[83-1b. Pirimicarb tech.: Chronic feeding - dog/1971 - revised 1995]

9/20/1996

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SUPPLEMENTAL DATA EVALUATION REPORT
(HED DOC.#s: 001725, 001326 & 001323)

STUDY TYPE: 2-Year Feeding Study - Dog

OPPTS NUMBER: 870.4100 **GUIDELINE NUMBER:** 83-1(b)

DP BARCODE: D215390 **SUBMISSION CODE:** None

P. C. NO: 106101 **TOX. CHEM NO:** 359C

MRID NO.: 43641002 (previously 00113441)

TEST MATERIAL: PP062; Pirimicarb

SYNONYMS: 2-(Dimethylamino)-5,6-dimethyl-4-pyrimidinyl dimethylcarbamate

REPORT NUMBER: CTL/R/337 (previously HO/IH/R/337; Study No.: PD0108)

SPONSOR: Imperial Chemical Industries

TESTING FACILITY: Zeneca Central Toxicology Lab.*
Alderley Park, Macclesfield,
Cheshire, UK

*Previously known as
Imperial Chemical Industries
Industrial Hygiene Res. Labs.,

TITLE OF REPORT: Pirimicarb (PP062): 2-Year Feeding Study in Beagle Dogs

AUTHOR: M C E Hodge

STUDY COMPLETED: December 1971; reanalyzed May 4, 1995

EXECUTIVE SUMMARY: In this chronic study (MRID#43641002), 4 beagles/sex/dose, received either 0, 0.4, 1.8 or 4 mg/kg/day PP062 tech. (94% a.i.) in their diet for two (2) years.

At 4 mg/kg/day there were noted effects on bone marrow cytology as indicated by decrease in the Myeloid:Erythroid (M:E) ratios of 64% in females and 32% males. This change resulted mainly from increases in late normoblasts ($P < 0.01$, 65%) and proerythroblasts ($P < 0.01$, 3.5 fold) and the total erythroid mass (41%) in females with two of the four females contributing most of the change. Nonsignificant increases were noted for late normoblasts (29%) and proerythroblasts (12%) and total erythroid mass in males. Total myeloids decreased in females (49%) and males (19%). Metamyelocytes (89%, $P< 0.01$ in females and 63%, $P < 0.05$ in males) were most significantly reduced and the associated bands (56%, $P < 0.05$ in females and 39%, not significant in males) were also reduced. Myeloblasts (4 fold in females and 3 fold in males) were increased. Promyelocyte-neutrophils in males ($P < 0.05$, 3 fold) were increased. Metamyelocytes-neutrophils (60%, $P < 0.05$) and metamyelocytes-eosinophils (68%, $P < 0.05$) in females were decreased. **The systemic toxicity LOEL is 4.0 mg/kg/day, based on bone marrow cytology. The NOEL is 1.8 mg/kg/day.**

The study was done prior to implementation of GLP Guidelines, therefore, does not fall under purview of either GLP or Quality Assurance requirements. The study is classified as **Acceptable** and **satisfy** the regulatory requirements (83-1b) for a chronic toxicity study in dogs.

A. **MATERIALS:**

1. **Test compound:** PP062. Description - a colorless odorless crystalline solid, Batch # - not given, Purity is not given but based on 90-day dog studies purity of PP062 was established as 94%.

2. **Test animals:** Species: canines, Strain: Beagles (inbred), Age: not given; probably fully mature considering initial mean body weights of ≈ 11 kg for males and 9.6 kg for females, Source: Alderly Park, Cheshire, England.

B. **STUDY DESIGN:**

1. **Animal assignment**

   Thirty-two male and female Beagle dogs, prescreened for clinical and hematological abnormalities and protected against distemper and dewormed against nematodes, were assigned to the following test groups in pairs over a period of four (4) weeks:

TABLE 1. ANIMAL ASSIGNMENT

TEST GROUP	DOSE (MG/KG)	NO. OF ANIMALS	
		♂	♀
I (Cont)	0	4	4
II (LTD)	0.4	4	4
III (MTD)	1.8	4	4
IV (HTD)	4.0	4	4

Whether randomization procedure was used to assign animals to different cells was not described, however, the starting group mean weights of males (10.4 to 10.9 kg) and females (9.3 to 9.6 kg) suggest that an adjustment for weights was made.

2. **Diet preparation**

The compound was diluted in corn oil (Dispersol OG) and ball milled for 24 hours. The animals were dosed (1 ml/kg) by adding an appropriate amount of suspension to daily canine diet. The frequency of suspension preparation was not specified.

3. Animals received 45 g meat preparation ("Kennomeat", Scottish Animal Products Ltd.), 226 g dry pelleted diet ("Kennel Kernals", James & Co., Hungerford) and a 56 g dog biscuit at 12 noon daily and a 56 g dog biscuit in the afternoon. Water was provided ad libitum.

4. **Statistics** - The data were analyzed by ANOVA and covariance using the GLM procedure in SAS (1989). Least-square means for each group were calculated using the LSMEAN option in SAS PROC GLM. Unbiased estimates of differences from control were provided by the difference between each treatment group least-square mean and control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.

Bodyweights, hematology, clinical chemistry and erythrocyte and plasma cholinesterases were considered by analysis of covariance on initial pre-treatment values. Urinalysis and serum folate and iron levels were considered by analysis of variance. Organ weights were considered by analysis of variance and analysis of covariance on final bodyweight.

5. The study was done in 1971 i.e., long before the implementation of GLP Guidelines; therefore, do not fall

under purview of either GLP or Quality Assurance requirements.

C. **METHODS AND RESULTS:**

1. **Observations:** Animals were observed daily for clinical signs of toxicity.

   No treatment-related clinical abnormalities were reported. All animals survived the experimental period.

2. **Body weight**

   All animals were weighed pre-treatment, and at weekly intervals for 12 weeks and then monthly for the remainder of the study period.

   Results - There were no treatment-related effects on bodyweight or bodyweight gains in males and females during the study. The overall mean bodyweight gain (kg) in males/females in the control, 0.4, 1.8 and 4.0 mg/kg/day was 2.5/2.9, 3.4/3.6, 3.0/3.0 and 2.6/4.2, respectively.

3. **Food consumption and compound intake**

   Food Consumption per se was not determined but assumed that food offered was consumed and as well as the compound in the food.

4. **Ophthalmological examination**

   Ophthalmological examination was not performed.

5. **Blood was collected** before treatment and at every 3 months for hematology from all animals. The CHECKED (X) parameters were examined. Serum iron and folate levels were determined at termination. Bone marrow was biopsied from iliac crest pre-treatment, and at 6, 12, 16, 20 and 24 months.

a. **Hematology**

X		X	
X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc. (MCHC)
	Erythrocyte count (RBC)		Mean corpusc. volume (MCV)
X	Platelet count		Reticulocyte count
	Blood clotting measurements	X	Mean cell diameter (MCD)
	(Thromboplastin time)	X	Erythrocyte sedimentation rate (ESR)
	(Clotting time)		
	(Prothrombin time)		

Results - Table 2 presents some selected, adjusted (the covariate adjustment was based on the separate sex pre-treatment group means) hematology parameters. In high-dose males, <u>% HCT</u> and <u>% Hgb</u> were reduced throughout the study but the differences were significant ($P \leq 0.05$) only at 3 months for HCT (11%) and at 15 months for HGB (16.9%). The decreases showed neither dose- nor time-response. These changes are consistent with suchronic dog studies (MRID # 43641001) but in this case they are weakly suggestive of treatment-effects. In males, the <u>mean cell diameters</u> decreased significantly ($P \leq 0.05$) at 3 and 6 month evaluations, which is inconsistent with the findings of subchronic studies and association with macrocytic anemia. The significance of decreased cell diameters are considered equivocal.

In females, neither HCT nor HGB were affected due to treatment. Mean cell diameter was significantly decreased at 6 months (1.9%) and 24 months (2.4%) in females on 4 mg/kg/day pirimicarb, however, the response showed no time or dose and were considered not to be compound-related.

TABLE 2. SELECTED, ADJUSTED HEMATOLOGY PARAMETERS1

PARAMETERS	MALES DOSE (MG/KG/DAY)				FEMALES DOSE (MG/KG/DAY)			
	0	0.4	1.8	4.0	0	0.4	1.8	4.0
PRE-TEST								
HGB (g/100ml)	13.4	13.2	13.3	13.1	13.5	13.9	13.3	13.3
HCT (%)	40.3	39.6	39.9	39.8	41.5	41.9	40.8	40.1
MCD (µm)	6.08	6.10	6.13	6.09	6.09	6.13	6.09	**6.16***
3 MONTHS								
HGB (g/100ml)	14.9	14.3	14.4	13.2	15.1	15.8	14.8	15.1
HCT (%)	43.9	43.1	43.5	**39.0***	43.7	46.9	45.5	46.0
MCD (µm)	6.31	6.10**	6.09**	6.06**	6.06	6.10	6.06	6.09
6 MONTHS								
HGB (g/100ml)	15.3	14.6	14.3	13.9	14.4	15.2	15.0	15.7
HCT (%)	45.9	45.6	44.0	42.3	44.2	46.0	42.9	47.6
MCD (µm)	6.21	6.05**	6.09*	6.08*	6.17	6.09	6.14	**6.05***
12 MONTHS								
HGB (g/100ml)	16.2	16.2	15.6	15.3	15.2	15.6	16.2	15.6
HCT (%)	48.4	47.8	47.5	45.0	44.5	46.9	46.8	46.7
MCD (µm)	6.23	6.10	6.21	6.10	6.18	6.10	6.16	6.13
18 MONTHS								
HGB (g/100ml)	16.3	16.1	16.5	15.2	16.0	16.1	15.2	16.8
HCT (%)	47.0	45.9	47.5	43.8	44.9	45.8	44.5	49.3
MCD (µm)	6.09	6.10	6.19	6.05	6.16	6.10	6.08	6.12
24 MONTHS								
HGB (g/100ml	15.8	15.5	16.6	14.2	14.5	16.5	15.4	15.6
HCT (%)	47.1	45.9	47.5	42.1	44.1	47.9	44.9	46.1
MCD (µm)	6.12	6.18	6.13	6.07	6.23	6.14	6.13	**6.08***

1 Copied from Report Table 2, P 81 - 97
N = 4, * = $P \leq 0.05$, ** = $P \leq 0.01$

Table 3 presents the **myeloid** (myeloblasts, promyelocytes, promyelocytes - neutrophils and eosinophils, metamyelocytes - neutrophils and eosinophils) and **erythroid** (proerythroblasts, early normoblasts, intermediate normoblasts, late normoblasts and megaloblasts) series. **Other cell** series (monocytes, plasmacytes and lymphocytes) are not in the table because the values were not adjusted and there was no trend indicating a possible effect.

Myeloid series generally decreased in the males and females in the high-dose group. At the 4 mg/kg/day dose the following treatment-related changes in bone-marrow cytology were observed:

-metamyelocytes: decreased significantly in males (62.8%, $P < 0.05$) and females (88.9%, $P < 0.01$).

-bands: decreased significantly in females (56.3%, $P < 0.05$). Decreased metamyelocytes and bands resulted in the decreased M:E ratio.

-Myeloblasts: increased significantly in males (1.6 fold, $P < 0.05$) and females (4 fold, $P < 0.01$).

-promyelocyte-neutrophils: increased significantly in males (3 fold, $P < 0.05$). Increased myeloblasts and promyelocyte-neutrophil an indicate bone-marrow stimulation.

In high-dose females promyelocyte neutrophils generally increased, but lacked dose-response, therefore, not considered to be of biological significance.

Erythroid - total erythroid mass increased in males and females. Following are the treatment-related effects at 4 mg/kg/day dose:

-late normoblasts: increased significantly in females (1.6 times, $P < 0.01$)

-megaloblasts: increased significantly in females (3.5 fold, $P < 0.01$).

In low- and mid-dose males, megaloblasts decreased significantly, which lacked dose response and considered to be of no biological significance.

The M:E ratio also decreased with myeloid series; only in the high-dose males and females the ratios are considered treatment-related. Based on the bone marrow cytology, the NOEL = 1.8 mg/kg/day and the LOEL = 4.0 mg/kg/day.

TABLE 3. TERMINAL BONE MARROW CYTOLOGY1

PARAMETERS	MALES DOSE (MG/KG/DAY)				FEMALES (MG/KG/DAY)			
	0	0.4	1.8	4.0	0	0.4	1.8	4.0
Myeloblasts	0.4	0.4	0.8	**1.3***	0.4	0.6	0.7	**1.7****
Promyelocytes	0.5	0.2	0.8	0.7	0.4	0.4	0.5	0.6
Promyelocytes - neutrophils	2.8	3.4	6.7	**8.3***	3.2	2.8	**9.5***	5.8
Promyelocytes - eosinophils	1.6	2.1	2.2	1.4	2.5	3.3	2.7	1.6
Metamyelocytes	7.8	8.8	5.8	**2.9***	9.0	9.2	5.9	**1.0****
Band Forms	20.8	18.7	15.4	12.7	20.6	18.0	15.1	**9.0***
Metamyelocytes - neutrophils	9.9	8.9	9.6	8.8	8.7	8.2	7.5	**3.5***
Metamyelocytes - eosinophils	1.0	1.4	0.7	0.3	2.5	1.9	1.0	**0.8***
TOTAL MYELOIDS	44.8	43.9	42.0	36.4	47.3	44.4	42.9	24.0
Proerythroblasts	0.9	0.9	1.0	1.2	1.0	0.6	0.9	1.6
Early Normoblast	2.6	3.1	2.8	3.8	3.2	3.4	3.5	2.7
Int. Normoblast	13.1	15.4	18.3	16.9	15.4	13.9	17.3	13.8
Late Normoblast	36.3	36.1	34.2	40.8	32.8	35.6	34.9	**54.1****
Megaloblast	1.7	**0.6***	**0.7****	2.2	0.8	0.9	0.9	**2.8****
TOTAL ERYTHROIDS	54.6	56.1	57.0	64.9	53.2	54.4	57.5	75.0
M:E	0.82:1.0	0.78:1.0	0.74:1.0	0.56:1	0.89:1.0	0.82:1.0	0.75:1.0	0.32:1

Values are adjusted means
1 Data extracted from Report Table 3, P 109 - 142
* = $P \leq 0.05$, ** = $P \leq 0.001$

b. Clinical Chemistry

X	Electrolytes:	X	Other:
	Calcium		Albumin
	Chloride		Blood creatinine
	Magnesium	X	Blood urea nitrogen
	Phosphorous		Cholesterol
X	Potassium		Globulins
X	Sodium	X	Glucose
	Enzymes		Total bilirubin
X	Alkaline phosphatase (ALK)		Total serum Protein (TP)
X	Cholinesterase (ChE)		Triglycerides
	Creatinine phosphokinase		Serum protein electrophores
	Lactic acid dehydrogenase (LAD)		
	Serum alanine aminotransferase (also SGPT)		
	Serum aspartate aminotransferase (also SGOT)		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
X	Serum folate		
X	Vitamin B_{12}		
X	Serum iron		

i) Serum folate and Iron:

Serum folate and iron levels were determined on all animals at termination of the study.

TABLE 4. MEAN SERUM IRON AND FOLATE LEVELS1

PARAMETERS	MALES DOSE (MG/KG/DAY)				FEMALES DOSE (MG/KG/DAY)			
	0	0.4	1.8	4.0	0	0.4	1.8	4.0
Folate (µg/100ml)	6.1 ± 2.8	6.2 ± 2.4	8.4 ± NA	7.8 ± 1.9	8.2 ± 3.3	6.5 ± 2.7	9.2 ± 3.0	5.6 ± 2.2
Iron (µg/100ml)	232.0 ± 56.3	213.0 ± 39.2	192.0 ± 30.1	178.5 ± 26.8	201.0 ± 49.7	234.0 ± 46.1	192.8 ± 21.6	181.0 ± 10.6

1 Copied from Report Table 2, p 107 - 108

Results: Table 4 presents mean serum iron and folate levels at 24 months. Serum folate and iron levels were not affected by treatment with pirimicarb. In high-dose males and females the serum iron levels decreased 23% and 10%, respectively; however, the effects were not reflected in peripheral blood i.e., hemoglobin levels.

ii) Cholinesterase

The plasma ChE, RBC AChE or brain ChE levels were not affected. The mean plasma ChE values ranged from 1.91 to 3.93 μmoles acetic acid/ml plasma/min; and mean RBC values ranged from 1.0 to 2.09. The mean male/female brain ChE levels in 4.0 mg/kg/day group were 11.6 ± 2.7/18.0 ± 2.1 compared to the 16.0 ± 2.6/11.9 ± 2.1 deltapH units/g brain/hr in the male/female controls. Since these determinations were based on one animal per sex, an 27% inhibition in male and of about 51% stimulation in female are not valid or might suggest inadequate methodology.

Bromsulphalein (B.S.P.) retention test. The B.S.P. retention values were not effected in the treated groups. They ranged from 1.6 to 3.5%/15 min.

6. **Urinalysis**

Urinalysis was done pre- and at three month intervals from all animals. **Urine collection procedure or conditions of collection were not described.** The CHECKED (X) parameters were examined.

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

Results - Urinalysis showed no changes attributable to treatment. Urine sp. gr. of treated males and females ranged 1.006 to 1.064 vs 1.006 to 1.058 of controls (normal 1.015 to 1.040). Urine pH of males and females ranged from 5.45 to 8.4 vs 5.9 to 7.9 of controls (normal 5.5 to 7.0).

7. **Sacrifice and Pathology**

Animals were sacrificed in pairs over a four week period using I.V. sodium pentobarbitone. All animals 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
	Esophagus		Bone marrow		Spinal cord (3 levels)
X	Stomach		Lymph nodes	X	Pituitary
X	Duodenum	XX	Spleen		Eyes (optic n.)
X	Jejunum	X	Thymus	Glandular	
X	Ileum	Urogenital		XX	Adrenal gland
	Cecum	XX	Kidneys		Lacrimal gland
X	Colon	X	Urinary bladder		Mammary gland
	Rectum	XX	Testes +		Parathyroids ++
XX	Liver +	XX	Epididymides	XX	Thyroids ++
	Gall bladder		Prostate	Other	
X	Pancreas		Seminal vesicle		Bone
Respiratory		X	Ovaries +	X	Skeletal muscle
	Trachea		Uterus		Skin
XX	Lung				All gross lesions and masses
	Nose				
	Pharynx				
	Larynx				

a. **Organ weight** - The adjusted (by analyses of covariance on final bodyweight, separately for males and females) organ to body wt. ratios were not affected.

b. **Gross pathology** - No effects of treatment were indicated.

c. **Microscopic pathology** - No changes attributable to the pirimicarb were reported.

D. **DISCUSSION:**

This study and 90-Day subchronic study (MRID #43641001) were conducted in 1971 prior to implementation of GLP guidelines. The study deviated from the guideline requirements (83-1b), but captured the main objective of chronic feeding study. These studies in combination provide required information and satisfy the regulatory requirements. Further, this DER reflects the corrected terminology of "megaloblasts" as "proerythroblasts". The sponsor rectified this terminology in the past and the Agency has accepted it (HED DOC. # 001323; August 1, 1980).

In this 2-year chronic study (MRID #43641002), 4 beagles/sex/dose, received either 0, 0.4, 1.8 or 4 mg/kg/day PP062 tech. (94% a.i.) in diet.

At 4 mg/kg/day there were noted effects on bone marrow cytology

as indicated by decrease in the Myeloid:Erythroid (M:E) ratio of 64% in females and 32% in males. This change resulted mainly from increases in late normoblasts ($P < 0.01$, 65%) and proerythroblasts ($P < 0.01$, 3.5 fold) and total erythroid mass (41%) in females with two of the four females contributing most of the change. Nonsignificant increases were noted for late normoblasts (29%) and proerythroblasts (12%) and total erythroid mass (19%) in males. Total myeloids decreased in females (49%) and males (19%). Metamyelocytes (89%, $P < 0.01$ in females and 63%, $P < 0.05$ in males) were most significantly reduced and the associated bands (56%, $P < 0.05$ in females and 39%, not significant in males) were also reduced. Myeloblasts ($P < 0.01$, 4 fold in females and $P < 0.05$, 3 fold in males) were increased. Promyelocytes-neutrophils in males ($P < 0.05$, 3 fold) increased. Metamyelocytes-neutrophils (60%, $P< 0.05$) and metamyelocytes-eosinophil (68%, $P < 0.05$) in females decreased. Increased cellularity of bone-marrow for myeloid series did not reflect in the peripheral blood. The study authors explained that this may be due to delayed maturation akin to "a compound-dependent, hemolytic anemia of the 'penicillin'." The study authors considered bone-marrow changes only in high-dose females were treatment-related. The study authors, however, failed to consider in males, the decreased (32%) M:E ratios and nonsignificant increase in late normoblasts and proerythroblasts and the total erythroid mass as related to treatment. TB-I considers that the magnitude of change in the aforementioned parameters are adequate to be biologically significant in males. **The systemic toxicity LOEL is 4.0 mg/kg/day based on bone marrow cytology. The NOEL is 1.8 mg/kg/day.**

The study is classified as **Acceptable** and **satisfy** the regulatory requirements (82-1b) for a chronic toxicity study in dogs.

Note: Megaloblasts have been changed to proerythroblats to correct for classification errors cited in HED Doc. #001323 dated August 1, 1980.

GREDDY\D215390\Pirimor/10/17/95
Final: 08/07/96

[83-1b. Pirimicarb tech.: Chronic feeding - dog/1971 - revised 1995]

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