8-12-94

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

AUG 12 1994
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# 325 PC Code 028201
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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

**MEMORANDUM**

**SUBJECT:** Propanil; One-Year Chronic Feeding Study in Dogs; Guideline Requirement: 83-1(b); Propanil Task Force; ID #: 028201; Reregistration Case #: 0226

Tox.Chem No.: 325
MRID No.: 42962901
DP Barcode No.: D196302
Submission No.: S452158

TO: Eric Feris, PM Team #71
Reregistration Branch
Special Review and Reregistration Division (H7508W)

FROM: William Dykstra, Ph.D., Toxicologist
Review Section I
Toxicology Branch I
Health Effects Division (H7509C)
William Dykstra 8/9/94

fa THRU: Roger Gardner, Section Head, Toxicologist
Review Section I
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Health Effects Division (H7509C)
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KB 8/10/94

**ACTION REQUESTED:** In response to the FIFRA '88 phase 4, the Registrant, The Propanil Task Force, has submitted a one-year chronic toxicity feeding study in beagle dogs with Propanil Technical, Code Blue. Toxicology Branch-I (TB-I) has been requested to review the study and determine its acceptability.

**CONCLUSIONS:** The study is acceptable as core-minimum data and fulfills the requirement for a one-year chronic toxicity study in dogs. There was no NOEL in the study. At the low-dose, there was mild (13% difference from controls) methemoglobinemia, due to the dichloroaniline moiety of the test substance, accompanied by

hemosiderin deposition in the kidneys of males and females and the liver of males. At the mid- and high-doses, the methemoglobinemia was moderate, macrocytic, and regenerative. There was clinical chemistry data available to suggest renal damage, but the only treatment-related organ weight changes were in the increased absolute and relative weight of the liver in both sexes. The histopathological findings at the mid- and high-dose consisted of hemosiderin deposition in the bone marrow, kidney, and liver. The incidence and grades of the lesions tended to be dose-related.

A DER is attached.

Reviewed by: William Dykstra Ph.D., Toxicologist
Section I, Tox. Branch I
for Secondary Reviewer: Roger Gardner, Section Head, Toxicologist
Section I, Tox. Branch I

William Dykstra 8/9/94
Pamela M. Hurley 8/9/94

DATA EVALUATION REPORT

STUDY TYPE: 83-1(b); One-Year Chronic Toxicity Study in Dogs

TOX. CHEM NO: 325

ACCESSION NUMBER: None MRID NO.: 429629-01

TEST MATERIAL: Propanil Technical, Code Blue, 98.5% Purity

SYNONYMS: Stam

STUDY NUMBER: WIL-141007

SPONSOR: Propanil Task Force, P.O. Box 301, Liberty, MO

TESTING FACILITY: WIL Research Labs, Ashland, OH

TITLE OF REPORT: One Year Oral Toxicity Study in Dogs with Propanil

AUTHOR(S): E. Crosby Tompkins

REPORT ISSUED: September 29, 1993

EXECUTIVE SUMMARY: Randomized groups of 4/sex/dose outbred beagle dogs were fed continuously via the diet at levels of 0, 200, 1600, and 3200 ppm (0, 5, 45, or 79 mg/kg/day for males and 0, 6, 42, or 85 mg/kg/day for females) of propanil technical, code blue, for 12 months. Criteria evaluated were clinical signs, body weight, food consumption, clinical pathology, gross necropsy observations, organ weights, and histopathology.

**No NOEL was established in the this study. Treatment-related and dose-related effects in both sexes consisted of decreased levels of RBC, hemoglobin, hematocrit and MCHC and increased levels of MCV, methemoglobin, and reticulocytes at the mid- and high-doses and to some extent at the low-dose at most sampling intervals. The macrocytic, regenerative, methemoglobinemia was considered moderate to severe at the mid and high-doses and mild (up to 13% difference from controls) at the low-dose.** Evaluation of reticulocyte smears from week 25 and 51 samples showed increased incidences of Heinz bodies in the mid and high-dose dogs of both sexes and low-

dose females at week 51.

**There was no NOEL for endogenous pigment (hemosiderin) found in the bone marrow, kidney, and liver at the mid- and high-dose of both sexes and at the low-dose in the kidney of males and females and the liver of males. The incidence and grades of hemosiderin deposition were dose-related.**

There were no mortalities and all dogs survived to terminal necropsy.

During the first week of dietary exposure to propanil technical, males showed statistically significant decreased weight gains of up to 500 grams at the high-dose. In females, all groups had decreased body weight gains during the first week, but the high-dose females were more than double the controls (-190 g in controls vs. -480 g in high-dose). For body weight means, decreases up to 10% in males and 4% in females were observed during the 4th week at the high-dose. Body weight gains returned to control levels by week 4 for high-dose females and week 7 for high-dose males. Mean body weights of high-dose dogs of both sexes were comparable to controls by week 26 in females and week 39 in males and mean body weight remained comparable between controls and high-dose dogs of both sexes for the remainder of the study. The decreases in body weight and body weight gain at the high-dose in both sexes are considered compound-related.

Food consumption in males was decreased in the high-dose during the first 6 weeks, whereas in high-dose females, food consumption was decreased only during the first week. These decreases in the food consumption of high-dose dogs are considered compound-related.

At the high-dose in both sexes, kidney damage was represented as statistically significant increases in serum BUN, creatinine, and potassium ranging from 13-58% above control levels at most sampling intervals. The presence of hemolytic anemia in both sexes at the mid and high-dose was seen in the statistically significantly elevated bilirubin levels at these doses in comparison to controls. Although the increases in bilirubin in treated groups were not large, the findings were consistent in both sexes over the course of the study.

In males, liver weight was statistically

significantly increased at the high-dose by 40% for the absolute weight and 38% for the relative weight. Low- dose and high-dose thymus weight was significantly reduced in males by 49% (absolute) and 51% (relative) at the low-dose and by 43% (absolute) and 43% (absolute) at the high-dose. High-dose thyroid weight was significantly increased in males by 48% (absolute) and 55% (relative).

In females, liver weight was significantly increased by 27% (relative) at the mid-dose and by 49% (absolute) and 48% (relative) at the high-dose. The increased relative and absolute liver weights in both sexes are considered compound-related. In the absence of clinical chemistry findings related to the thymus and thyroid, the changes in the weight of these organs in males are of uncertain toxicological significance.

Classification: **Core-Minimum**
(a) NOEL not established

Special Review Criteria (40 CFR 154.7) N/A

A. MATERIALS:

1. Test compound: Propanil Technical. Description - Light Brown to Dark Purple Granular Solid Powder, Batch # - Code Blue: Batch No. 01, Purity - 96.9 %- 98.5%.

2. Test animals: Species: Dog, Strain: Outbred Beagle, Age: 6 Months of age, Weight: 7.9-11.6 Kg (males) and 6.5-10.4 Kg (females), Source: Ridglan Farms, Inc., Mt. Horeb, WI.

B. STUDY DESIGN:

1. Animal assignment

Animals were individually caged and assigned randomly to the following test groups:

| Test Group | Dose in diet (ppm) | Main Study 12 months male | Main Study 12 months female | Interim Sac. months male | Interim Sac. months female |
|---|---|---|---|---|---|
| 1 Cont | 0 | 4 | 4 | | |
| 2 Low (LDT) | 200 | 4 | 4 | | |
| 3 Mid (MDT) | 1600 | 4 | 4 | | |
| 4 High (HDT) | 3200 | 4 | 4 | | |

2. Diet preparation

Diet was prepared weekly and stored at room temperature. Samples of treated food were analyzed for stability and concentration at the initiation and at weeks 0, 1, 2, 3, 7, 11, 24, 37, and 50.

Results - At the start of the study, homogeneity analyses showed that the mean concentrations for the 200 ppm group were 101% (top), 103% (mid), and 97.6% (bottom) of nominal levels. Both the 1600 and 3200 ppm groups had comparable homogeneity results. Samples of treated feed kept at room temperature for 10 days showed concentration analyses of 97.7% (low-dose), 88.9% (mid-dose), and 85.3% (high-dose) of nominal levels. A later dietary analysis of treated diets kept for 14 days at room temperature showed recoveries of 95.6% (low-dose), 96.3% (mid-dose), and 98.5% (high-dose) of nominal amounts. Periodic analyses of treated diets for concentration averaged 96.5% (low-dose), 98.3% (mid-dose), and 98.5% (high-dose) of nominal concentrations.

3. Animals received food (Purina Certified Canine Chow) and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data: Analysis of body weights, body weight changes, food consumption, clinical laboratory values, and absolute and relative organ weights were analyzed by a one-way analysis of variance followed by Dunnett's Test. All analyses were conducted using two-tailed tests for significance levels of 5% and 1% comparing the treatment groups to the vehicle control group by sex.

5. A signed quality assurance statement was present, signed by Deborah L. Little, Manager of Quality Assurance, and dated 9/29/93.

C. METHODS AND RESULTS:

1. Observations:

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

There were no mortalities and all dogs survived to terminal necropsy. There were increased incidences of soft stool in treated dogs of both sexes during the entire study, although the total incidences were not dose-related and did not result in increased incidences of diarrhea. In males, the incidences were 53, 340, 124, and 158 and in females, the incidences were 15, 63, 33, and 59, for the control, low, mid, and high-dose groups, respectively, in each sex. For all groups, control and treated, of both sexes, all 4 dogs were affected with soft stool, which is a common finding in laboratory beagles. This finding is not considered a significant toxic effect. There was an increased number of treated dogs in each sex at the high-dose (3 males and 3 females in the high-dose vs. 1/sex in the controls) with decreased urination and decreased defecation during the first 3 months of treatment. Most instances were limited to the first week of treatment which was associated with decreases in food intake and body weight gain in both sexes of high-dose dogs. Additionally, high-dose female dog #1184 had 5 incidences of dermal atonia from weeks 5-9 which was considered secondary to the decreases in food intake and body weight.

2. Body weight and Body Weight Gain

Animals were weighed weekly for the entire study. During the first week of dietary exposure to propanil technical, males showed statistically significant decreased weight gains of up to 500 grams at the high-dose. In females, all groups had decreased body weight gains during the first week, but the high-dose females were more than double the controls (-190 g in controls vs. -480 g in high-dose). For body weight means, decreases up to 10% in males and 4% in females were observed during the 4th week at the high-dose. Body weight gains returned to control levels by week 4 for high-dose females and week 7 for high-dose males (data not shown). At weeks 11-12, as shown, weight gains were comparable between controls and high-dose dogs of both sexes. Mean body weights of high-dose dogs of both sexes were comparable to controls by week 26 in females and week 39 in males and mean body weight remained comparable between controls and high-dose dogs of both sexes for the remainder of the study. The decreases in body weight and body weight gain at the high-dose in both sexes are considered compound-related. There were no treatment-related effects on body weight and body weight gain in the mid and low-dose groups of both sexes in comparison to controls during the study.

**BODY WEIGHT GAIN**

**MALES (Grams)**

**WEEKS**

| Dose | 0-1 | 2-3 | 11-12 | 25-26 | 38-39 | 51-52 |
|---|---|---|---|---|---|---|
| Contr. | 29 | 209 | 298 | 34 | 161 | 53 |
| Low | 109 | 290 | 344 | 84 | 134 | -10 |
| Mid | 155 | 311 | 329 | -16 | 49 | -23 |
| High | -412** | -40* | 192 | 58 | 356 | 12 |

**FEMALES (Grams)**

**WEEKS**

| Dose | 0-1 | 2-3 | 11-12 | 25-26 | 38-39 | 51-52 |
|---|---|---|---|---|---|---|
| Contr. | -190 | 285 | 168 | 144 | 271 | -207 |
| Low | -33 | 250 | 240 | 117 | 274 | -158 |
| Mid | -153 | 224 | 312 | -41 | 259 | -71 |
| High | -480 | 37 | 183 | -199 | 291 | -112 |

* $p \leq 0.05$

** $p \leq 0.01$

## BODY WEIGHT

### MALES (Grams)

| | WEEKS | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 1 | 4 | 12 | 26 | 39 | 52 |
| Dose | | | | | | | |
| Contr. | 9069 | 9097 | 9858 | 10980 | 11345 | 11282 | 11367 |
| Low | 9160 | 9269 | 9989 | 11326 | 12043 | 12342 | 12303 |
| Mid | 9183 | 9338 | 10257 | 11698 | 12308 | 12349 | 12456 |
| High | 9282 | 8870(3%) | 8905(10%) | 9731 | 10937 | 11341 | 11507 |

### FEMALES (Grams)

| | WEEKS | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 1 | 4 | 12 | 26 | 39 | 52 |
| Dose | | | | | | | |
| Contr. | 8036 | 7846 | 8286 | 9053 | 9350 | 9580 | 9623 |
| Low | 7839 | 7806 | 8482 | 9347 | 10658 | 10579 | 10498 |
| Mid | 7537 | 7384 | 7762 | 8383 | 8658 | 9033 | 8823 |
| High | 8304 | 7824 | 7936(4%) | 8599 | 9395 | 9881 | 9614 |

3. Food consumption and compound intake

Consumption was determined daily and mean daily diet consumption was calculated. Food consumption in males was decreased in the high-dose during the first 6 weeks, whereas in high-dose females, food consumption was decreased only during the first week. These decreases in the food consumption of high-dose dogs are considered compound-related. Intake of propanil averaged 5, 45, and 79 mg/kg/day in male groups and 6, 42, and 85 mg/kg/day in female groups for the low, mid, and high-dose groups, respectively.

**FOOD CONSUMPTION**

**MALES (Grams/Kg/Day)**

| | WEEKS | | | | | |
|---|---|---|---|---|---|---|
| | 0-1 | 3-4 | 5-6 | 11-12 | 25-26 | 51-52 |
| Dose | | | | | | |
| Contr. | 30 | 37 | 33 | 29 | 24 | 26 |
| Low | 31 | 34 | 31 | 29 | 24 | 23 |
| Mid | 37 | 39 | 36 | 31 | 25 | 27 |
| High | 23 | 26** | 24* | 26 | 25 | 24 |

**FEMALES (Grams/Kg/Day)**

**WEEKS**

| Dose | 0-1 | 3-4 | 5-6 | 11-12 | 25-26 | 51-52 |
|---|---|---|---|---|---|---|
| Contr. | 28 | 31 | 27 | 27 | 27 | 22 |
| Low | 35 | 41 | 38** | 31 | 26 | 24 |
| Mid | 27 | 36 | 32 | 28 | 26 | 23 |
| High | 21 | 31 | 27 | 29 | 24 | 26 |

* $p \leq 0.05$

** $p \leq 0.01$

4. Ophthalmological examination

Performed prior to study and week 52 on all animals. There were no compound-related ophthalmological findings at any treated dose level in comparison to controls for either sex.

5. Blood was collected before treatment and at study weeks 12, 25, 39, and 51 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 corpusc. HGB conc.(MCHC) |

| | | | |
|---|---|---|---|
| x | Erythrocyte count (RBC)* | x | Mean corpusc. volume (MCV) |
| x | Platelet count* | x | Reticulocyte count |
| x | Blood clotting measurements | | |
| x | (Thromboplastin time) | | |
| | (Clotting time) | | |
| x | (Prothrombin time) | | |
| x | Methemoglobin | | |
| x | Heinz Bodies | | |

* Required for subchronic and chronic studies

Results - Treatment-related and dose-related effects in both sexes consisted of decreased levels of RBC, hemoglobin, hematocrit and MCHC and increased levels of MCV, methemoglobin, and reticulocytes at the mid- and high-doses and to some extent at the low-dose at most sampling intervals. The macrocytic, regenerative, methemoglobinemia was considered moderate to severe at the mid and high-doses and mild (up to 13% difference from controls) at the low-dose. A comparison of the means of historical control data from WIL Labs for hematological parameters to the range of mean values in the 200 ppm dose group are shown below:

| | | | Means ± S.D. | |
|---|---|---|---|---|
| | | 200 ppm | 6-12 Months | 12-18 Months |
| **RBC** | **Males** | 6.49-7.77 | 6.76(5.5-8.0) | 7.50(6.2-8.8) |
| | **Females** | 6.09-6.78 | 6.92(5.5-8.4) | 7.33(6.3-8.4) |
| **Hg** | **Males** | 14.7-15.8 | 15.6(12.9-18.3) | 17.8(15.2-20.4) |
| | **Females** | 13.9-15.4 | 16.0(12.5-19.5) | 17.9(15.5-20.3) |
| **Ht** | **Males** | 44.9-54.2 | 47.9(38.6-57.2) | 49.0(40.7-57.3) |
| | **Females** | 42.2-47.8 | 49.6(38.9-60.3) | 48.4(39.8-57.0) |
| **MCV** | **Males** | 69.2-70.4 | 70.9(65.9-75.9) | 65.5(57.3-73.7) |
| | **Females** | 69.1-70.5 | 71.7(66.3-77.1) | 66.1(57.6-74.6) |

It is apparent from an examination of these data that the low-dose methemoglobinemia was more severe in females than in males.

There was an increase in Howell-Jolly bodies and macrocytes in both sexes at the mid and high-dose at all sampling intervals during the study. Also, 2/4 males at the low-dose had macrocytes at week 51. Howell-Jolly bodies were seen in one low-dose male at week 25 and one male and two females at week 51. However, Howell-Jolly bodies were seen

at pretest in two low-dose females also. Evaluation of reticulocyte smears from week 25 and 51 samples showed increased incidences of Heinz bodies in the mid and high-dose dogs of both sexes and low-dose females at week 51. Heinz bodies are aggregations of hemoglobin precipitated by oxidation.

**MALES**

**WEEK -1**

| Dose | RBC $10^6$/ul | Hg g/dL | Ht % | MCV $u^3$ | MCHC g/dL | MetH % | Ret % |
|---|---|---|---|---|---|---|---|
| Cont. | 6.08 | 13.5 | 41.0 | 67.8 | 32.8 | 0.0 | 1.0 |
| Low | 6.41 | 14.5 | 43.5 | 67.8 | 33.3 | 0.1 | 0.4 |
| Mid | 5.71 | 13.0 | 39.3 | 69.0 | 33.1 | 0.1 | 0.4 |
| High | 6.06 | 13.8 | 41.7 | 68.9 | 33.0 | 0.1 | 0.3 |

**FEMALES**

**WEEK -1**

| Dose | RBC $10^6$/ul | Hg g/dL | Ht % | MCV $u^3$ | MCHC g/dL | MetH % | Ret % |
|---|---|---|---|---|---|---|---|
| Cont. | 6.24 | 14.0 | 41.9 | 67.1 | 33.3 | 0.1 | 0.4 |
| Low | 6.40 | 14.2 | 43.8 | 68.5 | 32.5 | 0.0 | 0.7 |
| Mid | 6.19 | 14.4 | 43.3 | 69.8 | 33.3 | 0.1 | 0.7 |
| High | 6,04 | 13.6 | 41.1 | 68.2 | 33.1 | 0.0 | 0.3 |

**MALES**

**WEEK 12**

| Dose | RBC $10^6$/ul | Hg g/dL | Ht % | MCV $u^3$ | MCHC g/dL | MetH % | Ret % |
|---|---|---|---|---|---|---|---|
| Cont. | 6.70 | 15.1 | 46.3 | 69.1 | 32.7 | 0.0 | 0.8 |
| Low | 6.54 | 15.2 | 46.0 | 70.4 | 32.9 | 0.4 | 0.9 |
| Mid | 5.75* | 13.5 | 42.4 | 74.0* | 31.8* | 3.5** | 1.2 |
| High | 5.36* | 12.6** | 40.5 | 75.5** | 31.0* | 4.4** | 2.2** |

* $p \leq 0.05$

** $p \leq 0.01$

**FEMALES**

**WEEK 12**

| Dose | RBC $10^6$/ul | Hg g/dL | Ht % | MCV $u^3$ | MCHC g/dL | MetH % | Ret % |
|---|---|---|---|---|---|---|---|
| Cont. | 6.97 | 15.6 | 47.1 | 67.5 | 33.2 | 0.0 | 0.3 |
| Low | 6.09** | 13.9* | 42.2 | 69.3 | 33.0 | 0.4 | 0.6 |
| Mid | 5.77** | 13.6** | 42.1 | 73.0** | 32.2* | 3.2** | 1.2 |
| High | 5.36** | 12.4** | 39.4** | 73.4** | 31.6** | 6.2** | 1.3 |

* $p \leq 0.05$

** $p \leq 0.01$

**MALES**

**WEEK 25**

| Dose | RBC $10^6$/ul | Hg g/dL | Ht % | MCV $u^3$ | MCHC g/dL | MetH % | Ret % |
|---|---|---|---|---|---|---|---|
| Cont. | 6.85 | 15.6 | 47.0 | 68.7 | 33.1 | 0.0 | 0.2 |
| Low | 6.49 | 14.9 | 44.9 | 69.2 | 33.1 | 0.6 | 0.3 |
| Mid | 5.65** | 13.4** | 41.6* | 73.6* | 32.2** | 2.6** | 0.7* |
| High | 5.12** | 12.1** | 37.9** | 74.0** | 31.9** | 4.2** | 0.9** |

* $p \leq 0.05$

** $p \leq 0.01$

**FEMALES**

**WEEK 25**

| Dose | RBC $10^6$/ul | Hg g/dL | Ht % | MCV $u^3$ | MCHC g/dL | MetH % | Ret % |
|---|---|---|---|---|---|---|---|
| Cont. | 6.99 | 15.7 | 47.0 | 67.3 | 33.5 | 0.0 | 0.1 |
| Low | 6.20** | 14.2* | 42.8* | 69.1 | 33.0 | 0.6 | 0.2 |
| Mid | 5.63** | 13.4** | 40.7** | 72.3** | 32.8 | 3.2** | 0.7** |
| High | 5.27** | 12.2** | 38.6** | 73.2** | 31.7* | 6.3** | 0.6* |

* $p \leq 0.05$

** $p \leq 0.01$

**MALES**

**WEEK 39**

| Dose | RBC $10^6$/ul | Hg g/dL | Ht % | MCV $u^3$ | MCHC g/dL | MetH % | Ret % |
|---|---|---|---|---|---|---|---|
| Cont. | 7.19 | 15.4 | 49.4 | 68.7 | 31.2 | 0.3 | 0.2 |
| Low | 7.77 | 14.7 | 54.2 | 69.8 | 27.9 | 1.0 | 0.4 |
| Mid | 6.26 | 13.7* | 46.1 | 73.6** | 30.0 | 2.6** | 1.0* |
| High | 5.49* | 12.0** | 40.7* | 74.1** | 29.6 | 4.2** | 1.0** |

* $p \leq 0.05$

** $p \leq 0.01$

**FEMALES**

**WEEK 39**

| Dose | RBC $10^6$/ul | Hg g/dL | Ht % | MCV $u^3$ | MCHC g/dL | MetH % | Ret % |
|---|---|---|---|---|---|---|---|
| Cont. | 6.79 | 15.3 | 45.7 | 67.3 | 33.5 | 0.1 | 0.3 |
| Low | 6.16** | 14.3 | 43.3 | 70.3 | 33.2 | 0.7 | 0.6 |
| Mid | 5.69** | 13.4** | 41.0* | 72.0** | 32.7 | 3.8** | 0.7 |
| High | 5.16** | 12.0** | 38.0** | 73.6** | 31.6* | 7.0** | 0.9 |

* $p \leq 0.05$

** $p \leq 0.01$

**MALES**

**WEEK 51**

| Dose | RBC $10^6$/ul | Hg g/dL | Ht % | MCV $u^3$ | MCHC g/dL | MetH % | Ret % |
|---|---|---|---|---|---|---|---|
| Cont. | 7.52 | 17.4 | 52.0 | 69.0 | 33.5 | 0.0 | 0.4 |
| Low | 6.84* | 15.8* | 47.8 | 70.0 | 33.0 | 0.8* | 0.5 |
| Mid | 6.75** | 15.7* | 49.6 | 73.6** | 31.6** | 1.9* | 0.6 |
| High | 5.55** | 13.0** | 41.4** | 74.7** | 31.3** | 3.3** | 0.8 |

* $p \leq 0.05$

** $p \leq 0.01$

**FEMALES**

**WEEK 51**

| Dose | RBC $10^6$/ul | Hg g/dL | Ht % | MCV $u^3$ | MCHC g/dL | MetH % | Ret % |
|---|---|---|---|---|---|---|---|
| Cont. | 7.00 | 15.6 | 47.0 | 67.1 | 33.1 | 0.0 | 0.1 |
| Low | 6.78 | 15.4 | 47.8 | 70.5* | 32.3* | 0.9 | 0.5 |
| Mid | 6.43* | 15.1 | 47.0 | 73.0** | 32.2* | 2.7** | 1.0* |
| High | 6.21** | 14.6 | 45.7 | 73.7** | 31.8** | 4.8** | 1.0** |

* $p \leq 0.05$

** $p \leq 0.01$

b. Clinical Chemistry

| X | Electrolytes: |
|---|---|
| x | Calcium* |
| x | Chloride* |
| | Magnesium* |
| x | Phosphorous* |
| x | Potassium* |
| x | Sodium* |
| | Enzymes |
| x | Alkaline phosphatase (ALK) |
| | Cholinesterase (ChE)# |
| x | Creatine kinase*^ |
| | Lactic acid dehydrogenase (LAD) |
| x | Serum alanine aminotransferase (also SGPT)* |
| x | Serum aspartate aminotransferase (also SGOT)* |
| x | Gamma glutamyl transferase (GGT) |
| | Glutamate dehydrogenase |

| X | Other: |
|---|---|
| x | Albumin* |
| x | Blood creatinine* |
| x | Blood urea nitrogen* |
| x | Cholesterol* |
| x | Globulins |
| x | Glucose* |
| x | Total bilirubin |
| x | Total serum Protein (TP)* |
| | Triglycerides |
| | Serum protein electrophores |

* Required for subchronic and chronic studies
# Should be required for OP
^ Not required for subchronic studies

Results - At the high-dose in both sexes, kidney damage was represented as statistically significant increases in serum BUN, creatinine, and potassium ranging from 13-58% above control levels at most sampling intervals. The presence of hemolytic anemia in both sexes at the mid and high-dose was seen in the statistically significantly elevated bilirubin levels at these doses in comparison to controls. Although the increases in bilirubin in treated groups were not large, the findings were consistent in both sexes over the course of the study. The statistically significant increases in serum phosphorus in high-dose females in comparison to controls during the study were not considered compound-related, since the high-dose value for serum phosphorus at week -1 was statistically significantly increased as well. There were no consistent, compound-related effects in serum phosphorus in high-dose males during the study. The statistically significant 23% increase in serum phosphorus in high-dose males at week 39 was not observed at any other sampling period and was not considered toxicologically significant.

**MALES**

**WEEK -1**

| Dose | BUN mg/L | CREAT. mg/L | POTASS. meq/L | T.BILI. mg/L | PHOS. mg/L |
|---|---|---|---|---|---|
| Contr. | 10.9 | 0.7 | 4.93 | 0.1 | 5.9 |
| Low | 14.9 | 0.8 | 5.02 | 0.1 | 6.3 |
| Mid | 10.9 | 0.7 | 4.84 | 0.1 | 5.9 |
| High | 14.3 | 0.8 | 5.20 | 0.1 | 6.4 |

**FEMALES**

**WEEK -1**

| Dose | BUN mg/L | CREAT. mg/L | POTASS. meq/L | T.BILI. mg/L | PHOS. mg/L |
|---|---|---|---|---|---|
| Contr. | 12.7 | 0.8 | 4.85 | 0.1 | 5.4 |
| Low | 12.9 | 0.8 | 4.95 | 0.1 | 5.8 |
| Mid | 14.4 | 0.7 | 4.99 | 0.1 | 6.3* |
| High | 14.1 | 0.7 | 5.01 | 0.1 | 6.4* |

* $p \leq 0.05$

** $p \leq 0.01$

**MALES**

**WEEK 12**

| | BUN mg/L | CREAT. mg/L | POTASS. meq/L | T.BILI. mg/L | PHOS. mg/L |
|---|---|---|---|---|---|
| Dose | | | | | |
| Contr. | 12.5 | 0.7 | 4.64 | 0.1 | 4.9 |
| Low | 13.6 | 0.8 | 4.73 | 0.1 | 5.1 |
| Mid | 13.3 | 0.8 | 4.82 | 0.2** | 5.3 |
| High | 17.9** | 0.9* | 5.18** | 0.2** | 5.4 |

* $p \leq 0.05$

** $p \leq 0.01$

**FEMALES**

**WEEK 12**

| | BUN mg/L | CREAT. mg/L | POTASS. meq/L | T.BILI. mg/L | PHOS. mg/L |
|---|---|---|---|---|---|
| Dose | | | | | |
| Contr. | 13.2 | 0.8 | 4.62 | 0.1 | 4.4 |
| Low | 13.8 | 0.8 | 4.62 | 0.1 | 5.1 |
| Mid | 15.3 | 0.8 | 5.00 | 0.2* | 4.9 |
| High | 20.2** | 1.0 | 5.01 | 0.3** | 5.7** |

* $p \leq 0.05$

** $p \leq 0.01$

**MALES**

**WEEK 25**

| | BUN mg/L | CREAT. mg/L | POTASS. meq/L | T.BILI. mg/L | PHOS. mg/L |
|---|---|---|---|---|---|
| Dose | | | | | |
| Contr. | 13.0 | 0.9 | 4.52 | 0.1 | 4.3 |
| Low | 14.4 | 0.9 | 4.65 | 0.1 | 4.5 |
| Mid | 13.8 | 0.9 | 4.78 | 0.2** | 4.5 |
| High | 16.9 | 1.0 | 5.12** | 0.2** | 5.0 |

* $p \leq 0.05$

** $p \leq 0.01$

**FEMALES**

**WEEK 25**

| | BUN mg/L | CREAT. mg/L | POTASS. meq/L | T.BILI. mg/L | PHOS. mg/L |
|---|---|---|---|---|---|
| Dose | | | | | |
| Contr. | 16.0 | 0.9 | 4.52 | 0.1 | 4.3 |
| Low | 13.9 | 0.8 | 4.37 | 0.1 | 4.2 |
| Mid | 14.2 | 0.8 | 4.70 | 0.2 | 4.5 |
| High | 21.9** | 1.1* | 4.86* | 0.2* | 5.3* |

* $p \leq 0.05$

** $p \leq 0.01$

**MALES**

**WEEK 39**

| | BUN mg/L | CREAT. mg/L | POTASS. meq/L | T.BILI. mg/L | PHOS. mg/L |
|---|---|---|---|---|---|
| Dose | | | | | |
| Contr. | 11.7 | 0.7 | 4.49 | 0.1 | 3.4 |
| Low | 14.5 | 0.9 | 4.77 | 0.1 | 3.7 |
| Mid | 12.5 | 0.8 | 4.76 | 0.2 | 3.5 |
| High | 16.6* | 0.8 | 4.93* | 0.2** | 4.2** |

* $p \leq 0.05$

** $p \leq 0.01$

**FEMALES**

**WEEK 39**

| | BUN mg/L | CREAT. mg/L | POTASS. meq/L | T.BILI. mg/L | PHOS. mg/L |
|---|---|---|---|---|---|
| Dose | | | | | |
| Contr. | 15.2 | 0.8 | 4.48 | 0.1 | 3.2 |
| Low | 14.2 | 0.8 | 4.59 | 0.1 | 3.7 |
| Mid | 17.3 | 0.8 | 4.96 | 0.2 | 3.9 |
| High | 21.6** | 0.9 | 5.08* | 0.2** | 4.3 |

* $p \leq 0.05$

** $p \leq 0.01$

**MALES**

**WEEK 51**

| | BUN mg/L | CREAT. mg/L | POTASS. meq/L | T.BILI. mg/L | PHOS. mg/L |
|---|---|---|---|---|---|
| Dose | | | | | |
| Contr. | 14.3 | 0.8 | 4.88 | 0.1 | 4.9 |
| Low | 16.1 | 0.8 | 4.68 | 0.1 | 5.9* |
| Mid | 16.3 | 0.9 | 5.03 | 0.2** | 5.5 |
| High | 17.9 | 0.9 | 4.87 | 0.2** | 5.8 |

* $p \leq 0.05$

** $p \leq 0.01$

**FEMALES**

**WEEK 51**

| | BUN mg/L | CREAT. mg/L | POTASS. meq/L | T.BILI. mg/L | PHOS. mg/L |
|---|---|---|---|---|---|
| Dose | | | | | |
| Contr. | 19.4 | 0.8 | 4.44 | 0.1 | 4.8 |
| Low | 17.6 | 0.8 | 4.59 | 0.1 | 5.3 |
| Mid | 17.3 | 0.8 | 4.83 | 0.2* | 5.9 |
| High | 19.7 | 0.9 | 4.99* | 0.3** | 6.3* |

* $p \leq 0.05$

** $p \leq 0.01$

6. Urinalysis^

Urine was collected from fasted animals prior to study and at weeks 12, 25, and 51. The CHECKED (X) parameters were examined.

| X | | X | |
|---|---|---|---|
| x | Appearance* | x | Glucose* |
| x | Volume* | x | Ketones* |
| x | Specific gravity* | x | Bilirubin* |
| x | pH | x | Blood* |
| x | Sediment (microscopic)* | x | Nitrate |
| x | Protein* | | Urobilinogen |

^Not required for subchronic studies
* Required for chronic studies

Results - Urinalysis results did not show any statistically significant changes in treated dogs of either sex in comparison to controls at any sampling interval.

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.

| X | | X | | X | |
|---|---|---|---|---|---|
| | Digestive system | | Cardiovasc./Hemat. | | Neurologic |
| | Tongue | x | Aorta* | xx | Brain*+ |
| x | Salivary glands* | xx | Heart* | x | Periph. nerve*# |
| x | Esophagus* | x | Bone marrow* | x | Spinal cord (3 levels)*# |
| x | Stomach* | x | Lymph nodes* | xx | Pituitary* |
| x | Duodenum* | xx | Spleen | x | Eyes (optic n.)*# |
| x | Jejunum* | xx | Thymus* | | Glandular |
| x | Ileum* | | Urogenital | xx | Adrenal gland* |
| x | Cecum* | xx | Kidneys*+ | | Lacrimal gland# |
| x | Colon* | x | Urinary bladder* | x | Mammary gland*# |
| x | Rectum* | xx | Testes*+ | xx | Parathyroids*++ |
| xx | Liver *+ | xx | Epididymides | xx | Thyroids*++ |
| x | Gall bladder* | xx | Prostate | | Other |
| x | Pancreas* | | Seminal vesicle | x | Bone*# |
| | Respiratory | xx | Ovaries*+ | x | Skeletal muscle*# |
| x | Trachea* | x | Uterus* | x | Skin*# |
| x | Lung* | | | x | All gross lesions and masses* |
| | Nose^ | | | | |
| | Pharynx^ | | | | |

| | Larynx^

* Required for subchronic and chronic studies.
^ Required for chronic inhalation.
# In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement.
+ Organ weight required in subchronic and chronic studies.
++ Organ weight required for non-rodent studies.

a. Organ weight - In males, liver weight was statistically significantly increased at the high-dose by 40% for the absolute weight and 38% for the relative weight. Low-dose and high-dose thymus weight was significantly reduced in males by 49% (absolute) and 51% (relative) at the low-dose and by 43% (absolute) and 43% (absolute) at the high-dose. High-dose thyroid weight was significantly increased in males by 48% (absolute) and 55% (relative).

In females, liver weight was significantly increased by 27% (relative) at the mid-dose and by 49% (absolute) and 48% (relative) at the high-dose. The increased relative and absolute liver weights in both sexes are considered compound-related. In the absence of clinical chemistry findings related to the thymus and thyroid, the changes in the weight of these organs in males are of uncertain toxicological significance.

**ORGAN WEIGHTS**

**MALES**

| | **Liver (g)** | **%** | **Thymus (g)** | **%** | **Thyroid (g)** | **%** |
|---|---|---|---|---|---|---|
| Dose | | | | | | |
| Contr. | 284.66 | 2.498 | 9.68 | 0.085 | 1.0375 | 0.009 |
| Low | 323.55 | 2.628 | 4.91* | 0.042** | 1.1997 | 0.010 |
| Mid | 365.33 | 2.900 | 7.85 | 0.062 | 1.1685 | 0.009 |
| High | 398.83* | 3.456** | 5.51* | 0.048* | 1.5425* | 0.014* |

**FEMALES**

| Dose | Liver (g) | % |
|---|---|---|
| Contr. | 246.81 | 2.573 |
| Low | 274.94 | 2.626 |
| Mid | 289.67 | 3.265* |
| High | 368.41* | 3.819** |

* $p \leq 0.05$

** $p \leq 0.01$

b. Gross pathology - There were no compound-related gross findings in treated dogs of either sex in comparison to controls. The incidence of green discoloration of the suprapharyngeal lymph nodes was 3, 1, 3, and 2 in males and 1, 2, 4, and 4 in females of the control, low, mid, and high-dose groups, respectively. The incidence of white corticoid-medullary junction of the kidneys was 0, 0, 1, and 0 in males and 2, 0, 1, and 3 in females of the control, low, mid, and high-dose groups, respectively.

c. Microscopic pathology - There was no NOEL for endogenous pigment (hemosiderin) found in the bone marrow, kidney, and liver at the mid- and high-dose of both sexes and at the low-dose in the kidney of males and females and the liver of males. The incidence and grades of hemosiderin deposition were dose-related. The occurrence of aspermatogenesis in one high-dose male was not considered compound-related, since there was no evidence of Giant cells or other cells that would suggest that aspermatogenesis occurred after spermatogenesis.

1) Non-neoplastic - Iron-containing pigment (hemosiderin) was found in the bone marrow, kidney, and liver of both sexes at the mid- and high-dose and at the low-dose, as well, in the kidney and liver of males, and the kidney of females.

**MALES**

| Dose | Cont. | Low | Mid | High |
|---|---|---|---|---|
| **Bone Marrow** | | | | |
| **No. Examined** | 4 | 4 | 4 | 4 |
| endogenous pigment | 1 | 1 | 4 | 4 |
| **grades** | **1** | **1** | **1,1,2,2** | **1,2,2,2** |
| **Kidney** | | | | |
| **No. Examined** | 4 | 4 | 4 | 4 |
| endog. pig. proximal tubule | 0 | 4 | 4 | 4 |
| **grades** | | **1,1,1,1** | **1,2,2,3** | **2,2,3,3** |
| **Liver** | | | | |
| **No. Examined** | 4 | 4 | 4 | 4 |
| endog. pig. R-E cells | 0 | 1 | 2 | 4 |
| **grades** | | **1** | **1,1** | **2,2,2,2** |
| **Testes** | | | | |
| **No. Examined** | 4 | 4 | 4 | 4 |
| aspermatogenesis, severe | 0 | 0 | 0 | 1 |

**FEMALES**

| Dose | Cont. | Low | Mid | High |
|---|---|---|---|---|
| **Bone Marrow** | | | | |
| **No. Examined** | 4 | 4 | 4 | 4 |
| endogenous pigment<br>**grades** | 0 | 0 | 2<br>**1,1** | 4<br>**1,1,1,1** |
| **Kidney** | | | | |
| **No. Examined** | 4 | 4 | 4 | 4 |
| endog. pig.<br>proximal tubule<br>**grades** | 0 | 1<br>**1** | 4<br>**1,1,2,3** | 4<br>**2,2,2,3** |
| **Liver** | | | | |
| **No. Examined** | 4 | 4 | 4 | 4 |
| endog. pig.<br>R-E cells<br>**grades** | 0 | 0 | 4<br>**1,1,1,2** | 4<br>**2,2,2,2** |

**1 = minimal**
**2 = mild**
**3 = moderate**
**4 = severe**

2) Neoplastic - There were no tumors of any kind at any dose level.

D. DISCUSSION:

The dose levels of this study were based on preliminary data which suggested that the low-dose would be a NOEL. There was no NOEL in the study. At the low-dose, there was mild (13% difference from controls) methemoglobinemia, due to the dichloroaniline moiety of the test substance, accompanied by hemosiderin deposition in the kidneys of males and females and the liver of males. At the mid- and high-doses, the methemoglobinemia was moderate, macrocytic, and regenerative. There was chemical chemistry data available to suggest renal damage, but the only treatment-related organ weight changes were in the increased absolute and relative weight of the liver in both sexes. The histopathological findings at the mid- and high-dose consisted of hemosiderin deposition in the bone marrow, kidney, and liver. The incidence and grades of the lesions tended to be dose-related.

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