpoints reflecting hive condition, brood development, and nectar/pollen availability.
- Availability of raw data for conducting statistical analysis.
- Quantification of exposure to sulfoxaflor and metabolites in hive matrices (uncapped nectar, honey, bee bread)
- Sulfoxaflor was quantified in the solutions used to treat the crops for the exposure.

A number of limitations were noted, including:

- Relatively low number of replicates (n = 6) for each treatment and controls.
- Sulfoxaflor was detected in matrices of several control groups.
- Only one application method was tested.
- Not all colonies had enough eggs which led to a weaker analysis.
- Pupal samples were inadvertently analyzed instead of larval samples.
- Colony size was not equalized, and most hives did not meet the population criteria listed in the protocol.
- Overwintering survival was very poor in control hives which excludes use of that endpoint in analysis.

16. REVIEWER'S COMMENTS

Preliminary non-GLP assessments of hives took place from June 30-July 1 2016. Study initiation took place May 10, 2016. The start of the experimental phase was June 12, 2016. The end of the experimental phase was March 27, 2017. Study completion date was December 18, 2017.

Signed and Dated No Data Confidentiality, GLP, and Quality Assurance statements were provided. This study was conducted in accordance with OCSPP 850.3040, OECD guidance document No. 75 (2007), and OEPP/EPPO Guideline No. 170 (4), 2010.

17. REFERENCES

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All other references are standard guidelines and methodologies.

APPENDIX A

```
R version 3.5.2 (2018-12-20) -- "Eggshell Igloo"
Copyright (C) 2018 The R Foundation for Statistical Computing
Platform: x86_64-w64-mingw32/x64 (64-bit)

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'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.

[Workspace loaded from ~/.RData]

library("dplyr")
library("ggpubr")
library('DescTools')
Tunnel2<-
read.csv(file='C:/Users/mniesen/Documents/Rwork/Tunnel/USTunnel.
csv', header=TRUE)
Mort2<-
read.csv(file='C:/Users/mniesen/Documents/Rwork/Tunnel/USMortali
ty.csv', header=TRUE)
> with(Tunnel2, tapply(Adult.CC, Day, shapiro.test))
$`-4`
```

```
        Shapiro-Wilk normality test

data:  X[[i]]
W = 0.97986, p-value = 0.8932


$`8`

        Shapiro-Wilk normality test

data:  X[[i]]
W = 0.95238, p-value = 0.3049


$`25`

        Shapiro-Wilk normality test

data:  X[[i]]
W = 0.94868, p-value = 0.2536


$`66`

        Shapiro-Wilk normality test

data:  X[[i]]
W = 0.92106, p-value = 0.06164


$`288`

        Shapiro-Wilk normality test

data:  X[[i]]
W = 0.65444, p-value = 2.663e-06


> with(Tunnel2, tapply(Pollen, Day, shapiro.test))
$`-4`

        Shapiro-Wilk normality test

data:  X[[i]]
W = 0.83602, p-value = 0.001216


$`8`

        Shapiro-Wilk normality test
```

```
data:  X[[i]]
W = 0.68242, p-value = 5.95e-06


$`25`

        Shapiro-Wilk normality test

data:  X[[i]]
W = 0.87295, p-value = 0.006013


$`66`

        Shapiro-Wilk normality test

data:  X[[i]]
W = 0.92747, p-value = 0.0856


$`288`

        Shapiro-Wilk normality test

data:  X[[i]]
W = 0.59129, p-value = 4.945e-07
```

> bartlett.test(Adult.CC[Day=="-4"] ~ Trt[Day=="-4"], Tunnel2)

```
        Bartlett test of homogeneity of variances

data:  Adult.CC[Day == "-4"] by Trt[Day == "-4"]
Bartlett's K-squared = 1.7765, df = 3, p-value = 0.6201
```

> bartlett.test(Adult.CC[Day=="8"] ~ Trt[Day=="8"], Tunnel2)

```
        Bartlett test of homogeneity of variances

data:  Adult.CC[Day == "8"] by Trt[Day == "8"]
Bartlett's K-squared = 4.5066, df = 3, p-value = 0.2117
```

> bartlett.test(Adult.CC[Day=="25"] ~ Trt[Day=="25"], Tunnel2)

```
        Bartlett test of homogeneity of variances

data:  Adult.CC[Day == "25"] by Trt[Day == "25"]
Bartlett's K-squared = 1.4276, df = 3, p-value = 0.6991
```

> bartlett.test(Adult.CC[Day=="66"] ~ Trt[Day=="66"], Tunnel2)

```
        Bartlett test of homogeneity of variances
```

```
data:  Adult.CC[Day == "66"] by Trt[Day == "66"]
Bartlett's K-squared = 7.1962, df = 3, p-value = 0.0659

> bartlett.test(Adult.CC[Day=="288"] ~ Trt[Day=="288"], Tunnel2)

        Bartlett test of homogeneity of variances

data:  Adult.CC[Day == "288"] by Trt[Day == "288"]
Bartlett's K-squared = 12.899, df = 3, p-value = 0.004859


>
> bartlett.test(Pollen[Day=="-4"] ~ Trt[Day=="-4"], Tunnel2)

        Bartlett test of homogeneity of variances

data:  Pollen[Day == "-4"] by Trt[Day == "-4"]
Bartlett's K-squared = 12.968, df = 3, p-value = 0.004707

> bartlett.test(Pollen[Day=="8"] ~ Trt[Day=="8"], Tunnel2)

        Bartlett test of homogeneity of variances

data:  Pollen[Day == "8"] by Trt[Day == "8"]
Bartlett's K-squared = 32.661, df = 3, p-value = 3.798e-07

> bartlett.test(Pollen[Day=="25"] ~ Trt[Day=="25"], Tunnel2)

        Bartlett test of homogeneity of variances

data:  Pollen[Day == "25"] by Trt[Day == "25"]
Bartlett's K-squared = 5.0114, df = 3, p-value = 0.171

> bartlett.test(Pollen[Day=="66"] ~ Trt[Day=="66"], Tunnel2)

        Bartlett test of homogeneity of variances

data:  Pollen[Day == "66"] by Trt[Day == "66"]
Bartlett's K-squared = 2.3522, df = 3, p-value = 0.5026

> bartlett.test(Pollen[Day=="288"] ~ Trt[Day=="288"], Tunnel2)

        Bartlett test of homogeneity of variances

data:  Pollen[Day == "288"] by Trt[Day == "288"]
Bartlett's K-squared = 18.24, df = 3, p-value = 0.0003924

> DunnettTest(Pollen~Trt, data=Tunnel2[Tunnel2$Day=='66',])

  Dunnett's test for comparing several treatments with a control :
    95% family-wise confidence level
```

```
$Cont
            diff    lwr.ci    upr.ci    pval
T1-Cont 667.8333 160.8979 1174.769 0.00893 **
T2-Cont 722.5000 215.5645 1229.435 0.00463 **
T3-Cont 899.3333 392.3979 1406.269 0.00067 ***

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

>
> DunnettTest(Adult.CC~Trt, data=Tunnel2[Tunnel2$Day=='-4',])

  Dunnett's test for comparing several treatments with a control :
    95% family-wise confidence level

$Cont
            diff    lwr.ci    upr.ci    pval
T1-Cont -180.5000 -2848.240 2487.240 0.9963
T2-Cont -915.1667 -3582.907 1752.574 0.7184
T3-Cont -981.8333 -3649.574 1685.907 0.6763

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> DunnettTest(Adult.CC~Trt, data=Tunnel2[Tunnel2$Day=='8',])

  Dunnett's test for comparing several treatments with a control :
    95% family-wise confidence level

$Cont
          diff    lwr.ci    upr.ci    pval
T1-Cont -688.5 -2023.4067  646.4067 0.4338
T2-Cont -700.5 -2035.4067  634.4067 0.4204
T3-Cont  469.0  -865.9067 1803.9067 0.7046

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> DunnettTest(Adult.CC~Trt, data=Tunnel2[Tunnel2$Day=='25',])

  Dunnett's test for comparing several treatments with a control :
    95% family-wise confidence level

$Cont
            diff    lwr.ci    upr.ci    pval
T1-Cont -916.1667 -3201.688 1369.355 0.6203
T2-Cont -112.5000 -2398.021 2173.021 0.9986
T3-Cont  160.3333 -2125.188 2445.855 0.9959

---
```

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> DunnettTest(Adult.CC~Trt, data=Tunnel2[Tunnel2$Day=='66',])

  Dunnett's test for comparing several treatments with a control :
    95% family-wise confidence level

$Cont
              diff   lwr.ci   upr.ci   pval
T1-Cont -1053.6667 -4571.359 2464.026 0.7889
T2-Cont   387.6667 -3130.026 3905.359 0.9847
T3-Cont 1939.6667 -1578.026 5457.359 0.3822

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> #tunnel 2------------
> with(Tunnel2[Tunnel2$Day=="288",], pairwise.wilcox.test(Adult.CC, Trt, p.ad
j = 'bonf'))

        Pairwise comparisons using Wilcoxon rank sum test

data: Adult.CC and Trt

   Cont T1   T2
T1 1.00 -   -
T2 1.00 0.36 -
T3 1.00 0.24 1.00

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
6: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Tunnel2[Tunnel2$Day=="25",], pairwise.wilcox.test(Pollen, Trt, p.adj =
'bonf'))

        Pairwise comparisons using Wilcoxon rank sum test

data: Pollen and Trt

   Cont T1   T2

33

```
T1 1.00 -     -
T2 1.00 1.00 -
T3 1.00 0.25 1.00

P value adjustment method: bonferroni
```
> with(Tunnel2[Tunnel2$Day=="8",], pairwise.wilcox.test(Pollen, Trt, p.adj =
'bonf'))#check further Cont and T2

```
        Pairwise comparisons using Wilcoxon rank sum test

data:  Pollen and Trt

   Cont  T1    T2
T1 1.000 -     -
T2 0.077 0.077 -
T3 0.553 0.323 1.000

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
```
> with(Tunnel2[Tunnel2$Day=="-4",], pairwise.wilcox.test(Pollen, Trt, p.adj =
'bonf'))

```
        Pairwise comparisons using Wilcoxon rank sum test

data:  Pollen and Trt

   Cont T1 T2
T1 1    -  -
T2 1    1  -
T3 1    1  1

P value adjustment method: bonferroni
```
> with(Tunnel2[Tunnel2$Day=="288",], pairwise.wilcox.test(Pollen, Trt, p.adj
= 'bonf'))#check further Cont and T2

```
        Pairwise comparisons using Wilcoxon rank sum test

data:  Pollen and Trt

   Cont T1    T2
T1 1.00 -     -
```

```
T2 1.00 0.52 -
T3 1.00 0.24 1.00

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
6: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties

> with(Mort2, tapply(Mortality, Day, shapiro.test))
$`0`

        Shapiro-Wilk normality test

data:  X[[i]]
W = 0.80418, p-value = 0.0003451


$`1`

        Shapiro-Wilk normality test

data:  X[[i]]
W = 0.85712, p-value = 0.002972


$`2`

        Shapiro-Wilk normality test

data:  X[[i]]
W = 0.6898, p-value = 7.407e-06


$`3`

        Shapiro-Wilk normality test

data:  X[[i]]
W = 0.81674, p-value = 0.0005602
```

$`4`

        Shapiro-Wilk normality test

data: X[[i]]
W = 0.70806, p-value = 1.29e-05

$`5`

        Shapiro-Wilk normality test

data: X[[i]]
W = 0.69877, p-value = 9.704e-06

$`6`

        Shapiro-Wilk normality test

data: X[[i]]
W = 0.78945, p-value = 0.000199

$`7`

        Shapiro-Wilk normality test

data: X[[i]]
W = 0.62982, p-value = 1.353e-06

$`8`

        Shapiro-Wilk normality test

data: X[[i]]
W = 0.91106, p-value = 0.03719

$`9`

        Shapiro-Wilk normality test

data: X[[i]]
W = 0.84449, p-value = 0.00173

> bartlett.test(Mortality[Day=="2"] ~ Trt[Day=="2"], Mort2)

        Bartlett test of homogeneity of variances

```
data:  Mortality[Day == "2"] by Trt[Day == "2"]
Bartlett's K-squared = 27.025, df = 3, p-value = 5.816e-06

> bartlett.test(Mortality[Day=="3"] ~ Trt[Day=="3"], Mort2)

        Bartlett test of homogeneity of variances

data:  Mortality[Day == "3"] by Trt[Day == "3"]
Bartlett's K-squared = 12.017, df = 3, p-value = 0.007326

> bartlett.test(Mortality[Day=="4"] ~ Trt[Day=="4"], Mort2)

        Bartlett test of homogeneity of variances

data:  Mortality[Day == "4"] by Trt[Day == "4"]
Bartlett's K-squared = 25.451, df = 3, p-value = 1.243e-05

> bartlett.test(Mortality[Day=="5"] ~ Trt[Day=="5"], Mort2)

        Bartlett test of homogeneity of variances

data:  Mortality[Day == "5"] by Trt[Day == "5"]
Bartlett's K-squared = 11.966, df = 3, p-value = 0.0075

> bartlett.test(Mortality[Day=="6"] ~ Trt[Day=="6"], Mort2)

        Bartlett test of homogeneity of variances

data:  Mortality[Day == "6"] by Trt[Day == "6"]
Bartlett's K-squared = 12.051, df = 3, p-value = 0.007209

> bartlett.test(Mortality[Day=="7"] ~ Trt[Day=="7"], Mort2)

        Bartlett test of homogeneity of variances

data:  Mortality[Day == "7"] by Trt[Day == "7"]
Bartlett's K-squared = 17.676, df = 3, p-value = 0.000513

> bartlett.test(Mortality[Day=="8"] ~ Trt[Day=="8"], Mort2)

        Bartlett test of homogeneity of variances

data:  Mortality[Day == "8"] by Trt[Day == "8"]
Bartlett's K-squared = 1.6012, df = 3, p-value = 0.6591

> bartlett.test(Mortality[Day=="9"] ~ Trt[Day=="9"], Mort2)

        Bartlett test of homogeneity of variances

data:  Mortality[Day == "9"] by Trt[Day == "9"]
```

Bartlett's K-squared = 11.871, df = 3, p-value = 0.007839

```
> bartlett.test(Mortality[Day=="0"] ~ Trt[Day=="0"], Mort2)
```

       Bartlett test of homogeneity of variances

data: Mortality[Day == "0"] by Trt[Day == "0"]
Bartlett's K-squared = 38.879, df = 3, p-value = 1.842e-08

```
> bartlett.test(Mortality[Day=="1"] ~ Trt[Day=="1"], Mort2)
```

       Bartlett test of homogeneity of variances

data: Mortality[Day == "1"] by Trt[Day == "1"]
Bartlett's K-squared = 7.9219, df = 3, p-value = 0.04765

```
> with(Mort2[Mort2$Day=="0",], pairwise.wilcox.test(Mortality, Trt, p.adj = '
bonf'))
```

       Pairwise comparisons using Wilcoxon rank sum test

data: Mortality and Trt

```
   Cont  T1    T2
T1 0.013 -     -
T2 0.013 0.026 -
T3 0.030 0.030 1.000
```

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
```
> with(Mort2[Mort2$Day=="1",], pairwise.wilcox.test(Mortality, Trt, p.adj = '
bonf'))
```

       Pairwise comparisons using Wilcoxon rank sum test

data: Mortality and Trt

```
   Cont  T1    T2
T1 1.000 -     -
T2 0.180 1.000 -
T3 0.030 0.091 0.792
```

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :

```
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Mort2[Mort2$Day=="2",], pairwise.wilcox.test(Mortality, Trt, p.adj = '
bonf'))

        Pairwise comparisons using Wilcoxon rank sum test

data:  Mortality and Trt

   Cont T1   T2
T1 1.00 -    -
T2 1.00 1.00 -
T3 0.03 0.03 1.00

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Mort2[Mort2$Day=="3",], pairwise.wilcox.test(Mortality, Trt, p.adj = '
bonf'))

        Pairwise comparisons using Wilcoxon rank sum test

data:  Mortality and Trt

   Cont  T1    T2
T1 1.000 -     -
T2 0.466 0.156 -
T3 0.013 0.091 1.000

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Mort2[Mort2$Day=="4",], pairwise.wilcox.test(Mortality, Trt, p.adj = '
bonf'))

        Pairwise comparisons using Wilcoxon rank sum test
```

```
data:  Mortality and Trt

   Cont T1   T2
T1 0.18 -    -
T2 1.00 1.00 -
T3 0.18 0.03 1.00

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Mort2[Mort2$Day=="5",], pairwise.wilcox.test(Mortality, Trt, p.adj = '
bonf'))

        Pairwise comparisons using Wilcoxon rank sum test

data:  Mortality and Trt

   Cont T1 T2
T1 1    -  -
T2 1    1  -
T3 1    1  1

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Mort2[Mort2$Day=="6",], pairwise.wilcox.test(Mortality, Trt, p.adj = '
bonf'))

        Pairwise comparisons using Wilcoxon rank sum test

data:  Mortality and Trt
```

```
    Cont T1    T2
T1 1.00 -     -
T2 1.00 0.89 -
T3 1.00 1.00  1.00

P value adjustment method: bonferroni
```

Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
6: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Mort2[Mort2$Day=="7",], pairwise.wilcox.test(Mortality, Trt, p.adj = '
bonf'))

```
        Pairwise comparisons using Wilcoxon rank sum test

data:  Mortality and Trt

    Cont T1 T2
T1 1    -  -
T2 1    1  -
T3 1    1  1

P value adjustment method: bonferroni
```

Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
6: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Mort2[Mort2$Day=="8",], pairwise.wilcox.test(Mortality, Trt, p.adj = '
bonf'))

```
        Pairwise comparisons using Wilcoxon rank sum test

data:  Mortality and Trt
```

```
    Cont T1    T2
T1 1.00 -     -
T2 1.00 1.00 -
T3 0.65 1.00 0.46

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
   cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
   cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
   cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
   cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
   cannot compute exact p-value with ties
6: In wilcox.test.default(xi, xj, paired = paired, ...) :
   cannot compute exact p-value with ties
> with(Mort2[Mort2$Day=="9",], pairwise.wilcox.test(Mortality, Trt, p.adj = '
bonf'))

        Pairwise comparisons using Wilcoxon rank sum test

data:  Mortality and Trt

    Cont  T1     T2
T1 1.000 -      -
T2 0.026 0.116 -
T3 1.000 1.000 0.388

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
   cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
   cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
   cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
   cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
   cannot compute exact p-value with ties

> with(Mort2[Mort2$Day=="0",], pairwise.wilcox.test(Mortality, Trt, p.adj = '
bonf'))

        Pairwise comparisons using Wilcoxon rank sum test

data:  Mortality and Trt
```

```
    Cont  T1    T2
T1 0.013 -     -
T2 0.013 0.026 -
T3 0.030 0.030 1.000

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Mort2[Mort2$Day=="1",], pairwise.wilcox.test(Mortality, Trt, p.adj = '
bonf'))

        Pairwise comparisons using Wilcoxon rank sum test

data:  Mortality and Trt

    Cont  T1    T2
T1 1.000 -     -
T2 0.180 1.000 -
T3 0.030 0.091 0.792

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Mort2[Mort2$Day=="2",], pairwise.wilcox.test(Mortality, Trt, p.adj = '
bonf'))

        Pairwise comparisons using Wilcoxon rank sum test

data:  Mortality and Trt

    Cont T1    T2
T1 1.00 -     -
T2 1.00 1.00  -
T3 0.03 0.03 1.00

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
```

```
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Mort2[Mort2$Day=="3",], pairwise.wilcox.test(Mortality, Trt, p.adj = '
bonf'))

        Pairwise comparisons using Wilcoxon rank sum test

data:  Mortality and Trt

   Cont  T1    T2
T1 1.000 -     -
T2 0.466 0.156 -
T3 0.013 0.091 1.000

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Mort2[Mort2$Day=="4",], pairwise.wilcox.test(Mortality, Trt, p.adj = '
bonf'))

        Pairwise comparisons using Wilcoxon rank sum test

data:  Mortality and Trt

   Cont T1   T2
T1 0.18 -    -
T2 1.00 1.00 -
T3 0.18 0.03 1.00

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Mort2[Mort2$Day=="5",], pairwise.wilcox.test(Mortality, Trt, p.adj = '
bonf'))
```

```
        Pairwise comparisons using Wilcoxon rank sum test

data:  Mortality and Trt

    Cont T1 T2
T1 1     -  -
T2 1     1  -
T3 1     1  1

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Mort2[Mort2$Day=="6",], pairwise.wilcox.test(Mortality, Trt, p.adj = '
bonf'))

        Pairwise comparisons using Wilcoxon rank sum test

data:  Mortality and Trt

    Cont T1   T2
T1 1.00 -    -
T2 1.00 0.89 -
T3 1.00 1.00 1.00

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
6: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Mort2[Mort2$Day=="7",], pairwise.wilcox.test(Mortality, Trt, p.adj = '
bonf'))
```

```
        Pairwise comparisons using Wilcoxon rank sum test

data:  Mortality and Trt

    Cont T1 T2
T1 1     -  -
T2 1     1  -
T3 1     1  1

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
6: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Mort2[Mort2$Day=="8",], pairwise.wilcox.test(Mortality, Trt, p.adj = '
bonf'))

        Pairwise comparisons using Wilcoxon rank sum test

data:  Mortality and Trt

    Cont T1   T2
T1 1.00 -    -
T2 1.00 1.00 -
T3 0.65 1.00 0.46

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
6: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Mort2[Mort2$Day=="9",], pairwise.wilcox.test(Mortality, Trt, p.adj = '
bonf'))
```

```
        Pairwise comparisons using Wilcoxon rank sum test

data:  Mortality and Trt

   Cont  T1    T2
T1 1.000 -     -
T2 0.026 0.116 -
T3 1.000 1.000 0.388

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> DunnettTest(Mortality~Trt, data=Mort2[Mort2$Day=='0',])

  Dunnett's test for comparing several treatments with a control :
    95% family-wise confidence level

$Cont
             diff      lwr.ci   upr.ci    pval
T1-Cont  25.33333 -100.5760 151.2427 0.91951
T2-Cont 226.83333  100.9240 352.7427 0.00053 ***
T3-Cont 140.16667   14.2573 266.0760 0.02730 *

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> DunnettTest(Mortality~Trt, data=Mort2[Mort2$Day=='1',])

  Dunnett's test for comparing several treatments with a control :
    95% family-wise confidence level

$Cont
             diff      lwr.ci   upr.ci    pval
T1-Cont  31.33333 -49.871017 112.5377 0.64585
T2-Cont  82.66667   1.462317 163.8710 0.04525 *
T3-Cont 144.16667  62.962317 225.3710 0.00066 ***

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> DunnettTest(Mortality~Trt, data=Mort2[Mort2$Day=='2',])
```

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```
   Dunnett's test for comparing several treatments with a control :
      95% family-wise confidence level

$Cont
              diff     lwr.ci    upr.ci    pval
T1-Cont -4.166667 -67.33693  59.00359 0.9966
T2-Cont 42.500000 -20.67026 105.67026 0.2371
T3-Cont 49.500000 -13.67026 112.67026 0.1455

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

> DunnettTest(Mortality~Trt, data=Mort2[Mort2$Day=='3',])

```
   Dunnett's test for comparing several treatments with a control :
      95% family-wise confidence level

$Cont
              diff    lwr.ci   upr.ci    pval
T1-Cont  2.00000 -37.45297 41.45297 0.9984
T2-Cont 27.66667 -11.78630 67.11963 0.2101
T3-Cont 54.16667  14.71370 93.61963 0.0064 **

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

> DunnettTest(Mortality~Trt, data=Mort2[Mort2$Day=='4',])

```
   Dunnett's test for comparing several treatments with a control :
      95% family-wise confidence level

$Cont
               diff     lwr.ci   upr.ci    pval
T1-Cont -11.83333 -56.948473 33.28181 0.8443
T2-Cont   7.50000 -37.615140 52.61514 0.9516
T3-Cont  47.66667   2.551527 92.78181 0.0371 *

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

> DunnettTest(Mortality~Trt, data=Mort2[Mort2$Day=='5',])

```
   Dunnett's test for comparing several treatments with a control :
      95% family-wise confidence level

$Cont
              diff     lwr.ci   upr.ci    pval
T1-Cont -2.166667 -32.25886 27.92552 0.9956
T2-Cont  1.666667 -28.42552 31.75886 0.9980
T3-Cont 18.500000 -11.59219 48.59219 0.3005
```

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> DunnettTest(Mortality~Trt, data=Mort2[Mort2$Day=='6',])

  Dunnett's test for comparing several treatments with a control :
    95% family-wise confidence level

$Cont
              diff      lwr.ci    upr.ci    pval
T1-Cont -1.666667 -19.803146 16.46981 0.9909
T2-Cont  7.833333 -10.303146 25.96981 0.5677
T3-Cont  9.166667  -8.969813 27.30315 0.4494

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> DunnettTest(Mortality~Trt, data=Mort2[Mort2$Day=='7',])

  Dunnett's test for comparing several treatments with a control :
    95% family-wise confidence level

$Cont
              diff      lwr.ci    upr.ci    pval
T1-Cont  3.00000 -26.73737 32.73737 0.9881
T2-Cont 10.50000 -19.23737 40.23737 0.7016
T3-Cont 14.83333 -14.90404 44.57071 0.4596

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> DunnettTest(Mortality~Trt, data=Mort2[Mort2$Day=='8',])

  Dunnett's test for comparing several treatments with a control :
    95% family-wise confidence level

$Cont
              diff     lwr.ci    upr.ci    pval
T1-Cont 1.166667 -6.372302  8.705635 0.9602
T2-Cont 0.000000 -7.538968  7.538968 1.0000
T3-Cont 6.166667 -1.372302 13.705635 0.1237

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> DunnettTest(Mortality~Trt, data=Mort2[Mort2$Day=='9',])

  Dunnett's test for comparing several treatments with a control :
    95% family-wise confidence level
```

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```
$Cont
              diff     lwr.ci     upr.ci   pval
T1-Cont  -9.166667 -23.62420   5.290870 0.2785
T2-Cont -18.333333 -32.79087  -3.875796 0.0114 *
T3-Cont  -9.833333 -24.29087   4.624204 0.2300

---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

> with(Brood2T2, tapply(BI, Day, shapiro.test))
```
$D12

        Shapiro-Wilk normality test

data:  X[[i]]
W = 0.62236, p-value = 1.108e-06


$D16

        Shapiro-Wilk normality test

data:  X[[i]]
W = 0.63669, p-value = 1.63e-06


$D21

        Shapiro-Wilk normality test

data:  X[[i]]
W = 0.59028, p-value = 4.821e-07


$D6

        Shapiro-Wilk normality test

data:  X[[i]]
W = 0.77458, p-value = 0.0001164
```

> with(Brood2T2, tapply(CI, Day, shapiro.test))
```
$D12

        Shapiro-Wilk normality test

data:  X[[i]]
W = 0.71738, p-value = 1.724e-05
```

$D16

       Shapiro-Wilk normality test

data: X[[i]]
W = 0.24341, p-value = 3.888e-10

$D21

       Shapiro-Wilk normality test

data: X[[i]]
W = 0.68053, p-value = 5.628e-06

$D6

       Shapiro-Wilk normality test

data: X[[i]]
W = 0.87622, p-value = 0.00698


```
> with(Brood2T2, tapply(TR, Day, shapiro.test))
```
$D12

       Shapiro-Wilk normality test

data: X[[i]]
W = 0.62243, p-value = 1.111e-06

$D16

       Shapiro-Wilk normality test

data: X[[i]]
W = 0.63662, p-value = 1.627e-06

$D21

       Shapiro-Wilk normality test

data: X[[i]]
W = 0.60854, p-value = 7.704e-07

$D6

```
        Shapiro-Wilk normality test

data:  X[[i]]
W = 0.64286, p-value = 1.93e-06
```

```
> with(Brood2T2[Brood2T2$Day=="D6",], pairwise.wilcox.test(BI, Trt, p.adj = '
bonf'))
```

```
        Pairwise comparisons using Wilcoxon rank sum test

data:  BI and Trt

            CONTROL TREATMENT 1 TREATMENT 2
TREATMENT 1 1.00    -           -
TREATMENT 2 1.00    0.25        -
TREATMENT 3 1.00    1.00        1.00

P value adjustment method: bonferroni
```

```
> with(Brood2T2[Brood2T2$Day=="D12",], pairwise.wilcox.test(BI, Trt, p.adj =
'bonf'))
```

```
        Pairwise comparisons using Wilcoxon rank sum test

data:  BI and Trt

            CONTROL TREATMENT 1 TREATMENT 2
TREATMENT 1 1.00    -           -
TREATMENT 2 1.00    0.89        -
TREATMENT 3 1.00    1.00        1.00

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
6: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
```

```
> with(Brood2T2[Brood2T2$Day=="D16",], pairwise.wilcox.test(BI, Trt, p.adj =
'bonf'))
```

```
        Pairwise comparisons using Wilcoxon rank sum test

data:  BI and Trt
```

```
            CONTROL TREATMENT 1 TREATMENT 2
TREATMENT 1 1        -           -
TREATMENT 2 1        1           -
TREATMENT 3 1        1           1

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
   cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
   cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
   cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
   cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
   cannot compute exact p-value with ties
> with(Brood2T2[Brood2T2$Day=="D21",], pairwise.wilcox.test(BI, Trt, p.adj =
'bonf'))

        Pairwise comparisons using Wilcoxon rank sum test

data:  BI and Trt

            CONTROL TREATMENT 1 TREATMENT 2
TREATMENT 1 1.00    -           -
TREATMENT 2 1.00    0.65        -
TREATMENT 3 1.00    1.00        1.00

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
   cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
   cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
   cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
   cannot compute exact p-value with ties
> with(Brood2T2[Brood2T2$Day=="D6",], pairwise.wilcox.test(CI, Trt, p.adj = '
bonf'))

        Pairwise comparisons using Wilcoxon rank sum test

data:  CI and Trt

            CONTROL TREATMENT 1 TREATMENT 2
TREATMENT 1 1.00    -           -
TREATMENT 2 1.00    0.25        -
TREATMENT 3 1.00    1.00        1.00
```

```
P value adjustment method: bonferroni
> with(Brood2T2[Brood2T2$Day=="D12",], pairwise.wilcox.test(CI, Trt, p.adj =
'bonf'))

        Pairwise comparisons using Wilcoxon rank sum test

data:  CI and Trt

            CONTROL TREATMENT 1 TREATMENT 2
TREATMENT 1 1.00    -           -
TREATMENT 2 1.00    0.79        -
TREATMENT 3 1.00    1.00        1.00

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Brood2T2[Brood2T2$Day=="D21",], pairwise.wilcox.test(CI, Trt, p.adj =
'bonf'))

        Pairwise comparisons using Wilcoxon rank sum test

data:  CI and Trt

            CONTROL TREATMENT 1 TREATMENT 2
TREATMENT 1 1       -           -
TREATMENT 2 1       1           -
TREATMENT 3 1       1           1

P value adjustment method: bonferroni
> with(Brood2T2[Brood2T2$Day=="D16",], pairwise.wilcox.test(CI, Trt, p.adj =
'bonf'))

        Pairwise comparisons using Wilcoxon rank sum test

data:  CI and Trt

            CONTROL TREATMENT 1 TREATMENT 2
TREATMENT 1 1       -           -
TREATMENT 2 1       1           -
TREATMENT 3 1       1           1

P value adjustment method: bonferroni
Warning message:
In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Brood2T2[Brood2T2$Day=="D6",], pairwise.wilcox.test(TR, Trt, p.adj = '
bonf'))
```

Pairwise comparisons using Wilcoxon rank sum test

data: TR and Trt

```
            CONTROL TREATMENT 1 TREATMENT 2
TREATMENT 1 1.00    -           -
TREATMENT 2 1.00    0.54        -
TREATMENT 3 1.00    1.00        1.00
```

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Brood2T2[Brood2T2$Day=="D12",], pairwise.wilcox.test(TR, Trt, p.adj =
'bonf'))

Pairwise comparisons using Wilcoxon rank sum test

data: TR and Trt

```
            CONTROL TREATMENT 1 TREATMENT 2
TREATMENT 1 1.00    -           -
TREATMENT 2 1.00    0.89        -
TREATMENT 3 1.00    1.00        1.00
```

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
6: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Brood2T2[Brood2T2$Day=="D16",], pairwise.wilcox.test(TR, Trt, p.adj =
'bonf'))

Pairwise comparisons using Wilcoxon rank sum test

data: TR and Trt

```
          CONTROL TREATMENT 1 TREATMENT 2
TREATMENT 1 1        -           -
TREATMENT 2 1        1           -
TREATMENT 3 1        1           1

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
4: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
5: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
> with(Brood2T2[Brood2T2$Day=="D21",], pairwise.wilcox.test(TR, Trt, p.adj =
'bonf'))

        Pairwise comparisons using Wilcoxon rank sum test

data:  TR and Trt

          CONTROL TREATMENT 1 TREATMENT 2
TREATMENT 1 1        -           -
TREATMENT 2 1        1           -
TREATMENT 3 1        1           1

P value adjustment method: bonferroni
Warning messages:
1: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
2: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
3: In wilcox.test.default(xi, xj, paired = paired, ...) :
  cannot compute exact p-value with ties
```