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[Non-Guideline. Pirimicarb Tech.: 110-Week Feeding Study - Dogs/1973]

9/20/1996

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SUPPLEMENTAL DATA EVALUATION REPORT
(HED DOC.#: 001726)

STUDY TYPE: Special 2-Year Feeding Study - Dog

OPPTS NUMBER: N/A **GUIDELINE NUMBER:** N/A

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

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

MRID NO.: 43641004 (previously 00080515) and 43641005 (previously 00113443)

TEST MATERIAL: PP062; Pirimicarb

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

REPORT NUMBER: CTL/P/117B (Study No.: UD0028)
and
CTL/P/61 (previously HO/IH/P/61) and Study No.: XD0049

SPONSOR: Zenenca Inc.*

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

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

TITLE OF REPORT: First Revision to a Pirimicarb-Induced Hemolytic Anemia in Dogs - Report of a Special Study

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STUDY COMPLETED: 1972 to 1974; reanalyzed April 21, 1995

EXECUTIVE SUMMARY: In this 110 week chronic study (MRID#s 43641004 and 43641005), four young adult Beagle dogs, two male (#s 146 and 992) and two female (#s 7 and 967), prescreened for clinical and hematological abnormalities and protected against distemper, were used in the study. In Group I, one male (#146) and one female (#7) received pirimicarb 25 and 50 mg/kg/day, respectively for 110 weeks. In Group II, one male (#992) and female dog (#967) received the above doses of pirimicarb, respectively, for about 50 to 56 weeks, treatment was withheld for ≈ 24 to 37 weeks and then dogs were challenged with low doses (1 and 2 mg/kg/day). These dogs (#992 and #967) were also supplemented with hematinics (Vitamin B_{12}, Vitamin B_6 and Folic acid) once the hemoglobin levels dropped to 50% of the initial levels. Urine, hematology, serum chemistries and blood biochemistries were done pre-treatment and at appropriate intervals during the study. Further, agglutination reaction for the presence of anti-body against RBC treated with pirimicarb was investigated using in Vitro and in Vivo systems. Similarly RBC agglutination in the presence of serum from pirimicarb treated animals was investigated.

One male (50 mg/kg/day) and one female (25 mg/kg/day) dog 12 weeks into the treatment developed anemia, characterized by falling hemoglobin levels, increased reticulocyte counts, change in size and shape of red cells. Bone-marrow cytology revealed erythroid hyperplasia with a marked left shift (M:E - 1:2.12 and 1:1.45, respectively). Supplementation with hematinics for 30 weeks did not reverse bone-marrow or hematological changes, however, withdrawal of all treatments promptly followed by complete hematological recovery. A male dog (50 mg/kg/day), after a prolonged rest, was challenged with low doses (1 to 2 mg/kg/day) of pirimicarb for 14 weeks without anemia recurring, however, a female dog, previously anemic, developed anemia which reversed following withdrawal of treatment. Presence of an antibody, reactive towards RBC from the same or other pirimicarb treated dogs, was shown in anemic dogs. The anemia in the affected dogs has been described as drug-induced auto-immune hemolytic anemia akin to penicillin induced anemia. Very high dosage and protracted administration are required to produce this effect. It was speculated that this anemia was of rare type and pirimicarb is unlikely to present any hazard to man. Urine, blood biochemistry or serum enzymes were not affected.

The study was designed as a non-guideline study to elucidate the cause of hemolytic anemia. The study is classified as **supplementary** and **does not satisfy** (due to limited design) the requirements (83-1b) for chronic toxicity study in dogs.

[Non-Guideline. Pirimicarb Tech.: 110-Week Feeding Study - Dogs/1973]

A. **MATERIALS:**

1. **Test compound:** PP062. Description - powder, Batch # - not given, Purity: 95%, Source: Plant Protection Limited, Jealott's Hill..

2. **Test animals:** Species: canines, Strain: Beagles, Age: young adults; Bodyweight and Source were not given.

B. **STUDY DESIGN:**

The study was designed to determine mechanism/cause of anemia in Beagle dogs fed pirimicarb in diet.

1. **Animal assignment**

Four young adult Beagle dogs, two male (#s 146 and 992) and two female (#s 7 and 967), prescreened for clinical and hematological abnormalities and protected against distemper, were used in the study. In Group I, one male (#146) and one female (#7) received 25 and 50 mg/kg/day pirimicarb, respectively for 110 weeks. In Group II, one male (#992) and female dog (#967) received the above doses of pirimicarb, respectively, for about 50 to 56 weeks, treatment was withheld for ≈ 24 to 37 weeks and then challenged with low doses (1 and 2 mg/kg/day) for 14 weeks. These dogs (#992 and #967) were also supplemented with hematinics (Vitamin B_{12}, Vitamin B_6 and Folic acid) once the hemoglobin levels dropped to 50% of the initial levels.

2. **Diet preparation**

The compound was suspended in Dispersol OG (was identified as corn oil in a different study) and sprayed onto the morning food of each dog in appropriate amounts, however, the amount of food offered to each dog was not provided.

3. **Statistics** - Data were not analyzed statistically.

4. <u>The studies were done during 1971 - 1974 i.e., long before the implementation of GLP Guidelines; therefore, does not fall under purview of either GLP or Quality Assurance requirements.</u>

C. **METHODS AND RESULTS:**

1. **Observations:** None described or reported as observed.

All animals survived the experimental period.

2. **Body weight** were not recorded.

3. **Food consumption**

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, urine and Bone-marrow was collected** before treatment and at two-week intervals for the first 12 weeks and subsequent sampling frequency was based on clinical lab. findings and/or therapeutic regimen.

For the animals # 7 and 146, the hematological and clinical chemistry evaluations included hemoglobin (%), white cell counts, reticulocytes % RBC, plateletts, myloid:erythroid, iron, folate, alkaline phosphatase, aspartate aminotransferase (AST, SGOT), alanine aminotransferase (ALT, SGPT), urea and lactate dehydrogenase (LDH). These tests were performed at 0, 20, 40, 60, 80 and 100 weeks.

For the animals # 992 and 967, the hematological and clinical chemistry evaluations included hemoglobin, white cell counts, reticulocytes % RBC, mean cell diameter, methemoglobin, methalbumin, Heinz bodies, serum folate, serum iron, LDH, serum bilirubin (free and conjugated), glutathione (GSH) levels, GSH stability, osmatic fragility, serum hepatoglobin, glucose 6-phosphate dehydrogenase, serum total protein and gamma globulin fraction. The above tests were done on anemic animals prior to initiation of hematinic therapy. The report did not provide any information how often these tests were done. In addition the report did not describe whether serum enzymes were done on the anemic dogs.

Agglutination reaction for the presence of anti-body against RBC treated with pirimicarb in Vitro and in Vivo systems. Similarly RBC agglutination in the presence of serum from pirimicarb treated animals was investigated.

Results - Dog #s 7 (50 mg/kg/day) and 146 (25 mg/kg/day) on pirimicarb for 110 weeks did not develop anemia and biochemical, hematological and clinical parameters were normal.

In animals #s 992 and 967 (50 and 25 mg/kg/day, respectively) anemia was evident in ten weeks into the treatment characterized by changes in the size and shape of red cells and few nucleated red cells in peripheral blood films. No abnormalities were reported in white cells and plateletts. Hematinic supplementation was initiated when the hemoglobin levels dropped to the 50% of initial levels. Both dogs were clinically healthy, however, bone-marrow cytology displayed marked erythroid hyperplasia (1:1.45 and 1:2.12, respectively) with a "left shift". Reticulocytosis and hemolysis was a prominent feature with falling hemoglobin levels. Supplementation with hematinics for about 30 weeks did not correct anemia; at this time all treatments including pirimicarb administration were discontinued. There was no change biochemical or serum enzyme levels.

Dog 992, after a prolonged rest following 50 mg/kg/day dose, was initially put on 1 mg/kg/day pirimicarb for 12 weeks, then increased to 2 mg/kg/day, did not produce anemia. Whereas, dog 967 in 25 mg/kg/day group, was placed on 2 mg/kg/day pirimicarb for 14 weeks developed anemia, though less severely. After a interval of recovery, dosing at the same dose anemia recurred.

Agglutination tests on the above dogs and as well as dogs from other pirimicarb studies, revealed presence of antibodies on the RBC of anemic dogs. This agglutination cannot be overcome by the addition of free pirimicarb. This led the investigators to believe that RBC from the treated dogs have become antigenic to elicit the production of its own antibody; therefore an immunological component was responsible for anemia and hemolysis. The anemia was reversible upon cessation of treatment. However, re-exposure of susceptible dogs to low dose of pirimicarb i.e., 2 mg/kg/day for 3 months was sufficient to produce anemia again.

D. DISCUSSION:

The study was conducted in 1974 prior to implementation of GLP guidelines and therefore, does not meet the guidelines requirements. This long-term (about 2-year) feeding study was designed to investigate the cause of hemolytic anemia observed in dogs treated with pirimicarb (MRID #43641002). Primary reason for reevaluating this study is to update old DERs and extract pertinent information which could be used to establish the **systemic and ChE NOEL and LOEL in nonrodent species. None of the studies in the data base individually are adequate to establish the toxicity end-points. However, in combination**

would provide required information and satisfy the regulatory requirements.

In this 110 week chronic study (MRID#43641004), four young adult Beagle dogs, two male (#s 146 and 992) and two female (#s 7 and 967), prescreened for clinical and hematological abnormalities and protected against distemper, were used in the study. In Group I, one male (#146) and one female (#7) received pirimicarb 25 and 50 mg/kg/day, respectively for 110 weeks. In Group II, one male (#992) and female dog (#967) received the above doses of pirimicarb, respectively, for about 50 to 56 weeks, treatment was withheld for ≈ 24 to 37 weeks and then challenged with low doses of pirimicarb (1 and 2 mg/kg/day). These dogs (#992 and #967) were also supplemented with hematinics (Vitamin B_{12}, Vitamin B_6 and Folic acid) once the hemoglobin levels dropped to 50% of the initial levels. Urine, hematology, serum chemistries and blood biochemistries were done pre-treatment and at appropriate intervals during the study. Further, agglutination reaction for the presence of anti-body against RBC treated with pirimicarb was investigated using in Vitro and in Vivo systems. Similarly RBC agglutination in the presence of serum from pirimicarb treated animals was investigated.

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Supplementary and **does not satisfy** the regulatory requirements (83-1b) for chronic toxicity study in dogs.

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Sign-off date:	09/20/96
DP Barcode:	d215390
HED DOC Number:	012061
Toxicology Branch:	tb1