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WASHINGTON, D.C. 20460

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MAY 21 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Chloropicrin (CP): Supplementary information for: One year dog feeding (capsule) study. (MRID 43196301), previous BC# D202210).

BC#222580
Chemical ID #081501
Chemical No. 214

TO: Larry Schnaubelt/Susan Jennings (PM 72)
Reregistration Review Branch
Registration Division (7508W)

FROM: Stanley B. Gross, PhD, DABT, CIH
Toxicologist/Hygienist
Toxicology Branch I
Health Effects Division (7509C)

Stanley B Gross 3/13/96

THRU: Joycelyn E. Stewart, PhD
Head, Section II, Toxicology Branch I
Health Effects Division (7509C)

JES 5/17/96

I. SUBMISSION/REQUEST.

In response to Toxicology Branch I DER (HED no. 011563, 8/25/94), the registrant has submitted additional data on capsule retention for the previously reviewed study:

Evaluation of chloropicrin in a one year oral (capsule) toxicity study in dogs. John A. Wisler, PhD, DABT, Study director. International Research and Development Corporation (IRDC), Study No. 656-005, April 1, 1994. Performed for The Chloropicrin Manufacturer's Task Force, Northbrook, IL. (MRID 43196301).

The study is now ACCEPTABLE; the revised DER for this study is attached.

REVISED EXECUTIVE SUMMARY:

Chloropicrin dissolved in corn oil was administered orally in gelatin capsules to beagle dogs (4/sex/group) in dosage groups of 0.1, 1.0 and 5.0 mg/kg. daily for one year. A control group

received the corn oil vehicle in capsules over the same period of time.

There were no mortalities, no abnormal changes in organ weights, gross observations or histopathology. Dogs in all groups experienced emesis and diarrhea throughout the dosing period. These findings increased with levels of chloropicrin administration. All dogs were reported to have injected sclera during the treatment period; however, ophthalmological examination and histopathological examination were not remarkable. Body weights differed among the experimental groups but none were related to their treatments with CP.

Conclusion. The NOEL is considered to be 1.0 mg/kg/day. The LOEL is considered to be 5.0 mg/kg/day based on increased vomiting and diarrhea and hematological and blood chemistry alterations.

Classification: The study is now ACCEPTABLE. The study was previously labeled supplementary (HED no. 011563) because of the concerns about verifying dosing. The increase in vomiting and diarrhea in all treatment groups compared to the control animals raises question concerning the retention of the dosing with chloropicrin. The registrant has since submitted information which indicated that all of the animals in the low and mid dose groups retained their capsules and the dogs in the high dose group retained 94-100% (one female lost 16%) of the capsules administered.

cp222580.m E2 May 9, 1996

Primary reviewer: Stanley B. Gross, PhD, DABT, CIH. 3/13/96
Secondary Reviewer: Joycelyn E. Stewart, PhD. 5/17/96
Section II, Toxicology Branch I (H7509C).

REVISED
DATA EVALUATION REPORT
(HED No. 011568)

STUDY TYPE: One year capsule (oral) dog toxicity study.

TOX. CHEM. No.: 214

MRID No.: 431963-01

TEST MATERIAL: Chloropicrin

SPONSOR: The Chloropicrin Manufacturer's Task Force, Northbrook, IL.

TESTING FACILITY: International Research and Development Corporation (IRDC)

STUDY NO.: 656-005

REPORT TITLE: Evaluation of chloropicrin in a one year oral (capsule) toxicity study in dogs.

AUTHOR(S): John A. Wisler, PhD, DABT, Study director.

REPORT ISSUED: April 1, 1994.

Quality Assurance statement was included: signed by Margery J. Wirth, dated 4/1/94.

EXECUTIVE SUMMARY.

Chloropicrin dissolved in corn oil was administered orally in gelatin capsules to beagle dogs (4/sex/group) in dosage groups of 0.1, 1.0 and 5.0 mg/kg. daily for one year. A control group received the corn oil vehicle in capsules over the same period of time.

There were a number of laboratory findings which when compared to controls were statistically altered in the high dose groups. These included reduction in hemoglobin and hematocrit levels, blood proteins (total protein, albumin and globulin) and calcium, two isoenzymes (aspartate aminotransaminase and alanine aminotransaminase). Total protein, albumin and globulin were also reduced in the mid-dose group. These clinical laboratory changes were not relatable to pathological changes.

There were no mortalities, no abnormal changes in organ weights, gross observations or histopathology. Dogs in all

groups experienced emesis and diarrhea throughout the dosing period. These findings increased with levels of chloropicrin administration. All dogs were reported to have injected sclera during the treatment period; however, ophthalmological examination and histopathological examination were not remarkable. Body weights differed among the experimental groups but none were dose related to their treatments with CP.

Conclusions. The NOEL is considered to be 0.1 mg/kg/day. The LOEL is considered to be 1.0 mg/kg/day based on gastrointestinal irritation which involved increased vomiting and diarrhea. Systemic LOEL was 5.0 mg/kg/day based on hematological and blood chemistry alterations.

Classification: The study is now ACCEPTABLE. The study was previously labled supplementary (HED no. 011563) because of the concerns about verifying dosing. The increase in vomiting and diarrhea in all treatment groups compared to the control animals raises question concerning the retention of the dosing with chloropicrin. The registrant has since submitted information which indicated that all of the animals in the low and mid dose groups retained their capsules and the dogs in the high dose group retained 94-100% of the capsules administered.

I. STUDY DESIGN:

A. Treatment Regime. Beagle dogs (4 animals/sex/dosage group) were given gelatin capsules containing CP in corn oil at dosages of 0, 0.1, 1.0 and 5.0 mg CP/kg . The animals were monitored for toxicity, clinical chemistry, hematology, urinalyses, body weight, food consumption, ophthalmological examination and gross and histological changes.

B. Dosage Selection. Dosage selection was determined from past (unidentified) data.

II. MATERIALS

A. Test compound: Chloropicrin (99% a.i.) obtained from Niklor Chemical Co., , Long Beach, CA. Lot Nos. 920130-1. Identified as a "liquid".

Vehicle: Corn oil in gelatin capsules obtained from Hunt Wesson, Fullerton, California and Bio Serv, French Town, NJ (Corn oil) and Jorgensen Laboratories, Loveland, CO (Gelatin Capsules).

B. Test animals: Male and female beagles approximately 4 months of age were obtained from Marshall Farms, North Rose, NY. Weight: males 10.7 to 14.8 kg; females 7.9 to 12.0 kg.

4

III. METHODS.

A. Preparation of CP Capsules. CP was dissolved in the corn oil in amounts to achieve dosing concentrations within a dosing volume of corn oil of 0.1 ml per kg body weight. The CP in the oil was sealed and then refrigerated until used for dosing. Capsule preparations were done on a weekly basis.

Variability and stability confirmation of the CP in the oil was determined after 14 days of refrigerated storage. Concentrations of CP administration to the dogs were verified weekly during weeks 1 through 4 and every 4 weeks thereafter to study termination. Analytical concentrations of CP were determined using gas chromatography.

B. Animal Handling. Animals were acclimated for approximately 3 weeks under standard laboratory conditions prior to the start of the study. The animals were immunized against distemper, parvovirus, parainfluenza, adenovirus type 2, Bordetella leptospirosis and rabies by breeder. Flotation tests were conducted on stool samples obtained from each dog. Clinical pathology, ophthalmoscopic and complete physical examinations were conducted on each dog before being placed on study.

Housing. The dogs were individually housed in stainless steel cages under standard laboratory environmental conditions and were allowed to exercise for 30 minutes, three times a week.

Diets. The dogs were allowed food and water ad libitum. The basal diet was Certified Canine Chow #5007, (Purina Mills).

Clinical Observations Clinical observations were made twice daily. Body weights were obtained prior to study, weekly from week 1 to 14 and every 4 weeks thereafter until the end of the study. Food consumption was measured weekly during the study. Each animal was given a complete physical examination each week consisting of the determination of the general condition, heart and lung sounds and the examination of the head, neck, thorax, abdomen, external reproductive organs, skin and extremities.

C. Clinical Laboratory Analyses. Hematology, blood chemistries, urinalysis and ophthalmological examinations were performed on each dog prior to the start of the dosing with CP, and at 3, 6, 9 and 12 months during the study. Blood samples were obtained from the jugular vein following an overnight fasting period during which food and water were removed.

1) Hematology. The CHECKED (X) parameters were examined.

X Hematocrit (HCT)

X Hemoglobin (HGB)	X Leukocyte differential count
X Leukocyte count (WBC)	X Mean corpuscular HGB (MCH)
X Erythrocyte count (RBC)	X Mean corpuscular HGB conc.(MCHC)
X Platelet count	X Mean corpuscular volume (MCV)

2) Urinalysis - Urine sample were obtained by placing each animal in a metabolic collection cage and collecting urine overnight while the animals fasted. The following urinary analyses were determined:

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	X	Urobilinogen

* Includes crystals (triple phosphate, uric acid, calcium oxalate, amorphous, and other) and cellular formed elements including squamous and renal epithelial cells, leukocytes, RBC, sperm, yeast and bacteria and other.

3) Clinical Chemistry. The following CHECKED (X) analyses were carried out using jugular blood obtained at 3, 6, 9 and 12 months:

Electrolytes:

X Calcium
X Chloride
X Magnesium
X Phosphorus
X Potassium
X Sodium

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

Other:

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

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

D. Ophthalmological examinations. All animals received an indirect ophthalmoscopic examination of the cornea, conjunctiva, sclera, iris and fundus during the acclimation period and at months, 3, 6, 9 and 12.

E. Sacrifice and Pathology - At the end of the 12 month dosing period, all surviving animals were anesthetized with

sodium pentobarbital IV and exsanguinated. Terminal sacrifice involved the gross and histological examination of the following organs: -

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

The above organs marked with W (brain, liver, kidneys and ovary and testes) were weighed and compared to body weight changes.

F. Statistical Methods. Body weights, food consumption, clinical chemistries and organ weight data were analyzed for homogeneity of various using Bartlett's test, ANOVA and appropriate t-tests (Steel and Torrie). Dunnett's multiple comparison tables or pair-wise comparisons with Bonferroni corrections were used to determine the significant differences. Nonparametric analyses were conducted as appropriate by transforming the data into ranks prior to analysis as described by Conover and Iman. All statistical analyses were performed with $p < 0.05$ and $p<0.01$ levels of significance.

IV. RESULTS:

A. Analytical Concentrations. Replicate analyses of CP in corn oil for three target concentrations (1.0, 10 and 50 mg/ml) used to make final concentrations diluted with corn oil were exactly the same. These same solutions after 14 days storage under refrigeration were variable and somewhat reduced ranging from 95 to 97% of the previous concentrations. The concentration of the weekly corn oil solutions used during the study averaged 98 (89 to 109)%, 102 (90 to 114)% and 105 (96 to 114)% for the 0.1, 1.0 and 5.0 mg/kg/day dosage groups, respectively.

B. Clinical Observations. None of the animals died during the study.

Emesis and Diarrhea. Male and females in all treatment groups of animals showed signs of emesis (described using variously as emesis, food-like emesis, frothy emesis, etc.) and diarrhea (variously described as diarrhea, mucoid diarrhea, soft stool- mild to severe, etc.). The frequency of diarrhea and emesis is tabulated in Table 1 and shows increases over controls in a dose-response fashion. This table was compiled from the individual animal data in the study report.

TABLE 1. INCIDENCE OF EMESIS AND DIARRHEA**
(Frequency observed during the study)

DOSAGE GROUP					
----------MALES----------			----------FEMALES ----------		
Animal No.	Emesis	Diarrhea	Animal No.	Emesis	Diarrhea
			CONTROLS		
3199	1	5	3216	3	4
3201	7	4	3219	3	4
3205	4	4	3228	9	30
3213	2	2	3234	0	3
			0.1 MG/KG/DAY		
3196	10	6	3221	0	11
3197	7	2	3229	3	7
3200	11	2	3231	0	10
3215	1	4	3232	4	18
			1.0 MG/KG/DAY		
3203	6	14	3218	7	7
3210	2	0	3222	12	7
3211	25	6	3223	8	8
3214	20	28	3225	10	0
			5.0 MG/KG/DAY		
3198	79	16	3220	62	34
3206	82	12	3224	75	18
3209	46	10	3233	54	15
3212	84	14	3235	54	13

** Various types of emesis and diarrhea are combined in the table. Emesis includes designations of emesis, food-like emesis, frothy emesis, etc. "Diarrhea" listed here includes all the listings of "diarrheas" plus soft stool- moderate, severe; mucoid, moderate, severe. Not included were soft stools "slight" and soft stools- "slight to moderate".

Injected sclera were seen in all dogs; however,

ophthalmological and histopathological examinations were not remarkable. None of these signs or other signs (lacerations, skin problems or salivation) were relatable to the administration of CP.

C. Body Weight. Body weight changes were variable between the treatment groups but not relatable to the dosage of CP. Selected body weight data are shown in Table 2. Plots of body weight data presented in the report indicate there were consistent trends: males of the high dose group were consistently lower than the other groups; however, the controls were next lowest, and mid-dose group with the highest body weight curve. None of the weight gains were statistically significant.

TABLE 2. SELECTED BODY WEIGHT DATA (Kg)**.

	----Dosage Group (mg/kg/day)--------			
	Controls	0.1	1.0	5.0
	----------MALES----------			
Initial	12.8	13.1	12.6	12.6
Final	16.6	18.1	17.8	15.0
	----------FEMALES----------			
Initial	9.2	9.5	9.8	9.2
Final	11.6	13.5	14.2	11.6

** None of these results were statistically significant.

D. Food consumption. Selected data are shown in TABLE 3. Food consumption dropped with increasing age on the study and the results were highly variable. There were no trends between groups that were relatable to the administration of CP.

TABLE 3. SELECTED FOOD CONSUMPTION DATA (Gm/Kg/Day)**.

	----Dosage Group (mg/kg/day)--------			
	Controls	0.1	1.0	5.0
	----------MALES----------			
Initial	28.8	29.2	29.9	30.0
Final	18.2	20.5	23.4	19.2
	----------FEMALES----------			
Initial	33.4	34.5	38.8	39.2
Final	30.5	26.0	23.5	24.1

** None of the results were statistically different.

E. Hematology. There were several hematological parameters in the high dose group that were statistically elevated or decreased. These are summarized in TABLE 4. Mean corpuscular

hemoglobin and volumes were decreased in high dose group males and females over controls and other treatment groups. These changes were related to decreased Hb and Ht in both sex groups in the high dose group. Platelets were substantially increased in the high dose males (statistically) and females (not statistically) over the controls and low dose and mid dose treatment groups.

TABLE 4. SELECTED TERMINAL HEMATOLOGY DATA *

	----Dosage Group (mg/kg/day)--------			
	Controls	0.1	1.0	5.0
	----------MALES----------			
RBC's	7.50	7.19	7.73	8.04
Hb	17.3	17.1	17.6	16.2
Ht	49.8	49.7	51.1	47.8
MCV	66.6	69.1	66.0	59.5**
MCH	23.1	23.8	22.8	20.1***
Platelets	251	280	274	345**
	----------FEMALES----------			
RBC's	7.22	7.97	7.74	7.49
Hb	17.2	17.1	17.4	15.6
Ht	48.7	52.0	51.1	45.1
MCV	67.5	65.3	66.0	60.3***
MCH	23.0	22.4	22.8	20.9 ***
Platelets	288	258	330	372

* **Units:** RBC's (mill./cmm); Hb (g/dl); Ht (percent); MCV (microns); MCH (picograms); Platelets (thous./cmm).
** Significant (p,0.05)
*** Highly significant (p<0.01)

F. Clinical Chemistry. Several blood chemical parameters for terminal analyses are shown in TABLE 5. There were a number of laboratory findings which were statistically altered in the high dose groups but were not relatable to pathological changes: These included reduction in blood proteins (total protein, albumin and globulin) and calcium, two isoenzymes (aspartate aminotransaminase and alanine aminotransaminase). Total protein, albumin and globulin were also reduced in the mid-dose group. All of these statistically altered values fall with in the normal range for dogs and are not biologically significant. Although these values were statistically different from the controls at study termination of the study, many of them were oot altered over the time course of the study, adding to the interpretaion that they were biologically insignificant.

G. Urinalysis Urinary volumes varied considerably, ranging from below 100 ml to 300 ml. Specific gravity varied from 1.015 to greater than 1.50. pH varied from 6.0 to greater than 9.0. Protein in the urine varied from 10 to 300 ug/dl. Urobilinogen varied from 0.2 to 1.0 units/dL. Leukocytes were seen in the urine of many dogs

at 5 cell/HPF, glucose, ketone bodies, was generally absent. Of the formed elements, amorphous sediment was usually present and casts, uric-acid and calcium oxalate were absent. None of the above variations in urinary findings were treatment related.

H. Ophthalmological examinations. There were no agent related findings.

TABLE 5. SELECTED TERMINAL CLINICAL CHEMISTRY DATA *

	----Dosage Group (mg/kg/day)--------			
	Controls	0.1	1.0	5.0
	----------MALES----------			
Calcium	10.9	11.1	10.9	10.0**
Aspat. AT	29	26	23	11***
Alan. AT	30	27	28	15**
Tot. Prot.	7.3	7.4	6.4**	5.4***
Album.	3.5	3.5	3.3	2.6***
Globulin	3.8	3.9	3.2	2.8***
	----------FEMALES----------			
Calcium	10.8	10.8	10.6	9.9**
Aspat. AT	27	20	19	12
Alan. AT	24	32	25	14
Tot. Prot.	6.8	6.7	6.2	5.6**
Album.	3.4	3.4	3.3	2.6***
Globulin	3.4	3.4	2.9	3.0

* **Units:** Calcium (mg/dL); Aspartic aminotransaminase and Alanine aminotransaminase (IU/L); protein (gm/dL).
** Significant at $p<0.05$.
*** Highly significant $p<0.01$.

I. Gross Pathology Various incidental findings were seen in individual animals in all groups. Intestinal red patches were seen throughout different sections of the gastrointestinal tract in various animals in all experimental groups. None of the findings were related to the administration of the CP.

J. Organ weights None of the organ weight data revealed any significant differences between the control animals and the treated dogs.

K. Microscopic pathology The intestinal red patches were often seen histologically as congestion but were not relatable to the administration of CP. Two other incidental findings included trace tubular mineralization of the kidney, splenic hemorrhage (2-3/group), and occasional interstitial pneumonia (1-2 in each test group)s. There was no liver or adrenal pathology.

V. DISCUSSION.

A. Retention and Absorption of CP. Chloropicrin is a very irritating substance and it would be expected that it would cause vomiting and diarrhea. It induced vomiting when used as a war gas as discussed in the 1981 Registration Standard. Because of the ability to induce vomiting there is a question relating to the retention and absorption of CP that was swallowed in the capsule. The report did not indicate when emesis occurred relative to the dosing the animals with CP; however, the emesis was noted to be food-like and often frothy. Since this DER was sent to the registrant, the registrant submitted data in a 1/24/96 letter from John H. Butala in which all animals in the control, low and mid dose groups retained the administered capsule and the high dose group animals vomited the capsules 5, 6, 0 and 4% of the dose days for animal nos. 3198, 3206, 3209 and 3212 respectively.

B. NOEL/LOEL. The study investigators chose the NOEL = 1.0 mg/kg and LOEL = 5 mg/kg. Weekly vomiting in dogs is not unusual according to Toxicology Branch veterinarians (Drs. Melba Morrow and Guruva Reddy). Diarrhea and emesis was inconsistently increased in the low-dose and mid-dose test groups and obviously increased in the high-dose test group. Therefore a system LOEL = 5.0 mg/kg/day is set at the high-dose secondary to the hematological and clinical chemistry alterations and diarrhea and emesis and the NOEL = 1.0.