SHAUGHNESSY NO. _____   REVIEW NO. _____

EEB REVIEW

DATE: IN __3/9/90__   OUT: MAR 2 0 1990

FILE OR REG. NO. __5481-197_____

PETITION OR EXP. NO. _____

DATE OF SUBMISSION _____12/12/89_____

DATE RECEIVED BY ~~HED~~XREFED __3/8/90_____

RD REQUESTED COMPLETION DATE __6/11/90_____

EEB ESTIMATED COMPLETION DATE __6/11/90_____

RD ACTION CODE/TYPE OF REVIEW __660_____

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TYPE PRODUCT(S): I, D, H, F, N, R, S _____

DATE ACCESSION NO (S). _____413213-01_____

PRODUCT MANAGER NO. __74_____

PRODUCT NAME (S) _____PCNB_____

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COMPANY NAME _____Amvac Chemical Corp._____

SUBMISSION PURPOSE __Review of Daphnia Life Cycle Test___

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SHAUGHNESSEY NO.	CHEMICAL AND FORMULATION	% A.I.
	PCNB	technical

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Data Evaluation Report
Ecological Effects Branch

1. **Chemical:** Pentachloronitrobenzene (PCNB)

2. **Test Material:** PCNB technical grade, 99% purity
   lot no. GAB 39011 received 4/4/88

3. **Study Type:** 21 Day Chronic Toxicity Effects to <u>Daphnia</u>
   <u>magna</u> Life Cycle.

4. **Study Identification:**

   Study Author: Burgess, David
   Laboratory: Analytical Bio-Chemistry
   Laboratories, Inc.Columbia, MS.
   Study Dates: June 9-30, 1989
   Laboratory Identification: No. 36830
   Sponsor: AMVAC Chemical Corp., Los Angeles, CA.
   EPA Identification: MRID No. 413213-01

5. **Reviewed by:** Brian Montague, Fisheries Biologist
   Ecological Effects Branch
   Environmental Fate and Effects Division

6. **Approved by:** Ray Matheny, Supervisory Biologist
   Ecological Effects Branch, Section I
   Environmental Fate and Effects Division(H7507C)

   3/19/90

7. **Conclusions:** The study has followed acceptable protocol
   guidelines for 21 day life cycle testing. The
   MATC limits were determined to fall within the
   0.018 and 0.030 mg/L concentrations with a point
   estimate of 0.023 mg/L. No concentration related
   effect was observed in adult growth with
   concentrations under 0.10 mg/L. Only the 0.23
   mg/L concentration level showed significant
   mortality. The estimated EC_{50} was 0.14 mg/L.

8. **Recommendation:** N/A

9. **Submission Purpose:** Submitted to satisfy 72-4 registration guideline requirements.

10. **Test Protocol and Design:** Test Protocol was generally patterned after ASTM, OECD, and USEPA guidelines and is outlined in ABC protocol No. 7901.

    Test Organisms: Daphnia magna were obtained from in-house daphnia cultures maintained continuously since 1977. The organisms were cultured in water obtained from a surface impoundment source and combined with soft blended water to yield a water hardness of 160-180 mg/L of $CaCO_3$. Daphnids were cultured and tested at $20\pm2°C$ and provided with a 16D/8N photoperiod at a 50-70 footcandle intensity. The daphnids used were first instar (<24 hours) old.

    Test Dilution Water and Solution Preparation: The blended water had alkalinity of 136-178 mg/L as $CaCO_3$, a pH range of 7.9-8.1, conductivity of 400-460 μMHOS/cm, TOC of 1.5 ppm and is classified as hard water. Organophosphate and PCB content was under 0.50 ppb. Chlorinated hydrocarbons were all below 0.10 ppb and most were below 0.05 ppb.

    The diluter stock solution was prepared by addition of 0.3030 gm of PCNB to 100 ml of acetone producing a 3000 mg ai/L concentration. This stock solution was added to dilution water in the diluter mixing chamber to yield a maximum estimated concentration of 0.30 mg/L. Recoveries of PCNB in initial method validation studies averaged $101\pm6.7\%$ of the nominal.

    Test Materials and Design: Preliminary range finding tests at nominal concentrations of 0.03, 0.06, 0.13, 0.25 and 0.50 mg/L caused 30%, 0%, 10%,100%, and 100% mortality, respectively. The 30% mortality was no felt to be toxicant related.

    The definitive test concentrations were set at nominal levels of 0.018, 0.036, 0.075, 0.150 and 0.30 mg/L based on the preliminary test results. This represents a 50% dilution factor between concentrations.

    The diluter system was calibrated and allowed to operate 8 days prior to test initiation during which time analysis for actual concentration accuracy was conducted. Ten test daphnids were distributed randomly by group to each of the 4 replicate chambers in the 7 test groups, thus exposing 40 daphnids at each test concentration, control, and solvent control level. The 1 liter glass beakers used as test replicate chambers were covered with 50 mesh stainless steel screen. Dilution water and test solution were combined in the Mount and Brungs type diluter and then mixed appropriately and distributed to each of the chambers at an

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replicate chambers were covered with 50 mesh stainless steel
screen. Dilution water and test solution were combined in
the Mount and Brungs type diluter and then mixed
appropriately and distributed to each of the chambers at an
average flow rate of 4.0 ml/min. producing 5.7 volume
additions per 24 hour period. The test chambers were
partially immersed in a temperature controlled water bath
maintained at $20\pm2°C$ and monitored by a continuous thermal
data logger.

Analysis of test concentration levels was made from samples
removed 7 days before initiation, on the day of initiation,
and on days 4, 7, 14 and 21. The samples were analyzed by
gas liquid chromatography.

The organisms were fed three times daily with $2X10^8$ algal
cell suspensions. In addition they were supplemented once
per day with a Tetramin, yeast, vitamin mix at a solids
concentration of 1.8 mg/L. Daily observations of behavior
and mortality were recorded. Reproduction success was
measured every Monday, Wednesday, and Friday by offspring
counts. Offspring were removed by use of a 50 micron mesh
screen after parental daphnids were temporarily removed with
glass pipets. Chambers were cleaned with nylon bristle
brushes once per week. Diluter operation was observed and
recorded 2 times per day. At test termination adults were
measured by use of an eyepiece micrometer installed on a
dissecting scope.

Water quality was monitored in alternating replicates of the
control, high, medium and low concentrations on days 0, 4,
7, 14, and 21 for D.O. and pH. Temperature was not
monitored in test vessels, only in the water bath on a daily
basis.

Statistical analysis of the results used ANOVA, one way
ANOVA T-Test, Dunnett's multiple mean comparison test,
Toxstat, and Stephan's program in arriving at final analysis
of the test results.

11. **Reported Test Results:** Actual mean measured concentrations
for the 21 day test were 0.018, 0.030, 0.059, 0.10, and 0.23
mg/L and averaged 81% of the nominal level.

All adults in the 0.23 mg/L concentration died by day 3.

Slight variances were noted in body length of control adults
and all treatment level adults but the variance was slight.

Days to first brood showed no significant variation from the
two control groups.

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Reproduction of young was quite variable and a significant effect has been demonstrated with young per adult per reproduction day and decreased noticeably with increasing concentration as shown below.

Meas. Conc.	Young Per Adult Per Day	Days to First brood
Control	7.4	7.3
Solvent Control	7.5	7.0
0.018	7.2	7.0
0.030	5.3	7.0
0.059	2.1	7.0
0.100	0.23	7.0

12. **Study Author's Conclusions:** "One hundred percent mortality was observed in the 0.23 mg/L test level by day 3 of the study. A 21 day EC_{50} based on immobilisation was calculated to be 0.14 mg/L...... Based on the statistical analysis of survival, adult mean length, meantime in days to first brood and young/adult/reproduction day from this 21-day Daphnia magna dynamic life cycle study, the MATC limits were estimated to be the measured PCNB concentrations of 0.018 and 0.030 mg/L. The point estimate MATC value was calculated to be 0.023 mg/L."

13. **Reviewer's Discussion:** The study appears to have been designed and conducted in a scientifically sound manner. Results indicate little to no effect on growth in the 4 surviving test concentration levels.

Though a ten percent mortality was observed in the lowest concentration level it is not fellt to have been toxicant related. The EC_{50} calculations presented by the author were confirmed by the Agency toxanol program, however the confidence limits were not felt to be reliable in either the binomial or probit analysis methods.

A definite reduction in instar counts is shown in the 0.030, 0.059, and 0.10 mg/L concentration groups beginning on day 7 and increasing toward day 10.

Several deviations from protocol were noted. Two short blockages of the diluter caused a temporary reductions in the flow rate on day 2 and day 17. This resulted in 3 volume additions per vessel for the 24 hour period instead of the 5.2-6.1 additions recorded on other days. The diluter records show that normal flow rates were restored by the following day. ASTM guidelines suggest the use of 2 liter beakers not 1 liter beakers as employed in this study. Load factors were acceptable despite this factor. Dissolved

oxygen should be measured 2 times per week. It was measured once during the second and third weeks.

Statisical Analysis: Analysis of the variables by the Agency generally confirms the author's conclusions. Statistical analysis of survival by Dunnett's T-test and Duncan's Range Test showed variance with the highest concentration only. No significant variance between lower levels and controls was found.

Though some variances were found between treatment groups and controls in adult body length they were not correlated with increasing concentrations and therefore are not felt to be toxicant related.

The greatest statistical variances occurred in reproductive parameters. Five clear groupings were seen in the Duncan's Multiple Range test and were clearly related to increasing concentration levels. This variance is also confirmed in results using Dunnett's T-test. An effect on instar production rates has been clearly demonstrated.

Adequacy of Study:

   Classification: Core

   Rationale: The test has followed acceptable protocol guidelines. The reported results confirm the author's conclusions.

   Repairability: N/A

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8

NOTE: THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE
OF THE LOWER CONCENTRATIONS HAD ZERO MORTALITY.
THEREFORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

Montague PCNB 21 Life Cycle Daphnia
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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.23	40	40	100	0
.1	40	2	5	0
.059	40	0	0	0
.03	40	1	2.5	0
.018	40	4	10	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT
CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE
UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .143273

THE MOVING AVERAGE METHOD CANNOT BE USED WITH THIS DATA SET
BECAUSE NO SPAN WHICH PRODUCES MOVING AVERAGE ANGLES THAT
BRACKET 45 DEGREES ALSO USES TWO PERCENT DEAD BETWEEN 0 AND
100 PERCENT.

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
GOODNESS OF FIT PROBABILITY		
8	7.947053	41.58158
0		

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE   =   2.983965
95 PERCENT CONFIDENCE LIMITS =-5.427987   AND   11.39592

LC50 =   .1384317
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 =   5.195501E-02
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY
****

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
8	7.947053	41.58158	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED

7

9

OBS	TRT	SURV	LENGTH	OFFSP
1	a	10	4.1	1077
2	a	9	4.1	1010
3	a	10	4.1	921
4	a	9	4.1	1082
5	b	10	4.1	1235
6	b	9	4.1	1144
7	b	10	4.1	1039
8	b	9	4.1	1068
9	c	9	4.2	1017
10	c	10	4.2	1055
11	c	8	4.2	916
12	c	9	4.2	921
13	d	9	4.3	746
14	d	10	4.3	793
15	d	10	4.3	773
16	d	10	4.3	778
17	e	10	4.3	303
18	e	10	4.3	373
19	e	10	4.3	280
20	e	10	4.3	326
21	f	10	4.2	49
22	f	9	4.2	34
23	f	10	4.2	31
24	f	9	4.2	24
25	g	0	.	.
26	g	0	.	.
27	g	0	.	.
28	g	0	.	.

8

10

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	7	a b c d e f g

Number of observations in data set = 28

General Linear Models Procedure

Dependent Variable: SURV

Source	DF	Sum of Squares	F Value	Pr > F
Model	6	314.35714286	191.35	0.0001
Error	21	5.75000000		
Corrected Total	27	320.10714286		

R-Square	C.V.	SURV Mean
0.982037	6.398038	8.17857143

General Linear Models Procedure

Dependent Variable: SURV

Source	DF	Type I SS	F Value	Pr > F
TRT	6	314.35714286	191.35	0.0001

Source	DF	Type III SS	F Value	Pr > F
TRT	6	314.35714286	191.35	0.0001

9

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General Linear Models Procedure

Duncan's Multiple Range Test for variable: SURV

NOTE: This test controls the type I comparisonwise error rate,

not the experimentwise error rate

Alpha= 0.05   df= 21   MSE= 0.27381

Number of Means	2	3	4	5	6	7
Critical Range	0.769	0.807	0.834	0.850	0.862	0.872

Means with the same letter are not significantly different.

General Linear Models Procedure

Duncan Grouping		Mean	N	TRT
	A	10.000	4	e
	A			
B	A	9.750	4	d
B	A			
B	A	9.500	4	a
B	A			
B	A	9.500	4	b
B	A			
B	A	9.500	4	f
B				
B		9.000	4	c
	C	0.000	4	g

General Linear Models Procedure

Dunnett's T tests for variable: SURV

NOTE: This tests controls the type I experimentwise error for

comparisons of all treatments against a control.

Alpha= 0.05   Confidence= 0.95   df= 21   MSE= 0.27381
Critical Value of Dunnett's T= 2.790
Minimum Significant Difference= 1.0322
Comparisons significant at the 0.05 level are indicated by '***'.

General Linear Models Procedure

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
e	− a	−0.532	0.500	1.532	
d	− a	−0.782	0.250	1.282	
b	− a	−1.032	0.000	1.032	
f	− a	−1.032	0.000	1.032	
c	− a	−1.532	−0.500	0.532	
g	− a	−10.532	−9.500	−8.468	***

Dunnett's One-tailed T tests for variable: SURV

NOTE: This tests controls the type I experimentwise error for

comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 21 MSE= 0.27381
Critical Value of Dunnett's T= 2.448
Minimum Significant Difference= 0.9057

Comparisons significant at the 0.05 level are indicated by '***'.

General Linear Models Procedure

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
e	− a	−0.406	0.500	1.406	
d	− a	−0.656	0.250	1.156	
b	− a	−0.906	0.000	0.906	
f	− a	−0.906	0.000	0.906	
c	− a	−1.406	−0.500	0.406	
g	− a	−10.406	−9.500	−8.594	***

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: SURV

NOTE: This tests controls the type I experimentwise error for

comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 21 MSE= 0.27381
Critical Value of Dunnett's T= 2.448
Minimum Significant Difference= 0.9057

Comparisons significant at the 0.05 level are indicated by '***'.

General Linear Models Procedure

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
e – a	-0.406	0.500	1.406
d – a	-0.656	0.250	1.156
b – a	-0.906	0.000	0.906
f – a	-0.906	0.000	0.906
c – a	-1.406	-0.500	0.406
g – a	-10.406	-9.500	-8.594

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OBS	TRT	SURV	LENGTH	OFFSP
1	a	10	4.1	1077
2	a	9	4.1	1010
3	a	10	4.1	921
4	a	9	4.1	1082
5	b	10	4.1	1235
6	b	9	4.1	1144
7	b	10	4.1	1039
8	b	9	4.1	1068
9	c	9	4.2	1017
10	c	10	4.2	1055
11	c	8	4.2	916
12	c	9	4.2	921
13	d	9	4.3	746
14	d	10	4.3	793
15	d	10	4.3	773
16	d	10	4.3	778
17	e	10	4.3	303
18	e	10	4.3	373
19	e	10	4.3	280
20	e	10	4.3	326
21	f	10	4.2	49
22	f	9	4.2	34
23	f	10	4.2	31
24	f	9	4.2	24
25	g	0	.	.
26	g	0	.	.
27	g	0	.	.
28	g	0	.	.

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	7	a b c d e f g

Number of observations in data set = 28

NOTE: Due to missing values, only 24 observations can be used in this analysis.

Dependent Variable: LENGTH

Source	DF	Sum of Squares	F Value	Pr > F
Model	5	0.16000000	99999.99	0.0
Error	18	0.00000000		
Corrected Total	23	0.16000000		

R-Square	C.V.	LENGTH Mean
1.000000	0	4.20000000

Dependent Variable: LENGTH

Source	DF	Type I SS	F Value	Pr > F
TRT	5	0.16000000	99999.99	0.0

Source	DF	Type III SS	F Value	Pr > F
TRT	5	0.16000000	99999.99	0.0

13

15

Duncan's Multiple Range Test for variable: LENGTH

NOTE: This test controls the type I comparisonwise error rate,

not the experimentwise error rate

Alpha= 0.05   df= 18   MSE= 0

Number of Means   2 3 4 5 6
Critical Range   0 0 0 0 0

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	4.300	4	e
A			
A	4.300	4	d
B	4.200	4	c
B			
B	4.200	4	f
C	4.100	4	a
C			
C	4.100	4	b

Dunnett's T tests for variable: LENGTH

NOTE: This tests controls the type I experimentwise error for

comparisons of all treatments against a control.

Alpha= 0.05   Confidence= 0.95   df= 18   MSE= 0
Critical Value of Dunnett's T= 2.761
Minimum Significant Difference= 0

Comparisons significant at the 0.05 level are indicated by '***'.
General Linear Models Procedure

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
e - a	0.200	0.200	0.200	***
d - a	0.200	0.200	0.200	***
c - a	0.100	0.100	0.100	***
f - a	0.100	0.100	0.100	***
b - a	0.000	0.000	0.000	

Dunnett's One-tailed T tests for variable: LENGTH

NOTE: This tests controls the type I experimentwise error for

comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 18 MSE= 0
Critical Value of Dunnett's T= 2.407
Minimum Significant Difference= 0

Comparisons significant at the 0.05 level are indicated by '***'.

General Linear Models Procedure

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
e - a	0.200	0.200	0.200	
d - a	0.200	0.200	0.200	
c - a	0.100	0.100	0.100	
f - a	0.100	0.100	0.100	
b - a	0.000	0.000	0.000	

Dunnett's One-tailed T tests for variable: LENGTH

NOTE: This tests controls the type I experimentwise error for

comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 18 MSE= 0
Critical Value of Dunnett's T= 2.407
Minimum Significant Difference= 0

Comparisons significant at the 0.05 level are indicated by '***'.

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
e - a	0.200	0.200	0.200	***
d - a	0.200	0.200	0.200	***
c - a	0.100	0.100	0.100	***
f - a	0.100	0.100	0.100	***
b - a	0.000	0.000	0.000	

OBS	TRT	SURV	LENGTH	OFFSP
1	a	10	4.1	1077
2	a	9	4.1	1010
3	a	10	4.1	921
4	a	9	4.1	1082
5	b	10	4.1	1235
6	b	9	4.1	1144
7	b	10	4.1	1039
8	b	9	4.1	1068
9	c	9	4.2	1017
10	c	10	4.2	1055
11	c	8	4.2	916
12	c	9	4.2	921
13	d	9	4.3	746
14	d	10	4.3	793
15	d	10	4.3	773
16	d	10	4.3	778
17	e	10	4.3	303
18	e	10	4.3	373
19	e	10	4.3	280
20	e	10	4.3	326
21	f	10	4.2	49
22	f	9	4.2	34
23	f	10	4.2	31
24	f	9	4.2	24
25	g	0	.	.
26	g	0	.	.
27	g	0	.	.
28	g	0	.	.

Class Level Information

Class	Levels	Values
TRT	7	a b c d e f g

Number of observations in data set = 28

NOTE: Due to missing values, only 24 observations can be used in this analysis.

Dependent Variable: OFFSP

Source	DF	Sum of Squares	F Value	Pr > F
Model	5	3801226.87500	225.13	0.0001
Error	18	60785.75000		
Corrected Total	23	3862012.62500		

R-Square	C.V.	OFFSP Mean
0.984261	8.206438	708.12500000

Dependent Variable: OFFSP

Source	DF	Type I SS	F Value	Pr > F
TRT	5	3801226.87500	225.13	0.0001

Source	DF	Type III SS	F Value	Pr > F
TRT	5	3801226.87500	225.13	0.0001

Duncan's Multiple Range Test for variable: OFFSP

NOTE: This test controls the type I comparisonwise error rate, not the experimentwise error rate

Alpha= 0.05 df= 18 MSE= 3376.986

Number of Means	2	3	4	5	6
Critical Range	86.20	90.49	93.46	95.15	96.48

Means with the same letter are not significantly different.

17

19

Duncan Grouping		Mean	N	TRT
A		1121.50	4	b
B		1022.50	4	a
B				
B		977.25	4	c
C		772.50	4	d
D		320.50	4	e
E		34.50	4	f

Dunnett's T tests for variable: OFFSP

NOTE: This tests controls the type I experimentwise error for

comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 18 MSE= 3376.986
Critical Value of Dunnett's T= 2.761
Minimum Significant Difference= 113.47

Comparisons significant at the 0.05 level are indicated by '***'.

TRT Comparison		Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
b	- a	-14.47	99.00	212.47	
c	- a	-158.72	-45.25	68.22	
d	- a	-363.47	-250.00	-136.53	***
e	- a	-815.47	-702.00	-588.53	***
f	- a	-1101.47	-988.00	-874.53	***

Dunnett's One-tailed T tests for variable: OFFSP

NOTE: This tests controls the type I experimentwise error for

comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 18 MSE= 3376.986
Critical Value of Dunnett's T= 2.407
Minimum Significant Difference= 98.91

Comparisons significant at the 0.05 level are indicated by '***'.

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
b − a	0.09	99.00	197.91	
c − a	−144.16	−45.25	53.66	
d − a	−348.91	−250.00	−151.09	***
e − a	−800.91	−702.00	−603.09	***
f − a	−1086.91	−988.00	−889.09	***

Dunnett's One-tailed T tests for variable: OFFSP

NOTE: This tests controls the type I experimentwise error for

comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 18 MSE= 3376.986
Critical Value of Dunnett's T= 2.407
Minimum Significant Difference= 98.91

Comparisons significant at the 0.05 level are indicated by '***'.

TRT Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
b − a	0.09	99.00	197.91	***
c − a	−144.16	−45.25	53.66	
d − a	−348.91	−250.00	−151.09	
e − a	−800.91	−702.00	−603.09	
f − a	−1086.91	−988.00	−889.09	

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19

21