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

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JUN 1 4 1993

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

**MEMORANDUM**

Subject: EPA ID# 018301, Chlorpropham: CIC Review of the subchronic toxicity study in rats with Chlorpropham (MRID# 418631-01).

Shaughnessy #: 018301.
Caswell#: 510A.
HED Project#: 1-1441.
DP Barcode: D164997.
Case#: 818637.
Submission#: S397214.

From: David G Anderson, PhD David G Anderson 11/23/92.
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Thru: Karen Hamernik, PhD
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Data Reviewed:
MRID# 418631-01. J.H. Wedig. October 31, 1991. 90-Day Toxicity Evaluation of Chlorpropham in the Rat. Conducted at T.P.S., Inc. for Chlorpropham Task Force, John Wise & Associates, Ltd. Lab ID 393G-102-034-89.

CONCLUSIONS: Chlorpropham was administered via the diet to 10 Sprague Dawley rats per sex per group for 90-days at target dose levels of 0, 17, 70, 300 and 1200 mg/kg/day. The nominal average daily intake values were 0, 15.9, 64.8, 276 and 1118 mg/kg/day in males and 0, 16.0, 65.0, 279 and 1144 mg/kg/day in females. Chlorpropham exhibited hematological toxicity with a NOEL of < 15.9 mg/kg/day and a LOEL of < 15.9 mg/kg/day. Methemoglobin levels were not determined.

NOEL: < 17 mg/kg/day (LDT).

LOEL: < 17 mg/kg/day - Equivalent to 15.9 mg/kg/day in males and 16 mg/kg/day in females. Treatment related changes in red blood cell morphology were observed: 6 of 20 animals had

target cells present and 9 of 20 animals had crenated cells. Neither cell types was observed in control animals. No other significant treatment related effects were observed.

At higher dose levels equivalent to 64.8 mg/kg/day in males and 65.0 mg/kg/day in females. Target cells were seen from 9 of 20 animals and crenation in 13 of 20 animals. One male showed darken spleen and slight congestion and 1 male showed increased hematopoiesis and lymphoid hyperplasia of the spleen.

At 276 mg/kg/day in males and 279 mg/kg/day in females, increased relative spleen weights and absolute spleen weights in females. Decreased erythrocyte count and hemoglobin concentration occurred in both sexes, but a slightly decreased hematocrit occurred in females only. Target cells occurred in 18 of 20 and crenated cells occurred in 19 of 20 animals. All rat showed cellularity of the bone marrow, 80-100% showed hematopoiesis, hemosiderosis and congestion of the spleen and 4 of 20 rat showed pigment accumulation in the liver.

At 1118 mg/kg/day in males and 1144 mg/kg/day in females, decreased body weights and increase liver and spleen weights occurred. Decreased erythrocyte counts, hematocrit, hemoglobin concentration and increased reticulocyte count and crenation and target cells occurred. Histologically detected hematopoiesis (primarily erythropoiesis) in the liver, spleen and bone marrow. Clinical chemistry indicated that the HDT animals had elevated cholesterol and that females had depressed plasma cholinesterase.

Core Classification: Supplementary. The study is not acceptable under guideline 82-1 for a 90-day study in rodents. A NOEL was not established and stability studies on the test material in the feed were not submitted. In addition, hematology should have included analysis of methemoglobin levels. However, a 90-day study in rats may be unnecessary, if an acceptable NOEL can be established from the chronic feeding study in rats when submitted.

CMemo on a CIC-DER for a 90-day toxicity study in rats/MRID# 418993-01/B:\CHLORV25.10A\CM90DMO1.192/DANDERSON/11/24/92.*

DOC930158

FINAL

# DATA EVALUATION REPORT

Chlorpropham

Study Type: Subchronic Oral Toxicity in Rats

**Prepared for:**

Office of Pesticide Programs
Health Effects Division
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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**Prepared by:**

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August 10, 1992

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Contract Number: 68D10075
Work Assignment Number: 1-43
Clement Number: 93-54
Project Officer: James Scott

Guideline Series 82-1 -- Subchronic Oral Toxicity
in the Rodent: 90-Day Study

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Date: 4/26/93

DATA EVALUATION REPORT

STUDY TYPE: Subchronic Oral Toxicity in Rats

TEST MATERIAL: Chlorpropham

Tox. Chem. Number: 510A

SYNONYMS: Isopropyl *N*-(3-chlorophenyl)carbamate

Shaughnessy Number: 018301

CAS NUMBER: 101-21-3

MRID Number: 418631-01

STUDY NUMBER: 393G-102-034-89

SPONSOR: Chlorpropham Task Force, John Wise and Associates, Ltd.

TESTING FACILITY: T.P.S., Inc.
10424 Middle Mt. Vernon Road
Mt. Vernon, Indiana 47620

TITLE OF REPORT: 90 Day Subchronic Toxicity Evaluation of Chlorpropham in the Rat

AUTHOR: J.H. Wedig, Ph.D., DABT, Study Director

REPORT ISSUED: October 31, 1991

CONCLUSIONS: Chlorpropham was administered via the diet to Sprague-Dawley rats for 3 months at target dietary dose levels of 0, 17, 70, 300, and 1200 mg/kg/day.

17 mg/kg -- Treatment-related changes in red blood cell morphology were observed: 6 of 20 animals had target cells present and 9 of 20 animals had crenated cells. Neither of these cell types was observed in any animals in the control group. No other treatment-related effects significantly different from controls were observed.

70 mg/kg -- The red blood cells of 13 of 20 rats in this group showed crenation, and 9 of 20 rats had target cells present. In addition, one male had a darkened spleen on gross examination, with slight congestion seen in the histopathological examination, and one male showed increased hematopoiesis and lymphoid hyperplasia in the spleen.

300 mg/kg -- A significant increase in spleen-to-body-weight ratio was observed in both sexes; in females, the absolute weight of the spleen was also significantly increased. Hematological evaluation showed

significant decreases in red blood cell count and hemoglobin concentration in both sexes, and significantly reduced hematocrit in females. Red blood cell morphology revealed increased target cells in 18 of 20 animals and crenated cells in 19 of 20 animals. Histopathological results supported the evidence of these effects on the hematopoietic system in the spleen, liver, and bone marrow, with 80-100% of the rats showing increased hematopoiesis, hemosiderosis, and congestion in the spleen, 4 of 20 rats showing accumulation of pigment in the liver, and all rats showing marked cellularity in the bone marrow.

1200 mg/kg -- Treatment-related effects observed at this dose included significantly decreased body weight and significantly increased spleen and liver weights in both sexes. Hematological analysis of animals at this dose level revealed significantly decreased red blood cell counts, hematocrit, and hemoglobin concentration, and significantly increased reticulocyte counts. Dose-related effects were observed in the red blood cell morphology in these animals, with 95% of animals at this dose showing crenated and target cells. Histopathological evaluation confirmed the increased incidence of red blood cell breakdown and resulting increased hematopoiesis (primarily erythropoeisis) observed upon microscopic examination of the spleen, liver, and bone marrow. Statistically significant changes in blood chemistry included increased total cholesterol in both sexes and decreased plasma cholinesterase in the female rats.

Based on the observed hematological effects, changes in the spleen and liver weights, and supporting histopathological findings, the LOEL for this study was 17 mg/kg/day (LDT). The NOEL for this study was less than 17 mg/kg/day. The effects of chlorpropham on plasma cholinesterase decrease showed a LOEL of 1200 mg/kg/day in female rats, and a NOEL of 300 mg/kg/day; in male rats, the NOEL for cholinesterase was at the highest dose tested, 1200 mg/kg/day.

Although the study did not demonstrate a NOEL, the need for a repeat study or a supplementary study at a lower dose level will be reviewed. At that time, a determination will be made whether a satisfactory NOEL can be established from the chronic study.

CORE CLASSIFICATION: Supplementary. The study does not establish a NOEL as required and stability data on the test material in the feed must be submitted. In addition, hematology should have included analysis of methemoglobin levels. The study may be acceptable under guideline 82-1 for a 90-day feeding study in rats, if an acceptable NOEL can be established from the chronic feeding study when submitted and evaluated.

## A. MATERIALS, METHODS, AND RESULTS

### 1. Test Article Description

Name: Chlorpropham

Formula: $C_{10}H_{12}ClNO_2$

Lot number: 14065 L 89

Purity: 96.2% in weeks 1-4, 97.1% ± 2% in weeks 5-13

Physical properties: White crystalline solid; mp 40.7-41.1° C

Stability: Not reported (see #3 below)

2. Rationale for Dose Selection

The target doses for this study were 0, 17, 70, 300, and 1200 mg/kg/day, as determined by the sponsor in previous studies. No further information was provided on these preliminary studies; however, the doses appear to have been appropriately selected based on the incidence of toxicity without excessive mortality observed at the highest dose and the lack of toxicity observed at the lowest dose.

3. Test Article Analyses for Purity and Stability

In the current study, test diets containing chlorpropham with target concentrations of 0, 17, 70, 300, and 1200 mg/kg/day were prepared weekly for all groups by mixing the appropriate amount of test material (chlorpropham as either a liquid or a recrystallized powder) with the basal diet (Purina® Certified Rodent Meal #5002) in Hobart® or Univex® mixers. The test material (dissolved in corn oil) and 1000-1500 g rodent meal were mixed and then added to the appropriate amount of rodent meal (1850-4400 g). Final batch sizes were 3-6 kg. Diets were stored in labelled plastic bags within tightly closed containers.

The test material was stored in a sealed container at room temperature. Purity of the test sample was 96.2% for weeks 1-4, then 97.1 ± 2% for weeks 5-13. The reason for this variation in test article purity is not discussed in the study report; instead, information on purity and stability is reported to be "on file" (with the Chlorpropham Task Force, John Wise and Associates). Because of the nature of the test article and the original containers in which it was supplied, the material needed to be melted (Stabil-Term cabinet, 45-47°C, for 16 hours) to facilitate removal from this container. Both liquid and recrystallized powder aliquots of the test material were used during the experiment. The protocol indicated that the nature of the test material (i.e., liquid or recrystallized powder) would be provided in the report data, but this information was not provided. Doses were calculated to correct the test material to 100% purity during each week's diet preparation.

For weeks 1-4, 8, and 12, the blended diets for all groups were analyzed with HPLC for test article concentration and homogeneity, with random samples from the top, middle, and bottom of the mixtures. All diet concentrations were within 10% of theory (target ppm concentration), with the exception of the diet prepared for female rats in the 70-mg/kg/day dose group during week 4 (130% of theory) and week 8 (115% of theory).

Tap water was analyzed by a separate lab (Lancaster Laboratories, Lancaster, MI), retrospectively, for possible levels of contaminants including pesticides, nitrates, heavy metals, and trihalomethanes. Concentrations of these contaminants are not reported in the study but are reported to be below levels capable of compromising the study.

In order to achieve the target dosage levels, the test diets were prepared with varying concentrations (ppm) of chlorpropham, with dosage volume (the amount added to the final feed batch) adjusted according to food consumption and body weight. Using the food consumption data of the previous week, the actual dosage received by each group was also calculated, retrospectively. Target dosage levels and actual measured mean dosage levels (calculated by reviewer; data extracted from Study 393G-102-034-89, Table 1) were as follows:

| | Target Dosage Level (mg/kg/day) | | | |
|---|---|---|---|---|
| | 17 | 70 | 300 | 1200 |
| Study Mean Dosage (mg/kg/day) | 16 | 65 | 277 | 1131 |
| Standard Deviation (mg/kg/day) | 0.83 | 3.8 | 14.6 | 66.6 |
| Percent of Target | 94 | 93 | 92 | 94 |

Mean dosage levels were within 8% of the target dosage, with all group mean weekly values within 16% of target.

4. Animals

Male and female Sprague-Dawley rats, approximately 4-5 weeks of age, were obtained from Charles River Laboratories, Inc. (Portage, MI), and acclimated for a period of 15 days. From this batch, 100 animals (50 males, 50 females) were selected to be in the study based on physical examination and body weights (selected males weighed between 180.0 and 225.6 g; selected females weighed between 138.0 and 173.6 g). The animals were assigned to the five study treatment groups using a computer-randomized list, with 10 animals/sex/dose; each rat was uniquely identified by ear punch and tattoo. At the start of the study, animals were approximately 6-7 weeks of age. Animals were housed individually in stainless steel cages. Food (Purina® Certified Rodent Meal #5002) and tap water were provided *ad libitum* throughout the study. Room temperature, recorded daily, ranged from 67°F to 76°F, and humidity, recorded weekly, ranged from 40% to 70%. Cycled lighting (12 hours light/12 hours dark) was provided, and the air supply was filtered with 10-15 changes/hour.

5. Statistical Methods

Body weights, food consumption, organ weights (relative and absolute), and results of hematological and clinical chemistry evaluations were analyzed using Dunnett's analysis of variance test, comparing each treatment group with the control group. The following results were not analyzed statistically: RBC morphology, urinalysis, and histopathology.

6. General Observations

(a) Mortality/moribundity/survival

Animals were observed for mortality and moribundity twice per day (AM and PM), 7 days per week.

All animals in treated and control groups survived to Day 90 of the experiment.

(b) Clinical signs

Animals were observed twice per day (AM and PM), 7 days per week, for general health, physical appearance, behavior, and pharmacologic or toxic effects. In addition, general physical assessments, including digital palpation for tissue masses or abdominal distention, were made weekly throughout the study and at study termination. The incidence, size, and location of masses were recorded.

No significant adverse clinical observations were noted. Incidental clinical signs observed included hair loss on limbs, crusty scales on nose or around eyes, and palpable masses. However, these findings were sporadic and not significantly different from control incidences, and so were not considered to be treatment related. No behavioral clinical signs were noted in any rats.

(c) Body weights/food consumption/feed efficiency/test article intake

Body weights--Body weights were measured prior to randomization into the study treatment groups, the day before the first test dose was administered, then weekly during the exposure period, and just prior to necropsy at study termination. Group mean summary data and individual body weight data were provided in the study report.

Treatment-related effects on body weight were seen in both sexes at the 1200-mg/kg dose level (Table 1). The statistically significant decrease in the group mean body weight in males in this group started at week 1 and continued through study termination, with the exception of week 2, in which the decrease was not statistically significant. Females in this group showed a significant decrease in body weight beginning in week 5 and continuing through study termination. The mean body weights were 17% and 18% decreased at week 13, compared to controls, and at week 12, gains were 23% and 34% lower than control gains in males and females, respectively. Cumulative weight gain was significantly decreased from week 5 (males) and week 4 (females). No significant body weight changes were seen at lower dose levels in either sex.



3

Food consumption--Measurements of food consumption were made during the acclimation period and weekly thereafter throughout the study. Both summary and individual data were provided in the study report. Food consumption data were used to calculate and readjust dietary admix levels (ppm) in order to achieve target dose levels (mg/kg).

No treatment-related statistically significant changes were noted with respect to food consumption.

Feed efficiency--Feed efficiency was not determined in this study.

Test article intake--Test article intake in mg/kg/day was calculated by the author based on test article concentration (ppm) in the diet preparation, diluted by the appropriate volume of feed (based on food consumption from the previous week, and body weight). The concentration of the test article was adjusted weekly, based on the previous week's food consumption data, in order to maintain the desired dose level on a mg/kg/day basis.

(d) Ophthalmoscopic examination

Ophthalmic examinations were conducted on all stock animals prior to study initiation and on all study animals prior to study termination. Lesions reported at study termination included one female in the 70-mg/kg group with lenticular opacity and one female in the 300-mg/kg group with hemorrhage in the anterior chamber. One control male had corneal opacity and hemorrhage in the anterior chamber. Because of the low incidence of these lesions, they were not considered to be treatment related.

7. Clinical Pathology

Hematological, blood chemistry, and urine analyses were performed at study termination on all treated and control animals. The animals were fasted overnight immediately prior to necropsy. Blood samples for hematology and clinical chemistry evaluation were collected from the vena cava at necropsy.

For urinalysis, the rats were hydrated with 20 ml/kg tap water via gavage, and placed in metabolism cages overnight, without feed or water. The analysis required a 1-ml sample; the parameters that were examined are indicated below with an X.

Individual data and group means for each exposure group and each sex were provided in the report. Significant results in the hematological and clinical chemistry evaluations are summarized in Tables 2-4.

(a) Hematology

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
X Reticulocyte count (RETIC)
X Leukocyte differential count*
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
X Red blood cell morphology

* Recommended by Subdivision F (November 1984) Guidelines

Treatment-related, dose-dependent changes included hemolytic and macrocytic anemia, with significantly decreased red blood cell count and hemoglobin concentration in both sexes exposed to 300 mg/kg or 1200 mg/kg. Hematocrit values were significantly decreased in both sexes at 1200 mg/kg and in females in the 300-mg/kg group. Mean corpuscular volume and hemoglobin concentration were significantly increased in the 1200-mg/kg dose group in both sexes, while mean corpuscular hemoglobin concentration (MCHC) was increased only in males and at all treatment doses. (These MCHC values in the treated males were reported to be within the expected range for untreated rats.) Compensatory reticulocyte counts were significantly increased in both sexes in the 300-mg/kg and 1200-mg/kg groups.

Red blood cell morphology revealed dose-response effects on hematopoiesis (primarily erythropoiesis), which was confirmed by histopathological evaluation of spleen, liver, and bone marrow. An increasing incidence of crenated and target red blood cells was observed in all dose groups (see Table 2). The incidence of crenated cells in males and females was 0%, 45%, 65%, 95%, and 95% in controls, 17, 70, 300, and 1200 mg/kg dose levels, respectively. The incidence of target cells in males and females was 0%, 30%, 40%, 90%, and 90% at similar respective dose levels. Neither of these cell types was present in any control animals. Macrocytic cells and anisocytosis were observed in both sexes in the highest dose group (1200 mg/kg). A summary of red blood cell morphology data is provided in Table 2.

Summary data on significant hematological changes are presented in Table 3.

(b) Blood (clinical) chemistry

Electrolytes

X Calcium*
X Chloride*
X Phosphorus*
X Potassium*
X Sodium*

Enzymes

X Alkaline phosphatase (ALP)
X Creatinine phosphokinase
X Serum alanine aminotransferase (SGPT)*
X Serum aspartate aminotransferase (SGOT)*
X Lactate dehydrogenase
X Cholinesterase (plasma)[1]

Other

X Albumin*
Blood creatinine*
X Blood urea nitrogen*
X Cholesterol
X Globulins
X Glucose*
X Total bilirubin*
X Total protein*
X Thyroxine

* = Recommended by Subdivision F (November 1984) Guidelines

Treatment-related, statistically significant changes (p=0.05) in blood chemistry included increased total cholesterol in both sexes in the 1200-mg/kg dose group, with male cholesterol levels 181% of control, and female levels 158% of control. Plasma cholinesterase levels in females in the 1200-mg/kg dose group decreased to 68% of the control value (percentage-of-control values calculated by reviewer; Study 393G-102-034-89, Table 11, providing group mean summary report of clinical chemistry values). Other significant values included elevated protein, albumin, and albumin:globulin ratio in males in the 1200-mg/kg group, and decreased calcium in males in the 300-mg/kg group; these effects were not considered biologically significant related to treatment because they were within the expected ranges for untreated rats. A summary of significant clinical chemistry changes is provided in Table 4.

(c) Urinalysis

X Specific gravity
X pH
X Glucose
X Protein
X Ketones
X Leukocytes
X Bilirubin
X Blood
X Urobilinogen

* = Recommended by Subdivision F (November 1984) Guidelines

There were no treatment-related changes revealed by urinalysis in this study.

[1]Cholinesterase was determined by the method of Ellman (Boehringer Mannheim Diagnostics, Reagent Set No. 124117).

8. Sacrifice and Pathology

Complete gross examinations were performed on all animals at study termination, following overnight fast. Male rats were sacrificed on day 90 of the experiment, and females were sacrificed on day 91. The order of sacrifice was randomized for all treatment and control animals. Animals were euthanized using carbon dioxide, and all animals were examined for gross tissue and organ changes. The organs and tissues listed below were collected and preserved in 10% phosphate buffered formalin. Organs indicated by XX were weighed prior to preservation, except for pituitaries and thyroids, which were weighed after preservation. With the exception of the lumbar portions of spinal cords, all tissues marked with an X below were processed, stained, and examined microscopically, for all animals in the 1200-mg/kg dose group and in the control group. For animals in the other three treatment groups, the liver, spleen, and sternum with bone marrow were processed and examined microscopically, along with any gross lesions from any tissues noted at necropsy.

Digestive System

XX Liver*
X Salivary glands*
X Esophagus*
X Stomach*
X Duodenum*
X Jejunum*
X Ileum*
X Cecum*
X Colon*
X Rectum
Gall bladder*
X Pancreas*

Respiratory

X Trachea*
X Lung*

Cardiovascular/Hematologic

X Aorta*
XX Heart*
X Bone marrow*
X Lymph nodes*
XX Spleen
X Thymus

Urogenital

XX Kidneys*
X Urinary bladder*
XX Testes*
X Epididymides
X Seminal vesicle
X Prostate
XX Ovaries
X Uterus
X Cervix

Neurologic

XX Brain
X Peripheral nerve (sciatic)*
X Spinal cord (three levels)
XX Pituitary*
X Eyes*

Glandular

XX Adrenals*
X Lachrymal gland
X Mammary gland
XX Thyroid*
X Parathyroids*

Other

X Bone (sternum) and marrow*
X Skeletal muscle*
X Skin
X All gross lesions and masses*

* = Recommended by Subdivision F (November 1984) Guidelines

(a) Macroscopic

Rats of both sexes in the two highest dose groups had discolored spleens (dark red or red/black): at 1200 mg/kg, 5/10 males and 9/10 females had darkened spleens; at 300 mg/kg, 3/10 males and 6/10 females had darkened spleens. One male in the 70-mg/kg group had a darkened spleen. No other consistent, treatment-related gross tissue changes were noted.

(b) Organ weights and body weight ratios

Spleen weights (absolute) and spleen weights relative to both body weight and to brain weight were significantly increased in the 1200-mg/kg dose group in both sexes. Liver weights (absolute) and relative liver weights also increased in this highest dose group, in both sexes. At 300 mg/kg, the ratio of spleen-to-body weight increased significantly for both males and females; the absolute spleen weight increased significantly only in females. In the 1200-mg/kg dose group, other organs for which there was a significant increase in weight relative to body weight included the brain, the male gonads, and the female heart, kidney, and thyroid. Table 5 summarizes the significant effects of chlorpropham treatment on organ weights.

(c) Microscopic

The spleen, liver, and bone marrow of animals in the two highest dose groups (300 mg/kg and 1200 mg/kg) showed treatment-related increased incidences of hematopoiesis (primarily erythropoiesis). Accumulation of pigment was noted in 18 of 20 rats in the 1200-mg/kg group and in 4 of the 20 rats in the 300-mg/kg group. Spleens of all animals in the 1200-mg/kg group showed hematopoiesis, hemosiderosis, and congestion; at 300 mg/kg, increased hemosiderosis, congestion, and hematopoiesis were observed in all animals. There was moderate cellularity (grade 3) in the bone marrow of controls and the two lowest dose group animals, and marked cellularity (grade 4) in the two highest dose group animals. In addition, at the 70-mg/kg dose level, 1 male had a darkened spleen with slight congestion, and 1 male showed increased hematopoiesis and lymphoid hyperplasia.

No other distinct or consistent compound-related effects were found. A summary of important histopathologic changes is provided in Table 6.

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A signed Good Laboratory Practice Compliance Statement, a signed Quality Assurance Statement, and a list of Quality Assurance inspections were included in the report.

B. DISCUSSION

Review of the final report and supporting data indicate that the conduct of the study was adequate and the reporting of the results was accurate. The design of the experiment was judged adequate to fulfill the intent of the guidelines.

The dietary levels selected were based on a preliminary study conducted by the study author. The details and results of this preliminary study were not provided; however, the doses appear to have been appropriately selected based on the incidence of toxicity without excessive mortality observed at the highest dose and the lack of toxicity observed at the lowest dose.

The hematopoietic system appeared to be the most sensitive target system in the current study. Dose-dependent effects on red blood cells were clearly seen in rats in the 300-mg/kg and 1200-mg/kg dose groups, with significant decreases in red blood cell counts, hemoglobin concentrations, hematocrit values, and increases in mean corpuscular volume and hemoglobin. At the highest dose, macrocytic cells and anisocytosis were also observed, and reticulocytes were significantly increased. Marked changes in the morphology of these red blood cells were observed, with an increasing incidence of crenated and target cells observed in all dose groups (30-45% incidence at 17 mg/kg, increasing to 95% incidence at 1200 mg/kg).

The destruction of red blood cells and the resulting increase in hematopoietic activity were evident grossly in the spleens of animals in the two highest dose groups, and also evident upon microscopic examination in the spleen, liver, and bone marrow in animals of these two dose groups (300 mg/kg and 1200 mg/kg). Animals at the 1200-mg/kg level showed significant increases in spleen and liver weights (both absolute weights and weights relative to body weight or brain weight), whereas only spleen weights were elevated at 300 mg/kg (relative to body weights in males and females; increased absolute weights were seen only in females at this dose). There were no significant changes in organ weights in animals in the 70-mg/kg dose group, but histopathological examination revealed some of the same effects seen at higher doses in 2 of the males.

Based on the observed hematological effects, changes in spleen and liver weights, and supporting histopathological findings, the LOEL for this study is 17 mg/kg/day. The NOEL for this study is less than 17 mg/kg/day. The effects of chlorpropham on plasma cholinesterase decrease showed a LOEL of 1200 mg/kg/day in female rats, and a NOEL of 300 mg/kg/day; in male rats, the NOEL for cholinesterase decrease was at the highest dose tested, 1200 mg/kg/day.

TABLE 1. Mean Body Weight at Selected Weeks in Rats Fed Chlorpropham for 13 Weeks[a]

| Dietary Level (mg/kg/day) | Mean Body Weight (g ± S.D.) at Week: 0 | 1 | 5 | 7 | 13 |
|---|---|---|---|---|---|
| | | | Males | | |
| 0 | 206.1±8.5 | 254.2±11.2 | 403.1±18.2 | 458.0±17.3 | 505.2±27.5 |
| 17 | 203.0±6.7 | 253.4±6.7 | 409.8±19.8 | 461.2±22.2 | 508.1±31.6 |
| 70 | 199.7±12.0 | 248.2±14.7 | 394.0±23.5 | 448.2±30.3 | 495.6±42.5 |
| 300 | 206.3±11.2 | 253.9±1.17 | 399.7±15.2 | 449.4±20.6 | 481.5±28.1 |
| 1200 | 199.9±9.0 | 241.3*±9.9 | 370.5**±31.1 | 406.1**±33.3 | 423.2**±39.1 |
| | | | Females | | |
| 0 | 156.1±9.4 | 174.8±9.9 | 238.0±19.4 | 260.7±21.5 | 287.8±26.7 |
| 17 | 160.6±4.6 | 183.3±9.7 | 248.2±24.1 | 267.0±24.9 | 294.5±34.0 |
| 70 | 153.6±6.5 | 176.0±9.9 | 231.7±16.4 | 252.6±13.0 | 270.1±20.0 |
| 300 | 162.4±7.9 | 186.6*±8.1 | 246.6±15.0 | 265.9±17.8 | 290.2±22.4 |
| 1200 | 155.0 ± 8.8 | 170.7±9.1 | 214.6*±12.8 | 233.0**±14.7 | 236.8**±18.1 |

[a]Data extracted from Study No. 393G-102-034-89, Tables 6 and 7, and Appendix II.

*Significantly different from control value, $p < 0.05$
**Significantly different from control value, $p < 0.01$

TABLE 2. Red Blood Cell Morphology in Rats Fed Chlorpropham for 13 Weeks[a]

| | RBC Morphology at Dose Level (mg/kg/day):[b] | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Males | | | | | Females | | | | |
| Finding | 0 | 17 | 70 | 300 | 1200 | 0 | 17 | 70 | 300 | 1200 |
| Crenated cells | 0 | 3 | 7 | 9 | 10 | 0 | 6 | 6 | 10 | 9 |
| Target cells | 0 | 3 | 3 | 9 | 10 | 0 | 3 | 6 | 9 | 9 |
| Macrocytic | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 3 |
| Aniocytosis | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 2 |

[a]Data extracted from Table 10 and Appendix III of the Study Report.

[b]Based on 10 animals/sex/dose group.

TABLE 3. Mean Hematology Data at Termination for Rats Fed Chloropham for 13 Weeks[a]

| Parameter | Mean Hematology Data (count ± S.D.) at Dose Level (mg/kg/day): 0 | 17[b] | 70 | 300 | 1200 |
|---|---|---|---|---|---|
| **Males** | | | | | |
| RBC ($10^6$/mm$^3$) | 8.76±0.46 | 8.96±0.57 | 8.32±0.54 | 7.54**±0.42 | 6.67**±0.47 |
| Hemoglobin(g/dL) | 16.3±0.6 | 16.5±0.6 | 15.6±0.6 | 14.7**±0.8 | 14.5**±0.7 |
| Hematocrit(%) | 43.6±2.3 | 45.6±1.7 | 43.1±1.6 | 41.5±2.0 | 41.1*±1.8 |
| MCH (pg) | 18.7±1.2 | 18.5±0.9 | 18.9±1.0 | 19.5±1.3 | 21.7**1.0 |
| MCV ($\mu^3$) | 49.9±2.1 | 51.1±3.1 | 52.0±2.4 | 55.2**±3.6 | 61.8**±3.3 |
| MCHC (g/dL) | 37.5±1.8 | 36.3*±0.5 | 36.3*0.7 | 35.4**±0.9 | 35.2**±0.5 |
| Reticulocytes (%) | 1.0±0.3 | 1.1±0.3 | 1.5±0.3 | 3.3**±1.0 | 8.1**±1.9 |
| **Females** | | | | | |
| RBC ($10^6$/mm$^3$) | 8.43±0.46 | 7.98±1.02 | 7.97±0.54 | 6.49**±0.38 | 5.76**±0.53 |
| Hemoglobin(g/dL) | 15.5±0.3 | 15.3±0.8 | 15.2±0.9 | 13.7**±0.5 | 13.4**±0.7 |
| Hematocrit(%) | 44.5±2.4 | 41.9±4.1 | 42.9±2.2 | 38.0**±1.9 | 37.6**±2.6 |
| MCH (pg) | 18.5±1.1 | 19.4±2.0 | 21.4±6.5 | 21.2±1.3 | 23.4**±1.4 |
| MCV ($\mu^3$) | 52.9±0.9 | 52.7±2.2 | 54.1±2.0 | 58.8**±2.0 | 65.3**±3.1 |
| MCHC (g/dL) | 35.2±1.9 | 36.7±2.4 | 35.4±1.2 | 36.1±1.9 | 35.8±1.3 |
| Reticulocytes (%) | 1.3±0.4 | 1.6±0.3 | 1.9±0.4 | 4.9**±1.3 | 8.4**±2.7 |

[a]Data extracted from Table 9 and Appendix III of the Study Report.

[b]Data for the 17-mg/kg/day (males and females) dose group were based on 9 samples rather than 10 because of the clotting of 1 sample during hematology determinations.

*Significantly different from control value, $p < 0.05$
**Significantly different from control value, $p < 0.01$

TABLE 4. Selected Clinical Chemistry Findings at Termination in Rats Fed Chlorpropham for 13 Weeks[a]

| Parameter | Mean Clinical Chemistry Data (count ± S.D.) at Dose Level (mg/kg/day): 0 | 17 | 70 | 300 | 1200 |
|---|---|---|---|---|---|
| | | | Males | | |
| Cholesterol (mg/dL) | | | | | |
| | 77.4±18.1 | 82.8±21.1 | 75.3±16.3 | 88.6±18.9 | 140.3*±22.3 |
| Plasma Cholinesterase (U/L) | | | | | |
| | 258.3±76.0 | 277.6±75.9 | 289.4±102.9 | 259.2±58.8 | 286.1±86.8 |
| | | | Females | | |
| Cholesterol (mg/dL) | | | | | |
| | 97.6±19.4 | 90.8±17.4 | 98.8±15.6 | 116.3±16.9 | 153.8*±19.5 |
| Plasma Cholinesterase (U/L) | | | | | |
| | 2041.5±382.6 | 2046.6±549.1 | 1813.6±614.0 | 1805.2±408.7 | 1394.4*±294.1 |

[a]Data extracted from Table 11 and Appendix IV of the Study Report.

*Significantly different from control value, $p < 0.05$

TABLE 5. Mean Terminal Organ Weights in Rats Fed Chlorpropham for 13 Weeks[a]

| Parameter | Absolute Organ Weight (g ± S.D.) at Dose Level (mg/kg/day): 0 | 17 | 70 | 300 | 1200 |
|---|---|---|---|---|---|
| | | | Males | | |
| Absolute Weight (g ± S.D.) | | | | | |
| Liver | 14.65±0.80 | 15.27±1.89 | 14.02±1.64 | 14.32±2.05 | 17.60**±2.16 |
| Spleen | 0.847±0.111 | 0.798±0.092 | 0.870±0.194 | 1.029±0.128 | 1.592**±0.316 |
| Organ/Body Weight Ratio (% ± S.D.) | | | | | |
| Liver | 2.90±0.12 | 3.00±0.30 | 2.82±0.19 | 2.97±0.33 | 4.15**±0.17 |
| Spleen | 0.167±0.017 | 0.157±0.013 | 0.177±0.041 | 0.214*±0.030 | 0.377**±0.075 |
| | | | Females | | |
| Absolute Weight (g ± S.D.) | | | | | |
| Liver | 7.44±0.97 | 7.95±1.02 | 7.21±0.56 | 7.93±0.83 | 8.95**±0.78 |
| Spleen | 0.434±0.059 | 0.442±0.047 | 0.473±0.063 | 0.670**±0.089 | 1.036**±0.134 |
| Organ/Body Weight Ratio (% ± S.D.) | | | | | |
| Liver | 2.73±0.27 | 2.85±0.28 | 2.82±0.18 | 2.90±0.21 | 4.01**±0.44 |
| Spleen | 0.160±0.024 | 0.161±0.027 | 0.186±0.027 | 0.246**±0.038 | 0.464**±0.068 |

[a]Data extracted from Table 12 and Appendix V of the Study Report.

*Significantly different from control value, $p < 0.05$
**Significantly different from control value, $p < 0.01$

TABLE 6. Selected Histopathological Findings in Rats Fed Chlorpropham for 13 Weeks[a]

| Organ/ Finding | Incidence of Occurrence at Dose Level (mg/kg/day): Males | | | | | Females | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 17 | 70 | 300 | 1200 | 0 | 17 | 70 | 300 | 1200 |
| Liver | | | | | | | | | | |
| Hematopoiesis | 0 | 0 | 1 | 6 | 9 | 0 | 0 | 0 | 5 | 9 |
| Pigment | 0 | 0 | 0 | 1 | 8 | 0 | 0 | 0 | 3 | 10 |
| Spleen | | | | | | | | | | |
| Increased hematopoiesis | 0 | 0 | 1 | 10 | 10 | 0 | 0 | 0 | 10 | 10 |
| Increased hemosiderosis | 0 | 0 | 0 | 8 | 10 | 0 | 0 | 0 | 10 | 10 |
| Congestion | 0 | 0 | 1 | 10 | 10 | 0 | 0 | 0 | 10 | 10 |
| Bone marrow | | | | | | | | | | |
| Marked cellularity | 0 | 0 | 0 | 10 | 10 | 0 | 0 | 0 | 10 | 10 |

[a]Data extracted from Table 15 and Appendix VII of the Study Report.