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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: EPA ID # 7969-53 & 7969-62. Review of a One-Year Feeding study in Dogs with Vinclozolin (technical). Guideline 83-1. Study Number 87/0447, October 1987 from BASF. MRID No.: 408595-01

Caswell Number 323C.
Project No.: 9-0237
Record No.: 233865 &
233867.

To: L. Rossi/
L. Schnaubelt PM 21
Registration Division (H7505C)

From: David G Anderson, PhD. *David G Anderson 4/10/89.*
Section 2, Toxicology Branch I (IRS)
Health Effects Division (H7509C)

Thru: Marion Copley, DVM *Andrew W. Sansworth for MC.*
Acting Head Section 2. *4/10/89*
Toxicology Branch I (IRS)
Health Effects Division (H7509C).

Enclosed is the DER for the "Report on the Study of the Toxicity of Vinclozolin in Beagle Dogs After a 12-Month Administration Via the Diet". Study No. 87/0447, October 1987, from BASF. Sponsored by BASF. MRID Number 408595-01.

The abstract of the study, the NOEL and LEL, and the additional information required of the sponsor to upgrade the study from core supplementary to core minimum are presented below.

It is recommended that the DER be submitted to the registrant for their information.

ABSTRACT:

Vinclozolin, technical, was administered in the food of 6 beagle dogs per group per sex for one-year. Body weights were determined weekly. Blood was drawn initially, at week 13, 26, and 52, and hematology and clinical chemistry conducted on the samples. Necropsy was conducted at 55 weeks and the organs and tissues were examined microscopically.

Body weights were not affected. Detection of potential

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effects on the appetite of the animals was limited because the amount of food consumed was limited to 700 g per animal. As a result potential effects on the efficiency of food utilization were also limited. However within these limitations, no effects were noted on body weight, body weight gain, or efficiency of food utilization.

Hematology indicated marginal anemia at the HDT in males and females. This marginal anemia is consistent with destruction of red blood cells. A combination of the increase in mean cell volume (MCV) and mean hemoglobin content per erythrocyte (MCH) in females at the HDT, and a dose related increase in severity of hemosiderin in the livers of males and females at the HDT are consistent with this slight chronic anemia. The MCV in females was statistically significantly elevated at week 13 (103%), 26 (103%), and 52 (105%) at the HDT. The MCH in females also was statistically significantly elevated at week 52 at the HDT. The reticulocytes in males and females may have been elevated at the HDT, in males at week 13 (214%), 26 (206%), 52 (261%), and in females at week 13 (381%), 26 (545%), and 52 (294%). Although statistical analyses were not conducted on the reticulocytes, the increase appeared to be treatment related. Grade 3 hemosiderin occurred only at the HDT in the livers of 4/6 males, and in 2/6 females at the MDT2 and 2/6 at the HDT. Lower grade levels of hemosiderin occurred in males and females at lower dose levels but there was no apparent dose relationship at these grade levels.

The reticulocytes were 4%-8% prior to treatment, but declined to 1.8% in controls at the study termination after one year. These values were much higher values than normal values obtained from the literature (1.8%-2.3%). Parasitic infestation in these animals (i.e. worms) may have been the cause of the high initial values for the reticulocytes. Although the report indicated that the animals were de-wormed periodically, it did not indicate the type of worm infestation.

Clinical chemistry data indicates that serum bilirubin was statistically significantly increased in males (134%-181%) at week 26 at the MDT2 and HDT, and in females (150%) at the HDT. The increased bilirubin may indicate hemolysis.

Except for the increased serum bilirubin levels, no other dose related or compound related effects were noted in the serum chemistry values. The increased serum bilirubin levels is also consistent red blood cell destruction. Several of the clinical chemistry values were statistically significantly different from initial values before treatment, and some were statistically significantly different from controls but exhibited no dose relationship. Other values were within the normal range.

Urine analyses revealed no dose related or treatment related effects.

Several absolute organ weights were statistically significantly elevated in males at the HDT (liver, spleen, testes, adrenal and thyroid), and in females the absolute adrenal weights were statistically significantly increased at the two

highest dose levels. In the case of the absolute weights for livers, adrenals, and thyroids of males possible dose related weight increases were noted at the MDI2 as well. In the case of the testes a dose related response may have occurred at all dose levels. The relative organ weights exhibited essentially the same findings as the absolute organ weights, except that the relative liver weight in the males was statistically significantly elevated at all dose levels and testes weights were statistically significantly elevated at the MDT2 and HDT. In females the relative adrenal weight was statistically significant only at the HDT. It was concluded that the lowest effect level for organ weight change was the MDT2 or the 150 ppm dose level. This was the dose level for a statistically significant increase in testes weight in males and a statistically significant increase in adrenal weight in females.

Histological findings were noted in the adrenals of males and females, in the spleen and liver, and in the testes and prostates of males. Histopathology of the adrenals of males and females were noted at the highest dose level, and in females at the two highest dose levels. The adrenals were enlarged physically and an accumulation of lipid was noted at the HDT. The formation of adrenal capsular nodules were noted in all groups and cortical nodules in most groups with no apparent dose related response. Hemosiderin was noted in the spleen and liver of males and females of all groups. The severity of the grade levels of the hemosiderin was increased and test compound related at the HDT in males and probably test compound related at the two highest dose levels in females. In males diffuse hyperplasia of the leydig cells of the testes was noted at the HDT, and prostate atrophy at the two highest dose levels.

Corneal opacity was seen in one dog. Cataracts were also seen in chronic rat studies at unspecified dose levels. It can not be determined from the data presented whether or not this corneal opacity was treatment related.

Other feeding studies in dogs indicated increased platelets and Howell-Jolly bodies at 1000 ppm in a 90-day study and increased absolute and relative adrenal weight at 300 ppm in a 6 month study. In a 90-day feeding study in rats an increase in Howell-Jolly bodies were noted in males and females and a platelet increase, and adrenal weight increase was noted in females dosed at 2000 ppm. In addition, slight decreases in erythrocytes, hemoglobin, and the hematocrit were noted in another 90-day feeding study in rats dosed at 1000 ppm and above. A transient decrease was seen in erythrocytes, hemoglobin, and the hematocrit in some animals dosed at 300 ppm. Thus, treatment with Vinclozolin can cause anemia at these dose levels in dogs and rats, but the evidence for anemia in the current study in dogs is not clear cut.

It should be noted that sexual ambiguity of the male neonate rat was found in a Japanese teratogenicity study and confirmed in additional studies. The effect has only been confirmed in studies conducted at 50 mg/kg/day and higher. The test substance probably

has hormonal or anti-hormonal activity. The hyperplasia of the Leydig cells is consistent with gonadotrophin stimulation, while the the prostate atrophy is consistent with an anti-androgen effect. However, there is insufficient information to determine the exact nature of the activity.

NOEL AND LEL:

Doses administered in the feed to beagle dogs 0, 35, 75, 150, 1500 ppm (Doses to males 0, 1.1, 2.4, 4.8, & 47 mg/kg/day; to females 0, 1.1, 2.5, 5.1, & 53 mg/kg/day.)

NOEL: 75 ppm or 2.4 and 2.5 mg/kg/day for males and females, respectively.

LEL: 150 ppm or 4.8 and 5.1 mg/kg/day for males and females, respectively. In males - increased bilirubin, increased relative testes weights, and prostate atrophy; In females - increased absolute adrenal weights, adrenal lipid accumulation, and marginally increased liver hemosiderin. In addition at the HDT in males, increased absolute and relative liver, spleen, testes, adrenal, thyroid weights, and increased diffuse hyperplasia of the Leydig cells, and lipid accumulation in the adrenal cortex, and increased platelets were noted; In females at the HDT, increased absolute and relative adrenal weight, slight increases in MCV and MCH were noted. Possible increased reticulocytes in HDT males and females.

ADDITIONAL INFORMATION NEEDED:

1. The quality assurance statement must be signed by an appropriate quality assurance official and submitted to the Agency.
2. The results of the data found in the blood smears other than the reticulocytes must be submitted.
3. The source of the dogs used in the study must be submitted.
4. The historical incidence of spontaneous corneal opacity in the strain of dogs used must be submitted.
5. If possible, please explain the high values for the reticulocytes noted in the dogs prior to initiation of treatment.
6. Were there any detectable differences between the control group and the treated group(s) in the seminal vesicles, seminiferous tubules or the epididymides.
7. The numbers in Table C of this DER are assumed to represent

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reticulocyte counts per 100, but this is not clear in the submitted report. On page 30 (bottom page number) of the submitted report, the unit for reticulocytes are given as unidentifiable symbol, which appears to be "%" sign and (10^{-3} erythrocytes). This should be clarified.

8. The sponsor should present historical control data on the reticulocytes from the colony of dogs used in this study.

Cover memorandum for the One-Year Feeding Study/Dog/83-1/87/0447-
B:\VINCLOZ3.23C\C_MEM__3.23C/D Anderson/2/28/89.

Primary reviewer: David G Anderson, PhD. *David G Anderson 4/10/84*
Section 2, Tox. Branch 1 (IRS) (H7509C).
Secondary reviewer: Marion P Copley, DVM. *Marion P Copley*
Section 2, Tox. Branch 1 (IRS) (H7509C). *for MC 007122*

DATA EVALUATION REPORT

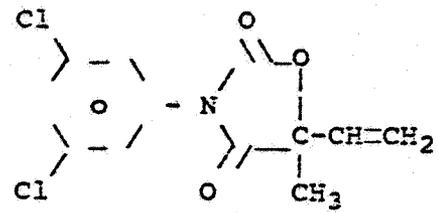
STUDY TYPE: One-Year Feeding Study/Dog/83-1/87/0447.

MRID No.: 408595-01. TOX. CHEM. No.: 323C

TEST MATERIAL: Vinclozolin.

SYNONYMS: 3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidine-2,4-dione.

STRUCTURE:



SPONSOR: BASF Corp. Chemicals Div., Agricultural Chemicals, 100 Cherry Hill Road, Parsippany, NJ 07054.

TESTING FACILITY: BASF Aktiengesellschaft, Dept. Toxicology, 6700 Ludwigshafen/Rhein, West Germany.

STUDY NO.: 87/0447

REPORT TITLE: Report on the Study of the Toxicity of Vinclozolin Beagle Dogs After a 12-Month Administration Via the Diet.

AUTHOR(S): J Hellwig.

REPORT ISSUED: October 1987.

CORE GRADE: Supplementary because additional information is needed.

CONCLUSIONS: Doses administered in the feed to beagle dogs 0, 35, 75, 150, 1500 ppm (Doses to males 0, 1.1, 2.4, 4.8, & 47 mg/kg/day; to females 0, 1.1, 2.5, 5.1, & 53 mg/kg/day.)

NOEL: 75 ppm or 2.4 and 2.5 mg/kg/day for males and females, respectively.

LEL: 150 ppm or 4.8 and 5.1 mg/kg/day for males and females, respectively. In males - increased bilirubin, increased relative testes weights, and prostate atrophy; In females - increased absolute adrenal weights, adrenal lipid accumulation, and

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marginally increased liver hemosiderin. In addition at the HDT in males, increased absolute and relative liver, spleen, testes, adrenal, thyroid weights; increased diffuse hyperplasia of the Leydig cells of the testes, and organ lipid accumulation in the adrenal cortex; and increased platelets were noted; In females at the HDT, increased absolute and relative adrenal weight, and slight increases in MCV and MCH were noted. There was a possible increase in reticulocytes in HDT males and females.

A. MATERIALS:

1. Test compound: Vinclozolin, technical. Description, whitish solid, Batch # N 173, ZNT substance no. 84/165, Purity \geq 98.9%.
2. Test animals: Species: Dogs, Strain: Beagle, Age: 6-9 months, Weight: males-9.6(8.0-11.9 kg), females-9.4(8.2-10.7 kg), Source: NOT SPECIFIED. Acclimatized for 1 week.
3. Environment: Dogs were housed singly in 6.0 m² area (inner kennel 1.5 m², outer kennel 4.5 m²). The inner kennel was heated during winter. Lighting was natural. Humidity: Not specified, but probably natural. Dogs were vaccinated with Stagleban P vaccine and de-wormed at regular intervals with piperazine.

B. STUDY DESIGN:

1. Animal Assignment - Animals were assigned randomly, (stratified by weight) to 5 groups, 1 control group and 4 dose groups of 6 animals/sex/group.

Test group	Dose in diet		Main Study		Bloods for clinical chem. & hematology, drawn at 0, 13, 26, and 52 weeks		
	(ppm)	(mg/kg)	M	F	M	F	
1. Cont.	0.0	0.0	0.0	6	6	6	6
2. Low (LDI)	35	1.1	1.1	6	6	6	6
3. Mid1(MDI1)	75	2.4	2.5	6	6	6	6
4. Mid2(MDI2)	150	4.8	5.1	6	6	6	6
4. High(HDI)	1500	47	53	6	6	6	6

2. Diet preparation - diet was prepared weekly, and stored at UNSPECIFIED temperature. Samples of treated food were analyzed for stability, homogeneity, and concentration at week 0, 13, 26, and 52 weeks.

Results - The percent of nominal of Vinclozolin in the diet varied between 83% and 102% with most of the values deviating less. In addition, the stability and homogeneity of the test substance in the diet was satisfactory.

3. Animals receive 700 g of food daily divided between morning and evening and water ad libitum.

4. Statistics - The following procedures were used in analyzing the numerical data: Conducted at the Computer Center of BASF Aktiengesellschaft. Statistical methods are specified on page 26, 27, and 28 (3.10.1-3.10.10) of the submitted report.

5. Quality assurance was not signed by M Fleig, Head of the Quality Assurance Unit.

C. METHODS AND RESULTS: Numbered tables were copied from the submitted report.

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality.

Results - Toxicity - No signs of toxicity were observed throughout the study which could be related to dose.

Mortality (Survival) - No unscheduled mortality was reported to have occurred in the study.

2. Body Weight - They were weighed initially and weekly for the duration of the study.

Results - Table A shows the initial body weight and body weight gain of the male and female dogs during the study. No significant dose or compound related effects were seen on body weight or body weight gain in males or females.

Table A.

Mean body weight and body weight gain in Kg, and % body weight gain for males(M) and females(F) during the study.

Test group	Sex	Body Wt. (initial)	Body Wt. (gain)	Body Wt. % gain
00.0 ppm	M	9.5	+ 2.8	+30
	F	9.2	+ 1.5	+16
35	M	9.7	+ 2.1	+22
	F	9.5	+ 2.2	+23
75	M	9.8	+ 2.1	+21
	F	9.4	+ 2.1	+22
150	M	9.6	+ 2.3	+24
	F	9.4	+ 1.8	+19
1500 ppm	M	9.5	+ 2.7	+28
	F	9.3	+ 1.7	+18

3. Food consumption and compound intake - All the food available to the animals was consumed.

Results - Food consumption - Consumption was constant during

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the study. Equal amounts of food were given for a total daily intake of 700 g. Both males and females ate essentially 100% of the food available to them.

Food efficiency - Not reported, or calculated.

Compound intake - Table B presents the consumption of the test substance.

Table B.

Mean daily consumption of the test compound from week 0 to 13, 13-26, 26-52, and over all consumption from week 0-52 for males (M) and females (F).

Test group	ppm	Test Compound Consumption (mg/kg/day)							
		Week 0-13		Week 13-26		Week 26-52		Week 0-52	
		M	F	M	F	M	F	M	F
Control	0000.								
LDT	35	1.2	1.2	1.1	1.1	1.1	1.1	1.1	1.1
MDT1	75	2.6	2.6	2.4	2.5	2.3	2.4	2.4	2.5
MDT2	150	5.2	5.4	4.8	5.3	4.5	4.8	4.8	5.1
HDI	1500	51.7	54.7	47.6	55.2	44.1	50.1	46.9	52.6

4. Ophthalmological examinations were performed initially, at 6 months, and at one year on all 6 animals/sex/group.

Results - Corneal opacity occurred toward the nasal quadrant of one eye of one male dog towards the end of the study at the HDI. It was reported that this type of lesion is found often in these animals. No Historical incidence data were reported. No other possible compound related effects were reported to have occurred.

5. Blood was collected before treatment and at 13 weeks, 26 weeks, and 52 weeks for hematological and clinical analysis from all 6 animals/sex/group. The following were considered in determining biological significance of these data; the degree of change, statistical significance, and dose relationship with controls and with the values before initiation of treatment. When a percent change is reported in parentheses, it refers to percent of control values.

The CHECKED (X) parameters were examined.

a. Hematology -

X Hematocrit (HCT)*	Total plasma protein (TP)
X Hemoglobin (HGB)*	X Leukocyte differential count
X Leukocyte count (WBC)*	X Mean corpuscular HGB (MCH)
X Erythrocyte count (RBC)*	X Mean corpuscular HGB conc. (MCHC)
X Platelet count*	X Mean corpuscular volume (MCV)
X Thromboplastin time	

Results - A statistically significant increase in the mean cell

volume (MCV), 104 %, over initial values occurred in males only at the HDI, but it did not appear to be treatment related. MCV increased with the time on study and each blood sampling period in controls, and the values at the HDI were not statistically significantly different from control values. The MCV was elevated in females at week 13 (103%), 26 (103%), and 52 (105%) at the HDI. These values were slightly but statistically significantly elevated over controls and initial values in the HDI group, and they were probably treatment related in females.

Mean hemoglobin content per erythrocyte (MCH) (103%) was statistically significantly elevated over controls at week 52 in HDI females. This value also was elevated over the initial values.

Platelets were statistically significantly elevated at week 13 (130%), 26 (135%), and 52 (133%) at HDI in males. These values were also statistically significantly elevated over initial values in this group, and were probably treatment related. The values in females were statistically significantly elevated over initial values in the HDI group, but not over the control group. The values in females did not appear to be treatment related.

Hemoglobin concentration, erythrocyte count, and hematocrit were all nominally lower (97%) than control values at week 52 in males at the HDI. The means of these values were at or close to one standard deviation from the control values, and did not appear to be dose related. The failure of the hematocrit, hemoglobin concentration, and the red blood cell count to be depressed is not inconsistent with a suggestion of slight chronic anemia at the HDI.

Marginal changes in the erythrocyte counts were stated in the submitted report to have occurred in male and females. A biologically significant decrease in the erythrocyte count in the HDI or any other dose group could not be verified. There were no statistically significantly values or biologically significant values in treated groups. Neither could a decrease in erythrocyte count in HDI females be confirmed. Values for females were statistically significantly lower compared with initial values, but they were nominally increased compared with control values, thus, the effect probably was not treatment related.

Reticulocytes were apparently elevated at the HDI at week 13 (214%), 26 (208%), and 52 (261%) in males, but not over initial values (Table C). In this case the increase over controls was considered to be biologically significant. The corresponding values in HDI females were at week 13 (381%), 26 (547%), and 52 (294%). These values were nominally increased over initial values as well as the control values. Thus the increase in the values in the females also were considered to be biologically significant. It should also be noted that the elevation in reticulocytes before treatment is consistent with anemia in the dogs at the time the study was initiated. It appears that the test material exacerbated the existing effect on the reticulocytes. The reticulocyte values were stated to be not meaningfully analyzable by statistics.

No treatment or dose relationship could be detected among

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the other parameters analyzed. Although many values were statistically significant, values were either not dose related or were within the normal range.

Table C.

Mean reticulocytes in male and female dogs.

Dose group	Study week							
	0		13		26		52	
	M	F	M	F	M	F	M	F
0.0	8.0	3.0	4.3	2.7	2.5	1.7	1.8	1.8
35 ppm	6.2	3.5	4.2	3.5	3.5	1.7	2.7	1.5
75 ppm	7.2	5.8	6.2	3.8	3.0	4.3	3.0	2.8
150 ppm	4.0	3.7	6.7	6.8	4.3	4.2	2.7	3.3
1500 ppm	6.0	4.3	9.2	10.3	5.2	9.3	4.7	5.3

The numbers in the above table are assumed to represent counts per 100, but this is not clear in the report. On page 30 (bottom page number), the unit for reticulocytes are given as unidentifiable simble, which appears to be "% sign and (10⁻³ erythrocytes). This should be clarified.

b. Clinical Chemistry

Electrolytes:

- X Calcium*
- X Chloride*
- Magnesium*
- X Phosphorus*
- X Potassium*
- X Sodium*

Other:

- X Albumin*
- X Blood creatinine*
- X Blood urea nitrogen*
- X Cholesterol*
- X Globulins
- X Glucose*

ENZYMES:

- X Alkaline Phosphatase (AP)
- Cholinesterase (CHE)
- Creatinine phosphokinase* (CP)
- Lactic acid dehydrogenase (LDH)
- X Serum alanine aminotransferase (also SGPT)
- X Serum aspartate aminotransferase (also SGOT)

- X Total bilirubin*
- X Total protein*
- X Triglycerides (TG)

Results - Total bilirubin was statistically significantly elevated at the 2 highest dose levels (134-186%) at week 26 in males, and at the HDI (150%) at week 26 in females. Other statistically significant changes in clinical chemistry parameters were sporadic and did not appear to be dose or treatment related.

6. Urinalysis - Urine was collected from fasted animals initially and at 13, 26, and 52 weeks. The CHECKED (X)

parameters were examined.

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Appearance*	X Glucose*
Volume*	X Ketones*
X Specific gravity*	X Bilirubin*
X pH	X Blood*
X Sediment (microscopic)*	X Nitrite
X Protein*	X Urobilinogen

Results - No dose or treatment related effects were found in any parameter analyzed.

7. Sacrifice and Pathology -

All animals that died and that were sacrificed on schedule were subject to gross pathological examination. Sacrifice was by exsanguination from the cervical and brachial vessels after anesthesia. The (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X DIGESTIVE SYSTEM	X CARDIVASC./HEMAT.	X NEUROLOGIC
Tongue	X Aorta*	XX Brain*
X Salivary glands*	X Heart*	X Periph nerve*
X Esophagus*	X Bone marrow*	X Spinal cord (3 levels)
X Stomach*	X Lymph nodes*	X Pituitary*
X Duodenum*	XX Spleen*	X Eyes(optic nerve)
X Jejunum*	X Thymus *	GLANDULAR
X Ileum*	UROGENITAL	XX Adrenal*
X Cecum*	XX Kidneys*	Lacrimal gland*
X Colon*	X Urinary bladder*	X Mammary gland*
X Rectum*	XX Testes*	X Parathyroids*
XX Liver*	Epididymides*	XX Thyroids*
X Gall bladder*	X Prostate	OTHER
X Pancreas*	Seminal Vesicle	X Bone*
RESPIRATORY	XX Ovaries	X Skeletal musc.*
X Trachea*	X Uterus*	X All gross lesions & masses.
X Lungs*		X Skin

Results -

a. Organ weights - Absolute and relative organ weights are presented in Tables 159-162 which were copied from the submitted report. In males the absolute weights of the following organs were statistically significantly increased at the HDT only: liver (130%), spleen (147%), testes (143%), adrenal (216%), and thyroid (129%). In males the relative organ weights statistically significantly increased for the following organs at the HDT only:

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spleen (150%), adrenal (218%), and thyroid (129%). The relative testes weights were statistically significantly elevated (123% and 144%) in the two highest dose levels, and at the remaining dose levels there was an increased trend in relative organ weight with increased dose level. The relative liver weights were statistically significantly increased at all dose levels. The absolute and relative adrenal and thyroid weights at the MDT2 were nominally elevated in males.

In females the absolute adrenal weights were statistically significantly elevated at the MDT2 (136%), and at the HDT (234%) only. Nominally increased adrenal weights occurred in a good dose related trend at the lower dose levels. The nominally increased absolute ovarian weights (116%) at the HDT were not statistically significant. Only relative adrenal weights (230%) in females were statistically significantly elevated at the HDT.

b. Gross pathology - The prostate of 1 MDT2 and of 2 HDT dogs were observed to be reduced in size. The adrenals of all males and of 5/6 females were observed to be enlarged at the HDT.

c. Microscopic pathology -

1) Non-neoplastic - Microscopically, lipid increase was found in the adrenals of all HDT males and in 5/6 HDT females. At least one nodule was detected under the adrenal capsule of each animal in the study, but the nodules in the cortex were found in only a few animals with no dose relationship. Five of six males at the HDT demonstrated diffuse hyperplasia of the testes (presumably of the Leydig cells). No comments were made on the state of the seminiferous tubules. Atrophy of the prostate was demonstrated at the MDT2 and the HDT. Hemosiderin was found in the liver and spleens of all animals of both sexes; but in the liver, the severity of the hemosiderin was found to be treatment related at the HDT in males and at the two highest dose levels in females.

2) Neoplastic - No neoplastic lesions were reported.

D. DISCUSSION AND ABSTRACT:

Vinclozolin, technical, was administered in the food of 6 beagle dogs per group per sex for one-year. Body weights were determined weekly. Blood was drawn initially, at week 13, 26, and 52, and hematology and clinical chemistry conducted on the samples. Necropsy was conducted at 55 weeks and the organs and tissues were examined microscopically.

Body weights were not affected. Detection of potential effects on the appetite of the animals was limited because the amount of food consumed was limited to 700 g per animal. As a result potential effects on the efficiency of food utilization were also limited. However within these limitations, no effects

were noted on body weight, body weight gain, or efficiency of food utilization.

Hematology indicated marginal anemia at the HDT in males and females. This marginal anemia is consistent with destruction of red blood cells. A combination of the increase in mean cell volume (MCV) and mean hemoglobin content per erythrocyte (MCH) in females at the HDT, and a dose related increase in severity of hemosiderin in the livers of males and females at the HDT are consistent with this slight chronic anemia. The MCV in females was statistically significantly elevated at week 13 (103%), 26 (103%), and 52 (103%) at the HDT. The MCH in females also was statistically significantly elevated at week 52 at the HDT. The reticulocytes in males and females may have been elevated at the HDT, in males at week 13 (214%), 26 (213%), 52 (261%), and in females at week 13 (381%), 26 (343%), and 52 (294%). Although statistical analyses were not conducted on the reticulocytes, the increase appeared to be treatment related. Grade 3 hemosiderin occurred only at the HDT in the livers of 4/6 males, and in 2/6 females at the MDT2 and 2/6 at the HDT. Lower grade levels of hemosiderin occurred in males and females at lower dose levels but there was no apparent dose relationship at these grade levels.

The reticulocytes were 4%-8% prior to treatment, but declined to 1.8% in controls at the study termination after one year. These values were much higher values than normal values obtained from the literature (1.8%-2.8%). Parasitic infestation in these animals (i.e. worms) may have been the cause of the high initial values for the reticulocytes. Although the report indicated that the animals were dewormed periodically, it did not indicate the type of worm infestation.

Clinical chemistry data indicates that serum bilirubin was statistically significantly increased in males (134%-181%) at week 26 at the MDT2 and HDT, and in females (150%) at the HDT. The increased bilirubin may indicate hemolysis.

Except for the increased serum bilirubin levels, no other dose related or compound related effects were noted in the serum chemistry values. The increased serum bilirubin levels is also consistent red blood cell destruction. Several of the clinical chemistry values were statistically significantly different from initial values before treatment, and some were statistically significantly different from controls but exhibited no dose relationship. Other values were within the normal range.

Urine analyses revealed no dose related or treatment related effects.

Several absolute organ weights were statistically significantly elevated in males at the HDT (liver, spleen, testes, adrenal and thyroid), and in females the absolute adrenal weights were statistically significantly increased at the two highest dose levels. In the case of the absolute weights for livers, adrenals, and thyroids of males possible dose related weight increases were noted at the HDT as well. In the case of the testes a dose related response may have occurred at all dose levels. The relative organ weights exhibited essentially the

same findings as the absolute organ weights, except that the relative liver weight in the males was statistically significantly elevated at all dose levels and testes weights were statistically significantly elevated at the MDT2 and HDT. In females the relative adrenal weight was statistically significant only at the HDT. It was concluded that the lowest effect level for organ weight change was the MDT2 or the 150 ppm dose level. This was the dose level for a statistically significant increase in testes weight in males and a statistically significant increase in adrenal weight in females.

Histological findings were noted in the adrenals of males and females, in the spleen and liver, and in the testes and prostates of males. Histopathology of the adrenals of males and females were noted at the highest dose level, and in females at the two highest dose levels. The adrenals were enlarged physically and an accumulation of lipid was noted at the HDT. The formation of adrenal capsular nodules were noted in all groups and cortical nodules in most groups with no apparent dose related response. Hemosiderin was noted in the spleen and liver of males and females of all groups. The severity of the grade levels of the hemosiderin was increased and test compound related at the HDT in males and probably test compound related at the two highest dose levels in females. In males diffuse hyperplasia of the leydig cells of the testes was noted at the HDT, and prostate atrophy at the two highest dose levels.

Corneal opacity was seen in one dog. Cataracts were also seen in chronic rat studies at unspecified dose levels. It can not be determined from the data presented whether or not this corneal opacity was treatment related.

Other feeding studies in dogs indicated increased platelets and Howell-Jolly bodies at 1000 ppm in a 90-day study and increased absolute and relative adrenal weight at 300 ppm in a 6 month study. In a 90-day feeding study in rats an increase in Howell-Jolly bodies were noted in males and females and a platelet increase, and adrenal weight increase was noted in females dosed at 2000 ppm. In addition, slight decreases in erythrocytes, hemoglobin, and the hematocrit were noted in another 90-day feeding study in rats dosed at 1000 ppm and above. A transient decrease was seen in erythrocytes, hemoglobin, and the hematocrit in some animals dosed at 300 ppm. Thus, treatment with Vinclozolin can cause anemia at these dose levels in dogs and rats, but the evidence for anemia in the current study in dogs is not clear cut.

It should be noted that sexual ambiguity of the male neonate rat was found in a Japanese teratogenicity study and confirmed in additional studies. The effect has only been confirmed in studies conducted at 50 mg/kg/day and higher. The test substance probably has hormonal or anti-hormonal activity. The hyperplasia of the leydig cells is consistent with gonadotrophin stimulation, while the the prostate atrophy is consistent with an anti-androgen effect. However, there is insufficient information to determine the exact nature of the activity.

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NOEL AND LEL:

Doses administered in the feed to beagle dogs 0, 35, 75, 150, 1500 ppm (Doses to males 0, 1.1, 2.4, 4.8, & 47 mg/kg/day; to females 0, 1.1, 2.5, 5.1, & 53 mg/kg/day.)

NOEL: 75 ppm or 2.4 and 2.5 mg/kg/day for males and females, respectively.

LEL: 150 ppm or 4.8 and 5.1 mg/kg/day for males and females, respectively. In males - increased bilirubin, increased relative testes weights, and prostate atrophy; In females - increased absolute adrenal weights, adrenal lipid accumulation, and marginally increased liver hemosiderin. In addition at the HDT in males, increased absolute and relative liver, spleen, testes, adrenal, thyroid weights, and increased diffuse hyperplasia of the Leydig cells, and lipid accumulation in the adrenal cortex, and increased platelets were noted; In females at the HDT, increased absolute and relative adrenal weight, slight increases in MCV and MCH were noted. Possible increased reticulocytes in HDT males and females.

ADDITIONAL INFORMATION NEEDED:

1. The quality assurance statement must be signed by an appropriate quality assurance official and submitted to the Agency.
2. The results of the data found in the blood smears other than the reticulocytes must be submitted.
3. The source of the dogs used in the study must be submitted.
4. The historical incidence of spontaneous corneal opacity in the strain of dogs used must be submitted.
5. If possible, please explain the high values for the reticulocytes noted in the dogs prior to initiation of treatment.
6. Were there any detectable differences between the control group and the treated group(s) in the seminal vesicles, seminiferous tubules or the epididymides.
7. The numbers in Table C of this DER are assumed to represent reticulocyte counts per 100, but this is not clear in the submitted report. On page 30 (bottom page number) of the submitted report, the unit for reticulocytes are given as unidentifiable symbol, which appears to be "% sign and (10⁻³ erythrocytes). This should be clarified.
8. The sponsor should present historical control data on the reticulocytes from the colony of dogs used in this study.

* Recommended by Subdivision F (Oct. 1982) guidelines for chronic studies.

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