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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

MAR 20 1990

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3/20/90  
CASWELL FILE

OFFICE OF  
PESTICIDES AND  
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Linuron Generic Reregistration - One-Year Dog Study

Caswell No. 528  
Record No. 260394

HED TOX Project No. 0-0804  
MRID No. 409526-01

FROM: Elizabeth A. Doyle, Ph.D. *E.A. Doyle* 3/5/90  
Review Section I, TOX Branch II (HFAS)  
Health Effects Division (H7509C)

TO: C. Peterson, PM #74  
Special Review and Reregistration Division (H7508C)

THRU: Michael Ioannou, Ph.D. *J.M. Ioannou* 3/5/90  
Section Head, Review Section I  
TOX Branch II (HFAS)  
Health Effects Division (H7509C)

and

Marcia van Gemert, Ph.D. *M van Gemert* 3/12/90  
Acting Branch Chief, TOX Branch II (HFAS)  
Health Effects Division (H7509C)

ACTION REQUESTED: Review toxicology data of a one-year dog study.

RECOMMENDATION: The study listed above was previously reviewed by S. Stolzenberg, Ph.D. of TOX Branch II (HFAS) in a memo issued in June, 1989. (See attached memo, HED document # 007284.) The cited review indicated the study was "Core - Minimum" with an LEL = 125 ppm and an NOEL = 25 ppm based upon hematological changes. No further review is deemed necessary.

SECTION HEAD



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

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JUN 29 1989

OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Linuron One-Year Dog study Call-In Data

Caswell No. 528  
Record No. 238020

HED TOX Project No. 9-0670  
MRID 409526-01

FROM: Sidney Stolzenberg, Ph.D.  
Review Section I, TOX Branch II (HFAS)  
Health Effects Division (H7509C)

*S. Stolzenberg 6/19/89*

TO: V. Walters, PM #25  
Registration Division (H7505C)

THRU: Michael Ioannou, Ph.D.  
Head, Review Section I  
TOX Branch II (HFAS)  
Health Effects Division (H7509C)

*J. M. Ioannou 6-20-89*

and

Marcia van Gemert, Ph.D.  
Acting Branch Chief, TOX Branch II (HFAS)  
Health Effects Division (H7509C)

*M. van Gemert 6/28/89*

Applicant: E. I. duPont de Nemours & Co.  
Agricultural Products Department  
Wilmington, DE 19898

ACTION REQUESTED: Review toxicology data of a one-year dog study.

Background Information: In a previous 2-year dog study with dietary doses of 0, 25, 125 and 625 ppm, performed by Hodge et al., 1968, an "abnormal blood pigment" was noted at 1 and 2 years. This was accompanied by decreases in erythrocyte count, hemoglobin and hematocrit. The pigment was postulated to be methemoglobin and sulfhemoglobin. A proposed 1-year dog feeding study with linuron regarding the nature and circulating levels of these pigments was submitted to EPA for comments and suggestions (See memo of J.N. Rowe to M. McDavit and R. Taylor, dated 11/21/86). The present 1-

year dog study includes a design to test for these pigments and to determine a NOEL level for these and other possible effects due to linuron administration.

### Summary and Conclusions

Doses tested were 0, 10, 25, 125, and 625 ppm which came to 0, 0.29, 0.79, 4.17, and 18.6 mg/kg/day in males and 0, 0.30, 0.77, 3.49, and 16.1 mg/kg/day in females. Compound-related hematological effects included increased methemoglobin and sulfhemoglobin pigments at 125 and 650 ppm and decreased erythrocyte count, hematocrit and hemoglobin at 650 ppm in males and females. The bone marrow after 1 year of treatment showed small increases in erythropoietic activity whereas the liver had small increases in hemosiderin deposits, observed microscopically. All of the above findings were interpreted to indicate that linuron at the highest doses caused a mild increase in red blood cell destruction. Other hematology effects noted were an increase in platelet count and in leukocyte count at the 625 ppm dose level. There was also an increase in cholesterol levels noted in males and females receiving 625 ppm. Absolute liver weight was increased (n.s.) in males receiving 625 ppm, relative liver weight was increased in males at 125 ( $P < 0.05$ ) and 625 ( $P < 0.05$ ) ppm. In females, no statistically significant effect on liver weights were observed.

### Recommendation:

Classification: Core Minimum

LEL = 125 ppm

NOEL = 25 ppm

Based on hematology changes.

Tox Chem No. 528 Linuron

EPA

File Last Updated \_\_\_\_\_

Current Date \_\_\_\_\_

Study/Lab/Study #/Date

Material

Accession No.

Results: ID50, LC50, PIS, NOEL, LEL

TOX Category

CORE Grade/ Doc. No.

1-year dog, feeding study  
Hawke/ Labs  
Report No. 181-88  
10/28/88

Linuron,  
technical,  
96.2%

409526-01

Doses tested in beagles, 4 of each sex per group, were 0, 10, 25, 125 and 625 ppm. LEL = 125 ppm. NOEL = 25 ppm. based on an increase in methemoglobin and sialthymoglobin levels in the blood. Other effects noted at 625 ppm included slightly decreased wbc, Hct and Hb in males, small increases in hemocritin deposition in Kupfer cells of liver, small increase in erythropoietin in bone marrow ~~and~~ in bone marrow cells, and an increase in ~~of~~ serum cholesterol levels at ~~the~~ 625 ppm in both sexes at the highest dose level.

Minimum

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Reviewed By: Sidney Stolzenberg, Ph.D.  
Section I, Toxicology Branch II - HFAS (H7509C) *S. Stolzenberg 6/19/89*  
Secondary Reviewer: Michael Ioannou, Ph.D.  
Section I, Toxicology Branch II - HFAS (H7509C) *JMI 6-20-89*

DATA EVALUATION REPORT

Study Type: 1-Year Dog

Guideline: 83-1

HED Project No.: 9-0670

MRID No.: 409526-01

Caswell No.: 528

ID No.: 035506

Test Material: Linuron

Synonyms: Lorox, INZ326-118, INZ-326

Laboratory Project ID No.: 181-88

Sponsor: E.I. du Pont de Nemours & Company, Inc.  
Wilmington, DE 19898

Testing Facility: Haskell Laboratory  
Newark, DE 19714

Title of Report: Chronic Toxicity Study with INZ326-118:  
One-Year Feeding Study in Dogs.

Author(s): L.A. Malley, Ph.D.

Report Issued: October 28, 1988

Conclusions:

Doses tested were 0, 10, 25, 125, and 625 ppm which came to 0, 0.29, 0.79, 4.17, and 18.6 mg/kg/day in males and 0, 0.30, 0.77, 3.49, and 16.1 mg/kg/day in females. Compound-related hematological effects included increased methemoglobin and sulfhemoglobin pigments at 125 and 650 ppm and decreased erythrocyte count, hematocrit and hemoglobin at 650 ppm in males and females. The bone marrow after 1 year of treatment showed small increases in erythropoietic activity whereas the liver had small increases in hemosiderin deposits, observed microscopically. All of the above findings were interpreted to indicate that linuron at the highest doses caused a mild increase in red blood cell destruction. Other hematology effects noted were an increase in platelet count and in leukocyte count at the 625 ppm dose level. These effects were suggested to be secondary to an increase in erythropoiesis. There was also an increase in

cholesterol levels noted in males and females receiving 625 ppm. Absolute liver weight was increased (n.s.) in males receiving 625 ppm, relative liver weight was increased in males at 125 ( $P < 0.05$ ) and 625 ( $P < 0.05$ ) ppm. In females, no statistically significant effect on liver weights were observed.

Classification: Core Minimum

LEL = 125 ppm

NOEL = 25 ppm

Based on hematology changes.

A. Materials:

1. Test Compound - Linuron; Haskell Sample No. 16,569; Purity 96.2%; List of contaminants was not submitted.
2. Test Animals - Species: Dog; Strain: Beagle; Age: 5 to 6 months; Weight: 5.2 to 7.8 kg; Source: Marshall Research Animals, North Rose, NY.

B. Study Design:

1. Animal Assignment - Animals were assigned to the following test groups:

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<u>Test Group</u>	<u>Dose in Diet (ppm)</u>	<u>Male</u>	<u>Female</u>
1. Control	0	4	4
2. INZ326-118	10	4	4
3. INZ326-118	25	4	4
4. INZ326-118	125	4	4
5. INZ326-118	625	4	4

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A computerized stratified randomization was used so that mean body weights within a sex were similar in all five groups.

2. Diet Preparation - Diet was prepared weekly and stored at refrigerated temperature. Samples of treated food were analyzed for stability and concentration.

Results - In tests for homogeneity of mixtures of all four diets, samples were taken from three levels of the mixing vessel: top, middle, and bottom. Results of analyses were for the most part within 6 percent of nominal amounts present in diets, with no differences at the three different levels.

Stability tests were also performed on all four dietary mixtures in storage samples prepared at the three time periods. This included fresh frozen, after storage at room temperature for 24 hours and 10 days, and after storage at refrigerated temperature for 10 days. Most measured concentrations were within 10 percent of nominal concentration for all four dose levels.

3. Animals received food and water ad libitum.
4. Statistics - Body weights, body weight gains, food consumption, organ weights, and clinical chemistry values

were analyzed by one-way ANOVA. When F tests among test groups were significant, pairwise comparisons between test and control groups were made with Dunnett's test. Significance was judged at 0.05 level. Bartlett's test for homogeneity of variances was performed on clinical lab data. If significant at  $p < 0.005$ , the Kruskal-Wallis test was employed, and the Mann-Whitney U test was used to compare means from control groups and treated groups.

5. Compliance - A signed Statement of Confidentiality Claim was provided.

A signed Statement of Compliance with EPA's GLP was provided.

A signed Quality Assurance Statement was provided.

6. Background for This Study - In a previous 2-year dog study with doses of 0, 25, 125, and 625 ppm in the diet, blood samples at 1 and 2 years indicated decreased red blood cell count, hemoglobin and hematocrit in males at 625 and possibly at 125 ppm. An abnormal pigment in the blood was observed which was postulated to be methemoglobin and sulfhemoglobin. In the present study, measurements of these two pigments were actually performed in all blood samples that were collected at 3, 6, 9, and 12 months.

C. Methods and Results:

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

Toxicity/Mortality (Survival) - No mortalities at any dose level were recorded.

No treatment related behavioral signs of toxicity were evident.

2. Body Weight - Animals were weighed weekly during the entire period of the study. There appeared to be a decrease in body weight gain in the 125 ppm treated males, which started at about day 50 of the study. Such a decrease in body weight gain was not observed in males treated with 625 ppm or in females of any dose group. Therefore, it is not considered compound related.
3. Food Consumption and Compound Intake - Consumption was determined and mean daily diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and body weight gain data.

No consistent effects were noted. Decreased intake in females at three time periods, weeks 17, 39, and 47, was considered to be "incidental."

Compound intake in mg/kg/day, averaged over the entire study and based on food intake, was calculated to be as follows by the investigators:

<u>Dose in ppm</u>	<u>Dose in mg/kg/day</u>	
	<u>Males</u>	<u>Females</u>
10	0.29	0.30
25	0.79	0.77
125	4.17	3.49
625	18.6	16.1

4. Ophthalmological Examinations - Performed on all animals prior to the start of the study and on day 352, near the end of the study.

A predosing ophthalmological report dated December 5, 1986 and a postdosing final ophthalmological report dated December 11, 1987, were both signed by James M. Clinton, D.V.M., Diplomate, American College of Veterinary Ophthalmologists. Indirect ophthalmoscopy was used.

It was concluded by Dr. Clinton that there was no evidence of ocular changes due to compound treatment.

5. Blood was collected twice before treatment and at 1, 3, 6, 9, and 12 months for hematology and clinical analysis from all animals. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB conc. (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count*	X	Reticulocyte count
	Blood Clotting Measurements	X	Methemoglobin
	(Thromboplastin time)	X	Sulfhemoglobin
	(Clotting time)		
	(Prothrombin time)		
X	Reticulocytes		

Methemoglobin and sulfhemoglobin were not measured at pretest and 1 month.

\*Required for subchronic and chronic studies.

Bone marrow smears were also obtained at the termination of the study.

Small decreases in erythrocyte count, hemoglobin, and hematocrit were observed in both sexes at 625 ppm throughout the study at virtually all time periods, but at no time point were any of these differences statistically significant for either sex. In females on 625 ppm, a decrease in reticulocyte count was seen only after 1 month ( $p < 0.05$ ). Effects on reticulocytes were inconsistent in males at 6 months; an increase at 25 ppm but a decrease at 125 ppm, probably sporadic.

The following is a summary of increased methemoglobin and sulfhemoglobin found in blood between 3 to 12 months of the study, extracted from the report.

Sample Time (Month)	Males								Females							
	3		6		9		12		3		6		9		12	
	125	625	125	625	125	625	125	625	125	625	125	625	125	625	125	625
Methemoglobin	+	+	+	+		+		+	+	+	+			+		+
Sulfhemoglobin		+		+	+	+		+	+		+		+	+		+

+Indicates significant increase ( $p < 0.05$ ) by Dunnett or Mann-Whitney U (+) criteria.

\*Significant increases in methemoglobin levels were also found in females treated with 10 and 25 ppm at 6 months.

Increased erythropoietic activity in bone marrow was seen in males and females at 625 ppm.

The changes in red cell parameters and bone marrow were considered small and the values found, according to the author, were usually within normal physiological range. The mean reticulocyte count at 1 month for the 625 ppm females exceeded the approximate upper canine reference limit for reticulocyte count.

Other effects considered compound-related included increased platelet count at 625 ppm in males at 3 months and in females at 3, 6, and 9 months. White cell count and neutrophils in females at 625 ppm were increased at 3, 6, and 9 months, and band neutrophils at 3 months.

b. Clinical Chemistry

<u>X</u>		<u>X</u>	
	Electrolytes:		Other:
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
X	Magnesium*	X	Blood urea nitrogen*
	Phosphorus*	X	Cholesterol*
X	Potassium*	X	Globulins
	Sodium*	X	Glucose*
	Enzymes	X	Total bilirubin*
X	Alkaline phosphatase (ALP)	X	Total serum protein*
	Cholinesterase		Triglycerides
X	Creatinine phosphokinase*		Serum protein electrophoresis
X	Lactic acid dehydrogenase	X	Creatinine
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
	Gamma glutamyl transferase (also SGOT)*		
	Glutamate dehydrogenase		

Phosphorus and magnesium were not measured at pretest and 1 month  
magnesium was not measured at 12 months.

\*Required for subchronic and chronic studies.

The only consistent change noted was an increase in cholesterol at 625 ppm in males after 1 (n.s.), 3 (p < 0.05), 6 (p < 0.05), 9 (p < 0.05), and 12 (p < 0.05) months. Increases in cholesterol in females receiving 625 ppm was also evident at all five time periods but was statistically significant only at 3 months.

6. Urinalysis - Urine was collected from fasted animals at 1, 3, 6, 9 and 12 months. The CHECKED (X) parameters were examined.

<u>X</u>		<u>X</u>	
X	Appearance*	X	Glucose*
X	Volume*	X	Ketones*
	Specific gravity*	X	Bilirubin*
X	pH	X	Blood*
X	Sediment (microscopic)*		Nitrate
X	Protein	X	Urobilinogen
X	Osmolality	X	Microscopic examination

\*Required for chronic studies.

No effects were noted in males or females.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

<p>X Digestive System Tongue X Salivary glands* X Esophagus* X Stomach* X Duodenum* X Jejunum* X Ileum* X Cecum* X Colon* X Rectum* XX Liver* X Gall bladder* X Pancreas* Respiratory X Trachea* X Lung* Nose Pharynx Larynx X Tonsils</p>	<p>X Cardiovasc./Hemat. X Aorta* XX Heart* X Bone marrow* X Lymph nodes* X Spleen* X Thymus* Urogenital XX Kidneys* X Urinary bladder* XX Testes* X Epididymides X Prostate Seminal vesicle XX Ovaries* Uterus* X Vagina</p>	<p>X Neurologic XX Brain* X Perif. Nerve* X Spinal cord (3 levels)* X Pituitary* X Eyes (optic n.)* Glandular X Adrenals* Lacrimal gland X Mammary gland* XX Parathyroids*1/ XX Thyroids *1/ Other X Bone* X Skeletal muscle* X Skin* All gross lesions and masses*</p>
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\*Required for subchronic and chronic studies.

1/Collected and weighed together.

- a. Organ Weight - In males, increase in absolute liver weight at 625 ppm (n.s.) and in relative (to body weight) liver weights at 25 (n.s.), 125 (p < 0.05) and 625 (p < 0.01) ppm with a dose-related trend in relative weight. In females, both absolute and relative liver weights were increased at 625 ppm but neither of these were statistically significant.
- b. Gross Pathology - No effects were noted.
- c. Microscopic Pathology - A pathology report was signed by E.F. Stula, D.V.M., Ph.D., Senior Research Pathologist. The report stated that microscopic changes were found for the 625 ppm treated male and female dogs. In bone marrow, a "slight increase in hematopoiesis" and in liver, "a slight increase in hemosiderin deposition" were observed. It was concluded that both effects suggested the compound was causing hemolysis of erythrocytes with a resultant increase in hematopoietic activity.

Pearl's stain was used to stain liver and kidneys of all dogs

in order to determine hemosiderin deposition. The following is extracted from Tables 24 and 25 of the report (Microscopic Observations) for effects on the liver.

Brown Pigment (Hemosiderin) in Kupffer Cells

Dose Group	Males	Females
	<u>N</u>	<u>N</u>
0	1: graded mild	3: all graded minimal
10	1: graded minimal	2: 1 minimal and 1 mild
25	3: all graded minimal	4: 2 minimal, 2 mild
125	1: graded minimal	4: 3 minimal, 1 mild
625	4: 2 graded mild, 2 graded moderate	4: 2 mild and 2 moderate

Livers from four males and four females per group were examined.

In the kidneys, one or sometimes two dogs in each group had hemosiderin in the proximal tubular cytoplasm graded mild or moderate or severe in every male group including controls. In females these scores were generally slightly lower than for males. No compound-related effect was seen in kidneys of males or females.

In the bone marrow, the incidences of "increased hematopoiesis" were listed as follows (from Tables 24 and 25, Microscopic Observations, taken from the report):

Dose Group	Males	Females
	<u>N:</u>	<u>N:</u>
0	0	1: graded minimal
10	1: graded minimal	1: graded minimal
25	0	0
125	2: both graded minimal	2: 1 graded minimal, 1 moderate
625	3: 1 graded minimal, 2 moderate	4: 1 graded minimal, 3 moderate

No compound-related effect was evident in any other organ. No "primary tumors" or malignant tumors were seen in any organ of the dogs in this study.

DISCUSSION:

In a previous 2-year dog study with doses of 25, 125 and 625 ppm in the diet, an abnormal pigment was observed in the blood of animals of all dose groups. Decreased red cell count, hematocrit and hemoglobin levels were also seen in males of the high dose group. The abnormal pigment in blood was postulated to be two substances,

methemoglobin and sulfhemoglobin. In the present 1-year dog study, assays for these two pigments actually confirmed that one or both of the pigments were increased in both sexes at all time intervals, which included 3, 6, 9 and 12 months. These changes occurred only at the 125 and 625 ppm dose levels for both sexes. Microscopic pathology of the liver revealed an increase in the amount of hemosiderin deposited in the kupffer cells of male and female dogs receiving the 625 ppm dose. Slight decreases, not statistically significant, were seen in erythrocyte count, hemoglobin and hematocrit levels in dogs of both sexes that received the highest dose level. These changes were seen at all time periods, beginning at 1 month after dosing was started. A small increase in erythropoietic activity was evident in bone marrow of both sexes receiving 625 ppm. All of the above changes are consistent with a suggestion for a mild increase in red blood cell destruction. Also observed in the liver was a statistically significant increase in relative weight at the 125 and 625 ppm dose levels in males but not in females.

Increases in platelet count, leukocyte count and serum cholesterol levels were noted in dogs treated with the 625 ppm dose and are considered compound related. It was suggested that the two hematological changes were secondary to the increase in hematopoiesis. The increase in cholesterol may have been caused by the liver changes that were noted.

In the present dog study, no effects were seen on body weight gain or survival. However, clear changes were seen in hematology, serum chemistry and in hemosiderin deposition in liver. Also, the blood pigments observed in the previous 2-year dog study were identified. The NOEL for the previous 2-year dog study was found to be < 25 ppm (LDT), whereas in the present study the NOEL was found to be 25 ppm. In both studies, the NOEL was based predominantly on the blood pigments, which were found to be present after only one month of treatment in the present study.