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SHAUGHNESSY NO.

REVIEW NO.

EEB REVIEW

DATE: IN 4-25-88 OUT: 3-2-90

FILE OR REG. NO. 7969-62 and 7969-63 MRID

PETITION OR EXP. NO.

DATE OF SUBMISSION 4-8-88

DATE RECEIVED BY HED 4-22-88

RD REQUESTED COMPLETION DATE 8-9-88

EEB ESTIMATED COMPLETION DATE

RD ACTION CODE/TYPE OF REVIEW 400

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

DATE ACCESSION NO (S).

PRODUCT MANAGER NO. L. Rossi

PRODUCT NAME (S) Vinclozolin

COMPANY NAME BASF Corporation

SUBMISSION PURPOSE Submission of avian male fertility test to satisfy special request.

SHAUGHNESSEY NO.	CHEMICAL AND FORMULATION	% A.I.

DATA EVALUATION REPORT

Ecological Effects Branch

1. **Chemical:** Vinclozolin (Ronalin)

2. **Test Material:** Batch No. N 182 of Test Substance $C_{12}H_9NO_3CL_2$....9.4% purity

3. **Study Type** (1) Avian Reproduction with Mallard Duck *Anas platyrhynchos* - Special Test for male fertility.
   (2) Pathology Report

4. **Study Identification:**

   Study Director: Munk, Dr. R.
   Laboratory: BASF Aktiengesellschaft, Dept. of Toxicology, Ludwigshafen West Germany
   Study Dates: March 11 - May 27, 1987
   Study Identification: Project No. S72W466/8651 and Pathology Report 72W466/86051.
   Submitted By: BASF Corporation, Chemical Division
   EPA Identification: 7969-62, 7969-63

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

6. **Approved by:** Ray Matheny, Supervisory Biologist Ray Matheny 3/2/90
   Ecological Effects Branch, Section I
   Environmental Fate and Effects Division (H7507C)

7. **Conclusions:** The study has shown no reduction in male fertility at concentration levels of vinclozolin from 2.5 ppm to 50 ppm. The study has provided supplemental information which can be used in risk assessment procedures.

8. **Recommendations:** N/A

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9. **Purpose of Submission:** Submission of data requested for registration action.

10. **Study Design and Protocol**

Protocol was designed to comply with study design proposed by Dr. Pierre Mineau of the Canadian Toxic Chemicals Program, Wildlife Toxicology and Pathology Division, Ottawa, Ontario. The protocol was based on EPA Subdivision E guidelines, 71-4. The study was designed to investigate the possibility of reduced fertility in males after ingestion of Vinclozolin.

Test Organisms

The test animals were acclimated for a period of 2 weeks prior to initiation of treated food. The treated food was provided for 15 weeks thereafter. The mallards were obtained from Heinz Bohneu, Wildlife Breeding in Mulheim/Ruhr, West Germany.

The test birds were indistinguishable from wild birds. A total of 450 birds were obtained in 1986 at age of 14 days. Study was begun when the ducks approached 7-7.5 months of age. The birds were housed in a single room during acclimation; lighting was decreased from 16 to 6 hours of light from the time of arrival to December. Temperature was decrease from 25°C to 20-16°C. Commercial duck pellet feed was provided during acclimation. The test birds were identified by numbered leg bands which pertained to cage no. and concentration group. Cages were marked with identification shields which listed test group, and replicate number. Later eggs were collected and marked with date and cage number. Test birds were weighed 1 day prior to test initiation and randomly assigned to different test groups on basis of their body weights. 4 weeks prior to the test 6 birds were taken to the Governmental Veterinary Health Service and the inspection listed no abnormalities or disease problems in these birds. All 184 test birds were observed for health problems before introduction to treatment cages. Birds of abnormal weight were not utilized.

Test Design

Twenty three replicated pairs were used for each of the 3 treatment levels and the controls. The stainless and galvanized steel wire cages had wire floors with a 1.3 meter x .65 meter floor area and a height of 1.3 meters. No nesting materials were provided. 66 cages were placed in each of the 2 test rooms. Each room contained birds from all 4 test groups but the cages were not randomly arranged within the rooms. During weeks 2-15 granular feed was provided ad libitum in stainless steel containers inside the cages.

Municipal drinking water was available ad libitum throughout the study. Temperature was maintained through controlled air conditioning systems. Fluorescent tubes with timer switches provided the illumination at a level 90-125 lux in the cages. The lighting schedule was 6D/18N for weeks 0-2, 8D/16N for week 3 & 4, and 17D/7N from week 5 to week 15. Relative humidity range was from 50-80%.

Two separate batches of commercial feed were purchased during the study due to the non-perishable guarantee of 3 months. Vitamin A (13500 ivs), Vitamin D_3 (2000 iv), and Vitamin E (12 mg) supplements were added to the commercial mix.

Eggs were collected daily and the counts per replicate cage were maintained for each week. Eggs were stored at 16 ± 2°C on cardboard setting trays in 2 special refrigerators at a humidity of 70 to 90%. After 1 week each replicate's egg production was weighed and mean egg weight calculated. After weight measurement the eggs were examined by candling for cracking or shell malformation. Cracked or abnormal eggs were discarded. Normal eggs were placed in an Ehret Type KMB6/V commercial brooder with automatic egg rotation. Temperature and humidity were measured daily and were 37.8° ± 4°C and 60-70%, respectively. Eggs were rotated 180° every 1 1/2 hours. Eggs were incubated 7 days and after examination by candling for infertility or abnormalities they were discarded. Fertile and infertile egg production was recorded at this time.

Dosage determinations were made based on prior reproduction studies (Report HRC, Jan 25, 1982) where dose induced infertility appeared to have occurred in 5 ppm (82.5%) and 50 ppm (65.7%) dosages. A 15 ppm was suggested by the Canadian National Wildlife Research Center. As a precaution, 0, 2.5, 10 and 50 mg/kg levels were included to get a more accurate N.O.E.L. As 50 mg/kg was tolerated for 25 weeks in prior studies without visible toxic signs (other than the suspected ♂ fertility effects), it was felt that this was an adequate maximum dosage.

During the study parental birds were observed daily for signs of toxic effect. The birds were weighed on day 0 and at test termination. At termination of the study the remaining male birds were sacrificed by decapitation and female birds by CO_2 asphyxia. Blood samples were taken from the males for later possible analysis of testosterone and FSH content. However, analysis was not performed as the study director felt no effect on fertility had occurred.

Dosage Preparation

69 kg test diet preparations were made each week for each test

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level. Diet stability had been tested and verified at the 2.5 mg/kg concentration. Samples taken at 3 levels of the mix and stored showed homogeneity. Analytical verification of concentrations was made twice at initiation, twice at median point and once at the end of the feeding period. Analysis for undesirable contaminants in feed and drinking water were made but are included in the raw data which has not been included in the study report.

11. Reported Test Results

Mortality results show a loss of 2 males and 5 females during the course of the study. 1 male and 3 females were lost in the control group; 1 male died in the 2.5 ppm test level, and 2 females died in the 10 ppm level. No mortality occurred in the 50 ppm test level. The partner in each replicate mortality was sacrificed for necropsy. Behavioral observations prior to the death of these birds included apathy, prone position, ataxia, ruffled feathers, tumbling or soft feces. Two of the females died without any prior signs. Three mortalities occurred in pre-egg production phase (2♂ and 1♀) and 4 occurred during egg production (all ♀'s).

Feed consumption decreased from week 1 to week 4 in controls, 2.5 ppm and 10 ppm levels. However, mean consumption in the 50 ppm level appeared to remain consistent during pre-egg production. This trend continued during the 10 week egg production stage with the 50 ppm level consistently demonstrating higher food consumption than the 3 other levels. This did not appear to be caused by the mortality as the means for food consumption were based on surviving birds.

The 8 males in the 50 ppm level which were measured for body weight after 19 weeks all showed weight gains of 20-291 gms. Four out of 7 control males showed weight loss after 19 weeks of 2 to 86 gms. and 3 showed 77-167 gm gains. 3 males in the 2.5 ppm level and 1 male in the 10 ppm level showed losses in weight. Eleven showed gains in these groups after 19 weeks.

Females generally showed weight losses in the control (7 out of 7), 2.5 ppm (6 out of 8), 10 ppm (5 out of 7) test levels after 19 weeks. Five out of 8 showed weight losses in the 50 ppm level.

The report has included estimates of mean uptake of test compound based on average food consumption and percentage of compound within that test diet. In general, these computations show an increase in weekly uptake of the compound during the egg production period ranging from 0.49 gm/week at the 2.5 ppm level to 10.12 gms/week at the 50 ppm level.

The mean number of eggs laid by the test birds showed little

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variation among the different test concentrations. The highest mean egg production figures occurred between weeks 4 and 9.

The mean egg weight ranged from 59.8 gms. for the control group to 62.7 gm. for the 50 ppm test level. Little variation is seen in the weight of eggs produced 3 weeks after toxicant exposure.

The mean eggs cracked percentages were one percent for the controls, 4.3% for the 2.5 ppm level, 0.5% for the 10 ppm level and 1.7% for the 50 ppm level. These percentages appear to indicate no effect on shell development. An increase in the percentage of egg shell cracking was seen during the three-week post exposure period, but was only seen in the lowest and highest concentrations.

Egg production was not seen in 22 of the 92 replicates. The percentages of nonproducing replicate pairs to the total were distributed as follows: Controls - 24%, 2.5 ppm - 32%, 10 ppm - 13%, and 50 ppm - 30%.

Egg fertility was 68% for controls, 59% for the 2.5 ppm level, 72% for the 10 ppm level, and 58% for the 50 ppm level. No dosage related effect can be attributed to these figures.

During weeks 12-15 of the post exposure phase egg production was reduced. It was felt that this was due to the onset of molting phases in the test birds during this period.

As the test was designed to measure only fertility in males, the eggs were not carried to hatch stage. Therefore there are no data concerning eggs hatched or surviving chicks.

Analysis of dietary samples was carried out by Dr. Dreher of the BASF laboratory and indicated no significant variation between 10-day samples and 32-day samples in stability tests. Actual measured concentrations ranged from 84 to 138% of the nominal concentration levels. They averaged 96% of the nominal estimated concentration for the 15 samples taken. No samples were measured for the controls.

12. **Study Author's Conclusion:** "Under the conditions of this study there was no evidence that the dietary administration of vinclozolin at levels of 2.5, 10 and 50 mg/Kg diet had any adverse effects on any of the parameters examined and, in particular, on male fertility."

13. **Reviewer's Discussion:** As this study was specially designed to look only at the effects of Vinclozolin on male fertility, the normal guideline requirements for a reproduction study are not completely applicable. A special protocol was developed

to address this particular study and was reviewed by EEB in 1986 under Record No. 178642.

BASF Laboratories deviated from EEB and Canadian Wildlife Service recommendations in a few areas:

(1) It was suggested that a four-week exposure period was too short to adequately assess the impact of the multiple applications proposed for the chemical and therefore might not be sufficient to support all proposed use patterns. Exposure was only for a four week period prior to egg laying.

(2) No observation of spermatazoa was made as suggested by CWS. Apparently it was not felt to be necessary due to the lack of effect on actual egg production.

(3) Originally BASF protocol called for an eight-week substance feeding period during egg laying with a four-week withdrawal period. The study increased this to an 11-week substance feeding with a four-week withdrawal period. This may have improved the study.

BASF mentioned that temperature variation due to technical problems with the air conditioners may have caused temperatures to exceed the 21 degree C plus or minus two degrees limits a few hours per day, but it was not stated as to the extent or length of this variation. Humidity levels were 50 - 80% during the study. This is a wide range. EPA guidelines recommend 55% humidity.

Administration of the chemical for 10 weeks prior to egg laying is suggested in EPA guidelines. BASF administered the chemical for only four weeks prior to the egg laying period. It is questionable whether this was sufficient time for assessment of reproductive impairment which might occur with over 10 repeat applications as permitted on the label for this product.

Placement of individual cages within the holding rooms was not completely random in that the four groups were clumped together within the rooms despite even distribution of test levels into each of the two test rooms. A more random assignment would have alternated cages within the rooms. It has been noted that egg production (Groups 0-3) among groups placed nearest the entrance doors was notably lower than those located farther from the doors (Groups 1 and 2). Mortality was also unexpectedly high for control level birds (Group 0). As pathological examination revealed no physical reason for

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these deaths, the high mortality may indicate a possible stress situation.

The absence of fertilization and early embryonic death are difficult to determine and can be clariified further by breaking out of suspected eggs for further examination. Candling for blood vessels in the germinal disk or for totally clear eggs, though a good indications, could easily have been further confirmed.

Unfertilized eggs and replicates producing no eggs at all were higher than would normally be expected. They ranged from 13% (10 ppm grp.) to 32% (50 ppm group). The control level displayed 24% infertility.

Embryo viability based on the seven-day candling observations of the individual eggs would indicate a below normal percentage of embryos surviving from the eggs which were set (52 - 69%). The variance between treatment levels, however, indicates no real correlation between higher dosage and embryo viability. The number of eggs which were actually fertilized (controls 69%, 2.5 ppm - 59%, 10 ppm 75%, and 58% - 50 ppm) are also lower than might be normally expected with the exception of the 10 ppm test level. Again no direct correlation between test levels can be made. The high rate of infertility in all groups somewhat compromises any attempt to determine affect based on treatment concentrations.

Though male reproductive organs showed considerable size variance between the 57-day and 105- or 133-day sacrifice dates this is not unexpected as the reproductive condition of the males would generally decrease after the initial 10-week period. The reduction in testicle weight is therefore not felt to be due to any chemical effect. At first sacrifice (Day 57) there is no real statistical variance shown between treatment levels. At this time most of the males would have been approaching the height of breeding condition.

Male Reproductive Effect Parameters

	Test Group	Mean Wt.	Testicles
	Controls	22.2	20.362
57 days	Group I	20.56	22.37
sacrif.	Group II	17.66	18.04
	Group III	24.1	22.26
Second	Control	4.4	4.0
Sacrif.	Group I	2.68	2.6 (SD-23)
105	Group II	8.38	7.66 (SD=8.2)
days	Group III	4.9	5.3 SD=4.7-5.0)
Third	Control	2.8	2.8 (SD 5.96-6.1)
sacrif.	Group I	7.1	7.7 (SD 5.49-6.1)
133	Group II	6.3	7.8 (SD - 6.0-7.6)
days	Group III	6.0	5.5 (SD - 4.5 - 4.3)

Statistical Analysis: The analysis of the data showed no statistical variance between treatment levels in the number of eggs laid, number of eggs set, or number of viable 7 week embryos. A variance was detected in the number of eggs cracked, but this was felt to be due to 3 extreme values within the 2.5 ppm test level which did not reflect the average within this test concentration.

The mean food consumption and body weight data shown no statistical variance between the individual test groups.

Adequacy of Study:

Classification: Supplemental

Rationale: Male fertility testing does not fulfill any particular guideline requirement though the study has provided data useable for risk assessment.

Repairability: Not applicable

8. ANALYSIS OF VE/ES DATA

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7:56 Tuesday, February 27, 1990

OBS	TRT	EL	EC	ES	VE
1	a	13	0	13	0
2	a	7	0	7	0
3	a	64	0	64	59
4	a	3	0	3	0
5	a	44	0	44	31
6	a	37	0	37	36
7	a	40	0	40	0
8	a	16	0	16	9
9	a	9	0	9	0
10	a	35	0	35	33
11	a	9	0	9	0
12	a	3	0	3	3
13	a	41	1	40	17
14	a	56	0	56	17
15	a	36	2	34	32
16	a	13	1	12	3
17	a	37	1	36	0
18	a	62	1	61	51
19	a	31	0	31	26
20	a	44	0	44	39
21	a	13	0	13	7
22	b	0	0	0	0
23	b	24	0	24	0
24	b	13	0	13	12
25	b	51	0	51	43
26	b	40	0	40	35
27	b	61	0	61	56
28	b	16	0	16	16
29	b	56	13	43	20
30	b	38	7	31	25
31	b	83	10	73	56
32	b	54	0	54	0
33	b	35	0	35	34
34	b	44	0	44	0
35	b	42	0	42	0
36	b	12	0	12	9
37	b	32	0	32	0
38	b	49	2	47	44
39	b	56	1	55	48
40	b	26	1	25	6
41	b	61	0	61	22
42	b	7	0	7	6
43	c	16	1	15	15
44	c	10	0	10	10
45	c	44	1	43	42
46	c	10	0	10	7

47	c	62	0	62	40
48	c	13	0	13	0
49	c	51	0	51	47
50	c	47	0	47	4
51	c	32	0	32	31
52	c	62	0	62	60
53	c	55	1	54	53
54	c	88	0	88	51
55	c	53	0	53	1
56	c	11	0	11	10
57	c	12	0	12	12
58	c	58	0	58	55
59	c	43	0	43	18
60	c	14	0	14	10
61	c	0	0	0	0
62	c	0	0	0	0
63	c	18	0	18	0
64	c	56	0	56	51

8. ANALYSIS OF VE/ES DATA

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OBS	TRT	EL	EC	ES	VE
65	c	13	0	13	10
66	c	4	1	3	3
67	d	44	1	43	0
68	d	61	0	61	53
69	d	43	3	40	13
70	d	20	0	20	0
71	d	30	0	30	27
72	d	45	0	45	44
73	d	4	0	4	4
74	d	57	2	55	0
75	d	51	2	49	30
76	d	27	0	27	15
77	d	6	0	6	0
78	d	0	0	0	0
79	d	4	0	4	4
80	d	57	2	55	28
81	d	43	0	43	41
82	d	3	0	3	0
83	d	24	0	24	7
84	d	15	1	14	14
85	d	43	0	43	0
86	d	42	0	42	38
87	d	14	0	14	13
88	d	12	0	12	9
89	d	15	0	15	15

1. ANALYSIS OF EL DATA

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7:56 Tuesday, February 27, 1990

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	a b c d

Number of observations in data set = 89

1. ANALYSIS OF EL DATA

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7:56 Tuesday, February 27, 1990

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	1197.64835881	0.87	0.4626
Error	85	39227.25051760		
Corrected Total	88	40424.89887640		

R-Square	C.V.	RESP Mean
0.029627	67.20355	31.96629213

Source	DF	Type I SS	F Value	Pr > F
TRT	3	1197.64835881	0.87	0.4626

Source	DF	Type III SS	F Value	Pr > F
TRT	3	1197.64835881	0.87	0.4626

1. ANALYSIS OF EL DATA

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7:56 Tuesday, February 27, 1990

General Linear Models Procedure

Duncan's Multiple Range Test for variable: RESP

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

Alpha= 0.05 df= 85 MSE= 461.4971
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 22.17504

Number of Means	2	3	4
Critical Range	12.84	13.50	13.93

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	38.095	21	b
A			
A	32.167	24	c
A			
A	29.190	21	a
A			
A	28.696	23	d

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2. ANALYSIS OF EC DATA

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7:56 Tuesday, February 27, 1990

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	a b c d

Number of observations in data set = 89

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2. ANALYSIS OF EC DATA

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7:56 Tuesday, February 27, 1990

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	28.70067695	2.74	0.0480
Error	85	296.31055901		
Corrected Total	88	325.01123596		

R-Square	C.V.	RESP Mean
0.088307	302.1283	0.61797753

Source	DF	Type I SS	F Value	Pr > F
TRT	3	28.70067695	2.74	0.0480

Source	DF	Type III SS	F Value	Pr > F
TRT	3	28.70067695	2.74	0.0480

2. ANALYSIS OF EC DATA

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7:56 Tuesday, February 27, 1990

General Linear Models Procedure

Duncan's Multiple Range Test for variable: RESP

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

Alpha= 0.05 df= 85 MSE= 3.486007
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 22.17504

Number of Means	2	3	4
Critical Range	1.116	1.174	1.211

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	1.619	21	b
B	0.478	23	d
B B	0.286	21	a
B B	0.167	24	c

3. ANALYSIS OF ES DATA

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General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	a b c d

Number of observations in data set = 89

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7:56 Tuesday, February 27, 1990

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	913.24158467	0.69	0.5620
Error	85	37620.96066253		
Corrected Total	88	38534.20224719		

R-Square	C.V.	RESP Mean
0.023700	67.11062	31.34831461

Source	DF	Type I SS	F Value	Pr > F
TRT	3	913.24158467	0.69	0.5620

Source	DF	Type III SS	F Value	Pr > F
TRT	3	913.24158467	0.69	0.5620

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General Linear Models Procedure

Duncan's Multiple Range Test for variable: RESP

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

Alpha= 0.05 df= 85 MSE= 442.5995
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 22.17504

Number of Means	2	3	4
Critical Range	12.58	13.22	13.64

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	36.476	21	b
A A	32.000	24	c
A A	28.905	21	a
A A	28.217	23	d

General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	a b c d

Number of observations in data set = 89

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	632.72637076	0.57	0.6381
Error	85	31600.91407867		
Corrected Total	88	32233.64044944		

R-Square	C.V.	RESP Mean
0.019629	102.1459	18.87640449

Source	DF	Type I SS	F Value	Pr > F
TRT	3	632.72637076	0.57	0.6381

Source	DF	Type III SS	F Value	Pr > F
TRT	3	632.72637076	0.57	0.6381

4. ANALYSIS OF VE DATA

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General Linear Models Procedure

Duncan's Multiple Range Test for variable: RESP

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

Alpha= 0.05 df= 85 MSE= 371.7755
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 22.17504

Number of Means	2	3	4
Critical Range	11.53	12.12	12.51

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	22.083	24	c
A A	20.571	21	b
A A	17.286	21	a
A A	15.435	23	d

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General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	a b c d

Number of observations in data set = 89

NOTE: Due to missing values, only 85 observations can be used in this analysis.

General Linear Models Procedure

Dependent Variable: RESPONSE
Weight: WT

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	12581.1056678	2.65	0.0544
Error	81	128228.3291898		
Corrected Total	84	140809.4348576		

R-Square	C.V.	RESPONSE Mean
0.089348	46.33800	85.86421167

Source	DF	Type I SS	F Value	Pr > F
TRT	3	12581.1056678	2.65	0.0544

Source	DF	Type III SS	F Value	Pr > F
TRT	3	12581.1056678	2.65	0.0544

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7:56 Tuesday, February 27, 1990

General Linear Models Procedure

Duncan's Multiple Range Test for variable: RESPONSE

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

Alpha= 0.05 df= 81 MSE= 1583.066
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 21.21699

Number of Means	2	3	4
Critical Range	24.33	25.59	26.40

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	88.46	22	c
A A	86.91	21	a
A A	85.30	22	d
A A	83.02	20	b

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General Linear Models Procedure
Class Level Information

Class	Levels	Values
TRT	4	a b c d

Number of observations in data set = 89

NOTE: Due to missing values, only 85 observations can be used in this analysis.

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7:56 Tuesday, February 27, 1990

General Linear Models Procedure

Dependent Variable: RESPONSE
Weight: WT

Source	DF	Sum of Squares	F Value	Pr > F
Model	3	73088.4756710	0.84	0.4744
Error	81	2341499.4621626		
Corrected Total	84	2414587.9378337		

R-Square	C.V.	RESPONSE Mean
0.030270	337.2796	50.40973722

Source	DF	Type I SS	F Value	Pr > F
TRT	3	73088.4756710	0.84	0.4744

Source	DF	Type III SS	F Value	Pr > F
TRT	3	73088.4756710	0.84	0.4744

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7:56 Tuesday, February 27, 1990

General Linear Models Procedure

Duncan's Multiple Range Test for variable: RESPONSE

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

Alpha= 0.05 df= 81 MSE= 28907.4
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 21.21699

Number of Means	2	3	4
Critical Range	104.0	109.3	112.8

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	TRT
A	58.44	22	c
A			
A	49.67	21	a
A			
A	46.55	20	b
A			
A	46.15	22	d

^Z

VINCLOZOLIN
MALE FERTILITY

Male Body wt Day 0 and Terminal weights

SAS 8:12 Friday, March 2, 1990

1

OBS	TRT	INIT	FIN
1	a	1276	1152
2	a	942	1306
3	a	1146	1060
4	a	1180	1268
5	a	1171	1147
6	a	1181	1154
7	a	1199	1295
8	a	1251	1348
9	a	1228	1262
10	a	1142	1192
11	a	1148	1163
12	a	1365	1405
13	a	1166	1275
14	a	1208	1268
15	a	1129	1102
16	a	1032	1109

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2

OBS	TRT	INIT	FIN
17	a	1046	1207
18	a	1355	1330
19	a	1097	1272
20	a	1210	1208
21	a	1393	1283
22	b	1188	1304
23	b	1381	1469
24	b	1291	1224
25	b	1183	1262
26	b	1161	1247
27	b	1233	1339
28	b	1143	1171
29	b	1307	1432
30	b	1171	1196
31	b	1227	1239
32	b	1224	1173

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3

OBS	TRT	INIT	FIN
33	b	1253	1241

34	b	1365	1390
35	b	975	1180
36	b	1097	1210
37	b	1215	1165
38	b	1186	1170
39	b	1196	1342
40	b	1269	1278
41	b	888	1309
42	b	889	1037
43	b	1589	1605
44	c	1126	1053
45	c	1265	1346
46	c	1142	1213
47	c	1257	1346
48	c	1142	1213

OBS	TRT	INIT	FIN
49	c	1257	1326
50	c	1242	1173
51	c	1205	1170
52	c	1272	1220
53	c	1391	1495
54	c	1119	1088
55	c	1232	1238
56	c	1025	1190
57	c	1018	1034
58	c	1256	1299
59	c	1177	1170
60	c	1225	1045
61	c	1194	1315
62	c	1178	1179
63	c	1127	1184
64	c	1521	1391

OBS	TRT	INIT	FIN
65	d	1219	1147
66	d	1336	1384
67	d	1269	1348
68	d	1167	1120
69	d	1092	1382
70	d	1227	1301
71	d	1253	1279
72	d	1206	1226
73	d	1375	1470

74	d	1087	1278
75	d	1256	1127
76	d	1193	1300
77	d	1150	1118
78	d	1278	1344
79	d	1142	1213
80	d	1246	1064

6

OBS	TRT	INIT	FIN
81	d	1145	1210
82	d	1123	1152
83	d	1108	1193
84	d	1260	1345
85	d	982	1023
86	d	917	1066
87	d	1285	1318

General Linear Models Procedure

Dependent Variable: FIN

Parameter		Estimate		T for H0: Parameter=0	Pr > |T|	Std Error of Estimate
INTERCEPT		526.7600113	B	5.50	0.0001	95.75838957
TRT	a	-4.1233695	B	-0.15	0.8773	26.63357813
	b	28.6188522	B	1.09	0.2804	26.33626089
	c	-24.1139262	B	-0.90	0.3688	26.68135871
	d	0.0000000	B	.	.	.
INIT		0.5964460		7.54	0.0001	0.07912605

NOTE: The X'X matrix has been found to be singular and a generalized inverse was used to solve the normal equations.

Estimates followed by the letter 'B' are biased, and are not unique estimators of the parameters.

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General Linear Models Procedure
Least Squares Means

TRT	FIN LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0	LSMEAN Number
a	1235.51305	19.27518	0.0001	1
b	1268.25527	18.81864	0.0001	2
c	1215.52249	19.28213	0.0001	3
d	1239.63642	18.40844	0.0001	4

Pr > |T| H0: LSMEAN(i)=LSMEAN(j)

i/j	1	2	3	4
1	.	0.2280	0.4661	0.8773
2	0.2280	.	0.0536	0.2804
3	0.4661	0.0536	.	0.3688
4	0.8773	0.2804	0.3688	.

General Linear Models Procedure

Duncan Grouping	Mean	N	TRT
A	1271.95	22	b
A			
A	1235.13	23	d
A			
A	1228.86	21	a
A			
A	1223.24	21	c

^Z

Mean Food Consumption Values Day-7 Day 56 used

OBS	TREATMENT	REPS	RESPONSE 1	RESPONSE 2	RESPONSE 3	RESPONSE 4	RESPONSE 5	RESPONSE 6	RESPONSE 7	RESPONSE 8	RESPONSE 9	RESPONSE 10	RESPONSE 11	RESPONSE 12	RESPONSE 13	RESPONSE 14	RESPONSE 15	RESPONSE 16	RESPONSE 17	RESPONSE 18	RESPONSE 19	RESPONSE 20
1	a	166	203	.	199	181	.	109	149	.	116	185	.	117	164	.	166	170	.	240	179	
2	a	185	199	.	296	186	.	150	190	.	192	183	.	164	172	.	182	219	.	150	244	
3	a	231	162	.	249	332	.	209	242	.	180	247	.	170	146	.	118	147	.	163	192	
4	b	166	139	.	220	148	.	187	167	.	171	220	.	166	191	.	136	139	.	122	157	
5	b	154	152	.	198	122	.	177	159	.	187	219	.	124	127	.	174	237	.	252	120	
6	b	182	202	.	156	156	.	189	275	.	159	194	.	151	187	.	217	212	.	153	189	
7	b	151	120	.	119	184	.	116	140	.	161	154	.	122	135	.	150	161	.	223	144	
8	c	130	173	.	195	169	.	204	185	.	181	257	.	123	188	.	132	182	.	98	178	
9	c	123	155	.	166	152	.	165	153	.	213	172	.	167	167	.	210	217	.	145	240	
10	c	158	161	.	108	128	.	161	184	.	143	187	.	121	153	.	147	146	.	188	193	
11	d	194	240	.	255	230	.	166	156	.	142	149	.	134	130	.	125	174	.	101	190	
12	d	104	167	.	128	149	.	171	205	.	116	160	.	163	185	.	180	147	.	148	159	

General Linear Models Procedure

Dependent Variable: RESP

Source	DF	Type I SS	Mean Square	F Value	Pr > F
TRT	3	13060.01190	4353.33730	2.94	0.0350

Source	DF	Type III SS	Mean Square	F Value	Pr > F
TRT	3	13060.01190	4353.33730	2.94	0.0350

General Linear Models Procedure

Duncan's Multiple Range Test for variable: RESP

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

Alpha= 0.05 df= 164 MSE= 1483.015
WARNING: Cell sizes are not equal.
Harmonic Mean of cell sizes= 39.52941

Number of Means	2	3	4
Critical Range	17.21	18.10	18.67

Means with the same letter are not significantly different.

General Linear Models Procedure

Duncan Grouping	Mean	N	TRT
A	186.762	42	a
B	168.446	56	b
B			
B	167.095	42	c
B			
B	163.143	28	d

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
ENVIRONMENTAL RESEARCH LABORATORY
200 S.W. 35TH STREET
CORVALLIS, OREGON 97333

18 December 1989

Dan Rieder
Ecological Effects Branch
Environmental Fate and Effects Division
Office of Pesticide Programs
U. S. EPA
Washington, D. C. 20460

Dear Mr. Rieder;

I have reviewed the reports from the special avian reproduction study conducted with mallards using Vinclozolin, with attention to the apparent treatment effects on testes weight and size. Although the testes weights after the recovery period (R1) appeared significantly greater in the treatment groups than in the control group using parametric statistics, I do not believe this represents a chemical effect. These data represent testes measurements from birds that range from reproductively active (testes greater than about 15g) to inactive (testes < 1g) in each treatment group. Only a few pairs of birds were actively laying fertile eggs during this recovery period, while the remaining pairs appeared to be rapidly regressing from reproductive condition. From the temporal distribution of egg production (Fig. 5) it appears that peak production was reached 4 to 6 weeks after it began, with birds rapidly falling out of production thereafter. Consequently, detection of treatment effects on testes size and weight is greatly compromised by the variability in reproductive condition of males within each treatment. I believe the statistical difference observed after the recovery period is an artifact of variability in reproductive condition and the small sample size. The most sensitive test of treatment effects should have been when all males were in the same reproductive condition, such as at the first sacrifice (I1), when birds were in peak egg production. There was no detectable treatment effect on testes weight or size at I1.

There are two other comments about the report I will make for your consideration. The first has to do with the experimental design pertaining to cage arrangements in the test rooms. Replicates from each treatment group were properly divided between the two test rooms, but they are clumped within each room. This would not be such a concern if it could be demonstrated that cage position within a room had no bearing on the response variables. However, I noticed that total egg production in Groups 0 (total of 620) and 3 (660), that were near the door, was lower than Groups 1 (800) and 2 (772) at the back of the room. Also, two of the three birds that died before producing eggs were control birds next to the door. If the

position of the cage in the room does influence the response variables, then the distribution of treatment within each room should be a completely randomized or randomized block design. In the current design, cage position is confounded with treatment and there is no way to separate the influences of the two factors. I do not know if it would make a difference in this test, but it is a possible weakness of this design.

The second concern is with the high rate of infertility observed throughout the test. The high number of pairs producing only infertile eggs in all treatment groups, including controls, greatly compromises the ability to detect effects attributable to the chemical. Given the variability in the infertility rate among control birds, only a major chemical effect could have been statistically detected. As observed in the first test, it is possible to achieve >90% fertility in control pairs. The results of pathology do not indicate the occurence of treatment related reductions in spermatogenesis, but also can not explain the high rate of infertility in the test. Unfortunately it is not possible from the reports to match bird numbers with cage numbers so pathology reports can not be linked with egg production or fertility.

I hope these comments are useful. If you have any questions about these comments, please feel free to call.

Sincerely;

Richard Bennett

Richard S. Bennett
Research Ecologist
FTS 420-4582