



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

JUN 14 1993

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MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Subject: CIC Review of the 28-Day Range-finding Study in Dogs with Chlorpropham (MRID# 418990-01).

Shaughnessy #: 018301.
Caswell#: 510A.
HED Project#: 1-1499.
DP Barcode: D165225.
Submission#: S397521.
Contract#: 68D10075.
Work Assign.#: 1-43
Clement#: 91-144.

From: David G Anderson, PhD. *David G Anderson*
Section 3, Toxicology Branch-1
Health Effects Division (H7509C) *4/26/93.*

To: Walter Waldrop/Venus Eagle PM-71
Reregistration Branch
Special Review and
Registration Division (H7508C)

Thru: Karen Hamernik, PhD. *Karen Hamernik*
Acting Section Head
Section 3, Toxicology Branch-1
Health Effects Division (H7509C) *6/15/93*

A. CONCLUSIONS:

The report submitted with DP D168846 is supplementary because it is a range-finding study useful for setting dose level.

B. ACTION REQUESTED:

Review 28-Day Range-finding Study in Dogs with chlorpropham (MRID# 418990-01).

C. BASES FOR THE CONCLUSIONS:

The status of the required data for chlorpropham was reviewed in 1991 by HED



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Cover Memo for DER for a 28-Day Range-Finding/Dogs/Chlorpropham/1-1499/D165225/418990-01.

and will be reviewed again for a committee prior to writing the RED for chlorpropham sometime in 1994. The major data gaps were addressed in the registration standard for chlorpropham. Chronic and oncogenicity studies are being conducted.

The study submitted with the DP Barcode D165225 was reviewed by Clement International Corp. and conclusions are indicated below.

Wedig, JH, 28-Day Range-finding Evaluation of Chlorpropham in the Dog. June 19, 1990. Study No. 393I-501-620-89. MRID# 418990-01.

Chlorpropham was administered in the feed to 1 Beagle dog per sex per group at 0, 5, 50 or 500 mg/kg/day for 28 days for a range-finding study.

NOEL: < 5 mg/kg/day.

LEL: < 5 mg/kg/day for increases in cholesterol levels and at 50 mg/kg/day for histopathological changes in the thyroid. Body weight was decreased at the 50 mg/kg/day dose level. Absolute and relative spleen weights were increased as well.

Core classification: Supplementary because the study is a range-finding study.

Cmemo for CIC generated review of a 28-Day Study in Dogs/chlorpropham/418990-01/D165225/HED proj.# 1-1499/B:\CHLORV25.10A\CM28DRF.DOG/DANDERSON/10/8/92(Edited 4/26/93).*

DOC930006
FINAL

DATA EVALUATION REPORT

CHLORPROPHAM

Study Title:
28-Day Rangefinding Evaluation of Chlorpropham in the Dog

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

March 10, 1992

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Contract Number: 68D10075
Clement Number: 91-144
Work Assignment Number: 1-43
Project Officer: Mr. Jim Scott

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Signature: David G. Anderson
Date: 10/17/92

Section Head: Karen Hamernik, Ph.D.
Review Section I, Toxicology Branch I,
Health Effects Division

Signature: Karen Hamernik
Date: 4/26/93

DATA EVALUATION REPORT

STUDY TYPE: Four-week range-finding study in dogs

TEST MATERIAL: Chlorpropham

SYNONYMS: Chlorpropham~~g~~

MRID Number: 418990-01

STUDY NUMBER: 393I-501-620-89

SPONSOR: Chlorpropham Task Force
John M. Wise Associates
P.O. Box 301
Liberty, MO 64068

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

TITLE OF REPORT: 28-Day Rangefinding Evaluation of Chlorproham in Dogs

AUTHOR: J.H. Wedig

STUDY COMPLETED: June 19, 1990

CONCLUSIONS: Chlorpropham was fed to male and female beagle dogs at dose levels of 0, 5, 50 or 500 mg/kg/day for 28 days. Food consumption in the high-dose dogs was negligible when compared to control and other treated dogs during week 1 indicating unpalatability of the high-dose diets. Food consumption in the high-dose dogs returned to expected levels during weeks 2 and 3 but sharply declined at week 4. A decrease in overall body weight gain at week 4 was noted in the high-dose male and female. Moderate-to-severe histopathological changes in the thyroid (irregularly shaped follicles lined by medium to high cuboidal epithelium containing clear to pale staining colloid) were observed in the mid- and high-dose dogs. Absolute and relative spleen weights were decreased in the mid- and high-dose dogs. Slight splenic lymphoid atropy was noted in the high-dose dogs. An increase in total cholesterol levels was observed in the low-, mid-, and high-dose dogs. There were no significant effects on mortality, clinical signs, hematology, or ophthalmology.

The LOEL is 50 mg/kg/day based on histopathological changes in the thyroid. The NOEL is 5 mg/kg/day for this effect.

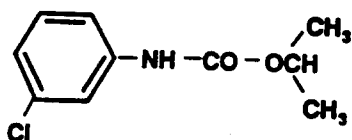
CORE CLASSIFICATION: Core Supplementary. This study provides supplementary information useful only for setting dose levels for a subchronic dog study.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: Chlorpropham

Formula: Isopropyl n-(3-chlorophenyl) carbamate



Lot number: 14065-L-89

Purity: 97.1 ± 2% (determined by sponsor)

Physical property: White-crystalline solid

Stability: Not reported

Storage: Closed containers at room temperature

2. Test Article Analyses for Purity and Stability

Test diets were prepared weekly, and concentrations were adjusted with each preparation in an effort to maintain dose levels of 5, 50, and 500 mg/kg/day. Prior to use, the crystalline chlorpropham was completely melted in a constant temperature cabinet at 45-47°C. In some instances a liquid aliquot containing test material was used, while in other instances the chlorpropham was allowed to harden and then ground into a powder with a mortar and pestle before addition to the test diets. Appropriate amounts of the material were then dissolved in mazola corn oil (doses calculated to correct the test material to 100%) and the solution was mixed with 1.5 kg of Purina dog chow meal diet no. 5007 (Lot no. DEC 07 891A) in a Hobart mixer for 15 minutes in order to prepare a preblend. Final test diet concentrations were achieved by mixing the preblend with the appropriate amount of dog chow in either a PK V-blender or a Univex M-20 Mixer. Control diets without test material were prepared in the same manner.

Samples of approximately 100 g were taken from the top, middle, and bottom of all test diets and analyzed by high pressure liquid chromatography for test article concentration at weeks 1, 2, 3 and 4. Mean results for verification of chlorpropham levels in the diets at

dose levels of 175-, 181-, 197-, 200-, 1094-, 1450-, 1700-, 1950-, 2000-, 2416-, 2500-, 1700-, 20000-, 21000-, 22553-, 25000-, and 28769-ppm diets were within ±5% of target dose. The distribution of the test article in the feed appeared to be fairly homogeneous. ~~The purity and stability of the compound were not determined in the study.~~ KB 4/23/93

3. Animals

Eight purebred beagle dogs (4 males/4 females) were received from Harlan Industries, Indianapolis, Indiana. Dogs were acclimated to laboratory conditions for at least one month. The dogs were selected from a pool of unspecified size based upon body weights and the results of physical examinations which included ophthalmological and clinical pathology examinations. Each dog was identified by an ear tattoo and a USDA tattoo number. At the initiation of dosing, the animals were approximately 7 months old and weighed from 11.05 to 13.05 kg (males) and 8.75 to 11.65 kg (females).

Animals were housed individually in metal cages suspended over flush pans in an animal room with a temperature of 64-80°F, relative humidity of 35-50%, filtered air supply (10-15 air changes/hour), and a 12 hour light/dark cycle. Minimum and maximum temperatures were recorded daily, and the humidity was recorded weekly. Clean cages were provided approximately every 2 weeks. Food (Purina dog chow meal) and water (fresh, tested tap water from a deep well) were provided *ad libitum*.

The dogs were randomly assigned to the following test and control groups:

Dose Levels (mg/kg/day)	Number of Animals	
	Males	Females
0 (control)	1	1
5 (low)	1	1
50 (mid)	1	1
500 (high)	1	1

4. General Observations

(a) Mortality/moribundity/survival

In addition to daily observations made for several hours postdosing, animals were observed twice daily (AM and PM) for mortality/moribundity.

No animals died during the study.

(b) Clinical observations

Dogs were inspected twice daily (AM and PM) for signs of toxicity. Animals were also observed for several hours postdosing throughout the 4-week period for general signs of toxicity.

No adverse clinical signs were observed during the study. High-dose animals of both sexes did not produce any stools for extended periods, but this is most likely due to their low food intake.

(c) Body weights, food consumption, and test article intake

Body weights--Individual body weights were measured during the pretest period, the day before the first exposure, weekly during the test period, and immediately prior to necropsy.

Table 1 summarizes body weight data. Slight decreases in body weights were noted in the control male, low-dose male and female and the high-dose male and female during week 1. Following week 1, the control male and the low-dose dogs gained weight. Overall body weight gains at week 4 were increased in the control, low-, and mid-dose animals. The high-dose male and female exhibited a slight weight gain at week 2; however, overall body weight gains at week 4 were decreased in these dogs. The overall body weight gain in the high-dose male at week 4 was -0.10 kg. The overall body weight gain in the high-dose female was -0.80 kg. The decreases in body weight gains in the high-dose dogs may be possibly attributable to the poor food intake in these dogs especially during weeks 1 and 4.

Food consumption-- Individual food consumptions were measured weekly.

Table 2 summarizes food consumption data. There were no effects upon food consumption in the low- and mid-dose animals. There appeared to be a palatability problem with the test diet at the high-dose level because high-dose animals consumed almost no food during the first week. Food consumption increased to near expected levels during the second and third weeks despite only slight reductions in the concentrations of chlorpropham in the diets. However, consumption in the high-dose dogs declined sharply again during the fourth week of exposure.

Compound consumption--Compound consumption was determined weekly.

Compound intake (mg/kg/day) was within $\pm 30\%$ of target doses in low- and mid-dose animals throughout the exposure period. Compound intake in the high-dose males was 14%, 131%, 89%, and 72% of nominal levels at weeks 1, 2, 3, and 4, respectively. Compound intake in the high-dose females was 10%, 94%, 72%, and 31% of nominal levels at weeks 1, 2, 3, and 4, respectively.

TABLE 1. Mean Body Weights (kg) in Dogs
Fed Chlorpropham for 4 Weeks^{a,b}

Weeks	Dose Level (mg/kg/day)			
	0	5	50	500
<u>Males</u>				
0	12.00	11.75	11.05	13.05
1	11.30	11.35	11.15	11.30
2	11.65	11.70	11.75	12.10
3	12.05	12.20	12.15	12.25
4	12.65	12.40	13.00	12.95
<u>Females</u>				
0	10.85	11.65	9.30	8.75
1	10.95	11.25	9.85	7.65
2	10.85	11.55	9.85	8.15
3	11.20	11.60	9.55	8.10
4	11.70	12.15	10.55	7.95

^aData extracted from Table 4 of the study report.

^bData based on 1 dog/sex/group.

TABLE 2. Mean Food Consumption (g/kg) in Dogs Fed Chlorpropham for 4 Weeks^{a,b}

Weeks	Dose Level (mg/kg/day)			
	0	5	50	500
<u>Males</u>				
1	22.30	30.25	36.85	2.41
2	31.31	27.35	38.66	32.76
3	26.86	27.97	32.51	26.15
4	21.23	20.00	25.57	17.98
<u>Females</u>				
1	27.89	29.41	26.12	2.18
2	23.00	29.42	23.61	23.56
3	28.89	21.83	17.95	17.16
4	21.00	18.68	22.22	6.16

^aData extracted from Table 6 of the study report.

^bData based on 1 dog/sex/group.

Compound consumption in the high-dose animals paralleled food consumption.

(d) Ophthalmoscopic examination

Ophthalmoscopic examinations were performed on all animals during the pretest period and at study termination.

There were no treatment-related ophthalmological effects observed.

5. Clinical Pathology

Blood samples were collected from the jugular vein of fasted dogs at pretest and during week four and analyzed for those hematology and clinical chemistry parameters which are indicated by an "X".

(a) Hematology

X Hematocrit (HCT)	X Leukocyte differential count
X Hemoglobin (HGB)	X Mean corpuscular HGB (MCH)
X Leukocyte count (WBC)	X Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC)	X Mean corpuscular volume (MCV)
X Platelet count	
X Cellular morphology	

The study author did not consider any of the changes in hematology parameters to be treatment-related. The reviewers noted a decrease in hemoglobin and hematocrit levels and red blood cell count in the high-dose male and high-dose female. However, no statistical analysis of the data can be performed because there was only one dog/sex in each dose group.

(b) Blood (clinical) chemistry

Electrolytes

X Calcium
X Chloride
X Phosphorus
X Potassium
X Sodium

Other

X Albumin
X Albumin/globulin ratio
X Blood creatinine
X Blood urea nitrogen
X Globulins
X Glucose
X Total bilirubin
X Total protein
X Cholesterol

Enzymes

X Alkaline phosphatase (ALP)
X Serum alanine aminotransferase (SGPT)
X Serum aspartate aminotransferase (SGOT)
X Creatinine phosphokinase
X Cholinesterase

The study author did not consider any of the changes in clinical chemistry parameters to be treatment-related. The reviewers note an increasing trend in cholesterol levels of both males and

females, but no statistical analysis of these data can be performed. Cholesterol levels in the high-dose dogs were nearly double the control values.

(c) Urinalysis

Urine samples were collected overnight during pretest and prior to termination following hydration by oral gavage with 20 mL water per kg of body weight. Urine samples were analyzed for those parameters indicated by an "X".

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

No treatment-related urinalysis effects were observed.

6. Sacrifice and Pathology

After 4 weeks of dosing, all animals were fasted overnight, euthanized by injection of sodium pentobarbital, exsanguinated via the carotid arteries, and necropsied. Organs and tissues were preserved in a solution of 10% phosphate buffered formalin, and those tissues and organs which are indicated by an "X" were sectioned, stained, and examined microscopically. Those organs indicated by "XX" were weighed also.

<u>Digestive System</u>	<u>Cardiovascular/Hematologic</u>	<u>Neurologic</u>
Salivary glands	Aorta	XX Brain
Esophagus	Heart	Peripheralnerve
Stomach	Bone marrow	(sciatic nerve)
Duodenum	Lymph nodes	Pituitary
Jejunum	XX Spleen	
Ileum		
Cecum		
Colon	<u>Urogenital</u>	<u>Glandular</u>
Rectum	XX Kidneys	Adrenals
XX Liver	Urinary bladder	X Thyroids
Gallbladder	XX Testes	
Pancreas	XX Epididymides	
	XX Ovaries	
	Uterus	
<u>Respiratory</u>		
Trachea		
Lung		
<u>Other</u>		
Bone (sternum and femur)		
X All gross lesions and masses		

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(a) Macroscopic

There were no apparent treatment-related gross findings.

(b) Organ weights and body weight ratios

There was an approximately 50% reduction in absolute and relative spleen weights in both the mid- and high-dose males and females when compared to controls. The author suggested that the decrease in spleen weights in these dogs might be a result of inanition and weight loss. However, the mid-dose animals did not display a decrease in body weight or food consumption, so it is possible that the decreases in spleen weights are treatment-related. Also, the decreases in the spleen weights in the high-dose dogs correlated to histopathological findings in this organ.

(c) Microscopic

Possible treatment-related histopathological findings were noted in the thyroids of the mid- and high-dose dogs. These findings were characterized by irregularly shaped follicles lined by medium to high cuboidal epithelium containing clear to pale staining colloid. Also, the severity of the thyroid findings was classified by the study report as moderate in the mid-dose male, and marked in the mid- and high-dose females and in the high-dose male. Histological findings in the thyroids of control and low-dose animals were classified as slight, i.e., characterized by the typical round follicles lined by low to medium cuboidal epithelium and containing relatively dense eosinophilic staining colloid. Slight splenic lymphoid atrophy with a decrease in the size and activity of the germinal centers was noted in the high-dose male and high-dose female.

B. REVIEWERS' DISCUSSION

The study protocol was, for the most part, acceptable for a 28-day repeated oral dosing study in dogs. ~~However, the purity and stability of the test material in the diet were not examined.~~ Also, detailed descriptions of gross and microscopic findings (presented in Appendix III of the Study report), as well as hematology and clinical chemistry findings, were limited to only a few animals.

15B
4/23/98

Unpalatability of the high-dose test diets appears to have been a problem in this study. Food intake of the high-dose dogs was negligible when compared with the control, low- and mid-dose dogs during the first week of treatment. Food intake of the high-dose dogs increased to near expected levels during the second and third weeks, but declined sharply during the fourth week of treatment. The body weight decrement noted in the high-dose male and female may have been due to the unpalatability of the test diet and the consequent decreased food intake.

The results of this study suggest that the thyroid may be a possible target organ. Histopathological findings in the thyroid were characterized by irregularly shaped follicles lined by medium to high cuboidal epithelium containing clear to pale staining colloid. The thyroid findings were graded as moderate in the mid-dose male, and marked in the mid- and high-dose females and in the high-dose male. However, thyroid function tests were not performed to determine if thyroid activity was indeed altered in these animals. Histologically the spleens from the high-dose dogs had slight lymphoid atrophy with a decrease in the size and activity of the germinal centers. Absolute and relative spleen weights were decreased in the mid- and high-dose males and females. Slight splenic lymphoid atrophy was noted in the high-dose male and female.

There were no significant effects on mortality, clinical signs, hematology, clinical chemistry, or ophthalmology.

Based on histopathological changes in the thyroid, the LOEL in males and females is 50 mg/kg/day. The NOEL is 5 mg/kg/day. This study provides supplementary information for setting dietary exposure levels to be used in a subchronic dog study.