RB-319
TAR-1230

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

001730

DATE: February 22, 1980

SUBJECT: Pirimicarb (4,5-Dimethyl-2-Dimethylamino-6-pyrimidinyl dimethylcarbamate) and all Registration/Petitions Related to its use. (PP#9F2235/9H5232; 9G2257/9H5238; 7F1915/9H5224; 9F2175; EPA Reg. Nos. 10182-7 and 10182-EUP-2) CASWELL #359C

FROM: Robert B. Jaeger RBJ 2/27/80
Toxicology Branch (TS-769)

TO: Marilyn Mautz
Product Manager#16

Recommendations/Conclusion

Data Requirements:

1. Rhesus Monkey - bone marrow cytology results and RBC survivability test are needed to further evaluate the effects seen in Rhesus monkeys. Should be obtained from the 17 week study.

2. Foxhound Study - NEL not determinable owing to the organ weight changes observed for spleen and liver at the lowest dose (2 mg/kg). Need histopath of liver and spleen (all doses), bone marrow cytology (including M:E ratio).

3. Beagle Dog - effects noted indicate an auto-immune hemolytic anemia which is extracorpuscular and which results in megaloblastic effects (secondary). The NEL for these effects is 0.4 mg/kg. TB agrees that hemolytic effects are similar to the penicillin type but show properties which are likewise dissimilar. The sensitization toward re-exposure has not been adequately evaluated. Evaluations are also needed for RBC survivability, Hb catabolism (i.e. urolilinogen in feces, bilirubin in serum), and qualitative evaluation of platelets and normoblasts in peripheral blood.

4. Plant Metabolite Feeding Studies in Rat - Insufficient pathology submitted, different sites were selected for blood collection (i.e. tail vein vs. heart puncture), no bone marrow cytology submitted, and insufficient biochemical evaluations to evaluate Hb catabolism. Also, TB is of the opinion that the proper species was not selected for evaluation of the plant metabolites. The Beagle Dog was obviously the most sensitive, yet the rat was selected. TB questions the selection of the rat(?) Therefore, the question of hemolytic anemia developing from exposure to selected plant metabolites has not been answered.

5. 2-Year Rat Feeding Study - The NEL is determined to be 175 ppm, based on data for females recently submitted with respect to growth depression. The LEL for growth depression is 250 ppm.

6. RCB deferrals:

(a) Nitrosamines in Cabbage - The risk analysis for DEN residues in edible portions of cabbage reveals from the Log Probit Model that the virtually safe level of DEN (mg/kg/day) is between an upper limit on risk of 5/100,000,000 and 1/10,000,000. Utilizing the One-Hit Model the following is obtained: virtually safe level of DEN (mg/kg/day) lies between an upper limit on risk of 1/10,000,000 and 5/10,000,000.

Obviously, to evaluate the risk for all tolerances requested. RCB will need to submit their nitrosamine residue evaluations for all RAC's involved, including meat and milk. See report for more detailed information.

(b) Toxicological Significance of Hydroxypyrimidine Metabolites - TB has determined that terminal residues in meat, milk and eggs occur at such a level that an adequate toxicological evaluation of the residue is needed. Therefore, a 90-day oral dosing study in the Beagle Dog is required to be performed using the major hydroxypyrimidine metabolite - metabolite VI. See report for more detailed considerations. Establishment of tolerances in terms of these metabolites is dependent upon the results of this study.

Conclusion: An adequate evaluation of the human health hazards is not possible until the deficiencies noted above are resolved.

DATA CONSIDERED IN SUPPORT OF PIRIMOR 50W

Acute Oral LD_{50} (Rat) (50% WP) (Core-Minimum)	male - 200 mg/kg female - 173 mg/kg
Acute Dermal LD_{50} (Rabbit) (50% WP) (Core-Minimum)	greater than 1000 mg/kg (highest level tested)
Primary Dermal Irritation (Rabbit) (Core-Minimum) (50% WP)	slight to mild edema and erythema
Primary Eye Irritation (Rabbit) (Core-Minimum) (50% WP)	mild (no corneal involvement)
Delayed Neurotoxicity (Hen) (Supplementary)	negative at highest dose of 25 mg/kg
Teratology (Rabbit) (Core-Minimum)	negative at 5 mg/kg
3-Generation Reproduction (Rat) (Core-Minimum)	no adverse effects on reproduction at 750 ppm (high dose); LEL is 250 ppm (growth depression in adult)

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Oncogenicity (Mouse) (Core-Minimum)	negative at highest dose of 1500 ppm
Mutagenicity-Dominant Lethal (Mouse)	negative at the high dose (20 mg/kg/day)
2-Year Rat Feeding	NEL 175 ppm (based on additional data submitted)
	LEL 250 ppm (growth depression); Not carcinogenic at 750 ppm (high dose)
2-Year Dog Feeding (Core-Minimum)	NEL 0.4 mg/kg;
	LEL 1.8 mg/kg based hemolytic anemia and erythyropoiesis effects (substantiated by Foxhound and Rhesus Monkey studies)
90/180-Day Dog Oral Dosing (Core-Minimum)	NEL 0.4 mg/kg (0.4 and 1.8 mg/kg for 90 days; 4.0 mg/kg for 180 days)
	LEL 1.8 mg/kg based on hemolytic anemia and erythropoiesis effects.

Acceptable Daily Intake Data

A. 2-Year Dog Feeding - NEL = 0.4 mg/kg (16 ppm)
S.F. = 100
ADI = 0.004 mg/kg/day
MPI = 0.240 mg/day/60 kg

Published Tolerances (PP#5F1608)

Potatoes	-	0.1 ppm
food factor	-	5.43
TMRC	=	0.00814 mg/day/1.5 kg
% ADI	=	3.37%

B. Proposed Tolerances (PP#9G2199)

Pecans	-	0.05 ppm
food factor		0.03
TMRC	=	0.00002 mg/day/1.5 kg
% ADI	=	.0019%

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Proposed Tolerances (9F2235/9H5232)

1.0 ppm - apples
0.05 " - cottonseed
2.0 ppm - apple pomace
2.0 ppm - apple pulp
0.2 ppm - cottonseed oil

Proposed Tolerances (9G2257/9H5238) EPA Reg.#10182-EUP-2

1.0 ppm - apples
0.05 " - cottonseed
0.5 " - cabbage
1.0 " - head lettuce
0.5 " - bell peppers
0.05 " - milk
0.05 " - meat, fat, meat by-products of cattle, goats, hogs, horses and sheep
0.05 " - poultry, eggs

2.0 ppm - apple pomace
2.0 " - apple pulp
0.2 " - cottonseed oil
2.0 ppm - cabbage wrapper leaves
2.0 ppm - lettuce wrapper leaves

Proposed Tolerances (7F1915/FAP 9H5224)

1.0 ppm - broccoli
0.5 " - brussel sprouts
0.5 " - cabbage
0.5 " - cauliflower
1.0 " - lettuce
2.0 " - peppers (chili)
0.5 " - peppers (bell)

20 ppm - broccoli trimmings
20 ppm - brussels sprouts trimmings
20 ppm - cabbage wrapper leaves
20 ppm - cauliflower trimmings
20 ppm - lettuce wrapper leaves

Proposed Tolerances (9F2175) EPA Reg.#10182-7

Alfalfa
0.05 ppm - Pecans

Reference is made to the memo of conference, 1/27/78, with ICI Americas, Inc. and EPA representatives; and to Dr. S-L Chan's review of PP#7F1915, dated 4/7/77. Certain problem areas were identified and ICI America agreed to clarify the issues raised by undertaking new studies and submitting additional specifically identified data which is needed. PP#9F2175 contains some of the information requested (Acc.#097847 and 097848) and this data has been reviewed with specific reference to problem areas highlighted in previous correspondence.

The specific areas of concern, which were addressed 1/27/78, are restated below:

1. Two-Year Dog Study

   (a) Interim bone marrow cytology needed to satisfy the requirements for this study.

   (b) Need to resolve the differences between the 90-day dog NEL and the 2-year dog NEL.

2. Rhesus Monkey Studies

   (a) Results of these studies are needed to answer any questions regarding the two-year dog study (i.e. in specific reference to hemolytic anemia).

3. Anemia from Metabolites

   (a) The question of possible development of hemolytic anemia resulting from exposure to two plant metabolites will be answered by the data from two 28-day rat feeding studies.

4. Two-Year Rat Study

   (a) A determination is to be made regarding the acceptability of this study (e.g. NEL not established) when considered in conjunction with the final results of NEL from the dog feeding studies.

5. Cholinesterase Sensitivity

   (a) Company needs to submit evidence which demonstrate plasma cholinesterase is more sensitive than RBC AChE towards Pirimicarb.

6. Mouse Carcinogenicity Study

   (a) This study was reevaluated by S-L Chan, 10/13/78, and was determined to be acceptable.

Before discussing the apparent hemolytic effects observed in dogs, we will address the data obtained using the Rhesus Monkey in answer to problem #2 above.

13-Week Oral - Rhesus Monkey (Huntingdon Res. Center)

Dose: 0 - 1M/1F
2 mg/kg/day - 2M/2F
25 mg/kg/day - 2M/2F

Age: 1 1/2 - 3 yrs. old

Body Weight: 2.4 - 4.5 kg

Vol. Admin.: 4 ml/kg B.W. (gavage)

Blood Samples: from femoral vein (food witheld for 16 hrs. prior to sampling)

Sternal Bone Marrow: predose, 4, 6, 8, 10 and 12 weeks

Pre-Dosing Evaluations: hematology, biochemistry, urinalysis, body weight, food/water consumption, Cholinesterase (plasma/RBC)

Results:

Cholinesterase: RBC - slight decrease at 2 mg/kg
moderate decrease at 25 mg/kg

Plasma - minor decrease at 25 mg/kg
marked decrease at 25 mg/kg

*No Biochemistry or Urinalysis values obtained during or after dosing.

*No Bilirubin values reported.

*No bone marrow smear results submitted (classification/morphology)

Direct Coombs Test results were all negative (used anti-human globulin and anti-monkey serum). There were no gross or histopathological effects related to dose administration.

M/E Ratio - 2 mg/kg - significantly less than one for 2/4 animals

25 mg/kg - not significantly different from control

NOTE: 1/6 of all bone marrow samples were contaminated with blood which indicated a somewhat poor technique, considering the importance of these values.

Hematology - No increase in reticulocytes; No dose-related effects on Hb (g%), PCV, MCHC or MCV; the number of RBC's was not apparantly effected by treatment.

Platelet counts were somewhat elevated above pre-dose values for 25 mg/kg group; these were not significant elevations however.

WBC - Eosinophils and neutrophils for 25 mg/kg were significantly increased above control during pre-dose, with eosins remaining increased following 2 weeks of treatment. Total WBC, as well as neutrophils and lymphocytes were significantly increased for both treatment groups at 2 weeks. Thereafter, the 25 mg/kg group showed continued significant elevations of neutrophils along with subsequent decrease in lymphocyte counts.

Conclusion

This study does not clarify the hemolytic anemia problem observed in dogs. The, fact that the M/E ratio was significantly effected (<1) for both 2 and 25 mg/kg groups together with the lack of bone marrow cytology and certain biochemical determinations are the reasons for this conclusion.

17-Week Oral - Rhesus Monkey (Huntingdon Res. Center)

Dose: 0 mg/kg - 4M/4F
2 " - "
7 " - "
25 " - "

Age: 1 1/2 - 3 yrs old

Body Weight: 2.0 - 3.6 kg

Vol. Admin.: 4 ml/kg/day

Food/Water Consumption: Daily

Body Wt. Determinations: Weekly

Hematology: Pre-dose, weekly, and 2, 4, 6 and 8 weeks post dosing.

Bone Marrow: Pre-dose, 4, 8, 13, 17 weeks and 4 and 8 weeks post-dose.

Cholinesterase (Plasma/RBC): Pre-dose, 2, 4, 6, 8, 10, 13, 15 and 17 weeks, and 2, 4, 6, 8 weeks post-dose.

Biochemistry: Pre-dose only

Clinical Symptons: Daily

Results

Body Weight - Slightly reduced in 25 mg/kg group; normal after recovery period.

Food Consumption - No differences from control .

Cholinesterase - RBC - Slight decrease at 25 mg/kg (normal in recovery)

Plasma - Slight decrease at 2 mg/kg

Moderate decrease at 7 mg/kg

Marked decrease at 25 mg/kg

Gross Pathology - Unremarkable

*Histopathology - Not performed; tissue preserved.

*No bone marrow cytology results submitted.

*No bilirubin results submitted.

Results of M:E ratio (sterum marrow) - historical - 1.37 (1.11-1.63):1

pre-dose - control - 1.10:1

2 mg/kg - 1.15:1

7 mg/kg - 1.10:1

25 mg/kg - 1.16:1

There were no compound related effects on the M:E ratio (4.4% of the samples were contaminated with blood).

Direct Coombs Test

Used: anti-monkey serum (Gibco)

anti-monkey globulin (Gibco)

anti-human globulin (Dade)

0 mg/kg - negative throughout

2 mg/kg - 1/8 positive (++) test week 9 thru 4 of recovery (both anti-human and anti-monkey globin).

7 mg/kg - 1/8 positive (+) test week 10-12 (anti-human globulin).

1/8 positive (+) test week 13 thru 4 of recovery (anti-human and anti-monkey globulin).

25 mg/kg - 1/8 positive (+) test week 16 (anti-human globulin).

Interpretation of Results - Anti-human globulin will cross-react with monkey; however, this is not ideal because you may lose some sensitivity (i.e. strong + will be detected, week + could be missed). The Direct Coombs' Test is a measure of in vivo coating of erythrocytes; if extracorpuscular factor (chemical) results in incomplete antibody uniting with RBC ("coated RBC"), then addition of anti-human/monkey globulin will result in a agglutination. It would appear that this did occur at the low and intermediate doses.

NOTE: Anti-monkey serum was evaluated pre-dose and test weeks 1-5. Anti-monkey globulin was not evaluated pre-dose, but was evaluated test weeks 3-17 and post-dose weeks 1-8.

Anti-human globulin was evaluated pre-dose and test weeks 1-17, post-dose weeks 1-8.

A positive control should have been used, but was not.

Urinalysis - There was an increase of Hb in the urine at 25 mg/kg in 4/8 monkeys. Indicative of Hb catabolism and often depends on Hb-Haptoglobin complex. Occurs if plasma Hb exceeds 100-130 mg/100 ml (man) or when plasma haptoglobin has been used up (man).

Hematology - No increase in retriculocytes. No apparent compound related effects on Hb (g%), PCV, MCHC, MCV or the number of RBCs.

Platelets were variably increased in the 25 mg/kg group above the pre-dose values throughout tne test and recovery phase.

WBC values were variably affected at all dose levels with increases reflected in total count as well as in lymphocytes and neutrophils above pre-dose values. Such occurrences were sporadic and not considered compound-related by the testing laboratory. However, hemolytic anemia does involve a left shift in leukocytes with moderately elevated neutrophilia not uncommon.

Conclusions

This study does not clarify the hemolytic anemia problem observed in dogs. The M:E ratio was not noticably affected by treatment.

However, the positive Direct Coombs' Test results, while not dose dependent, does implicate an immune response associated with RBCs in the monkey. The bone marrow cytology results and RBC survivablity test are needed to further evaluate this effect in Rhesus monkeys.

Rhesus Monkey - 4-Week Evaluation (Huntingdon Research Center)

No compound administration.

15M/15F

Age: 1.5 to 3 years old

Body Weight: 2.3 to 3.6 kg

Blood Samples: Weekly (vol. 4.5 ml)

Coombs' Test (Direct) - 2M/1F showed (+) to anti-human globulin but (-) the following day. No (+) reaction to anti-monkey globulin. This effect observed during week 1 and clear thereafter. The one (+) female also had slight hypochromasia.

Conclusion: None

Rhesus Monkey - 17-Week Oral Addendum (Hematology) (Central Toxicology Laboratory)

Objective: "to elucidate the occasional positive Direct Coombs' Test results noted in the Huntingdon Research Center Study".

Serological Investigations conducted after 11, 12, 14 and 17 weeks of dosing and after 5 weeks post-dosing.

Reagents used: Anti-human globulin (Dade & Ortho)
Anti-monkey globulin (Gibco)
Anti-monkey serum (Gibco)

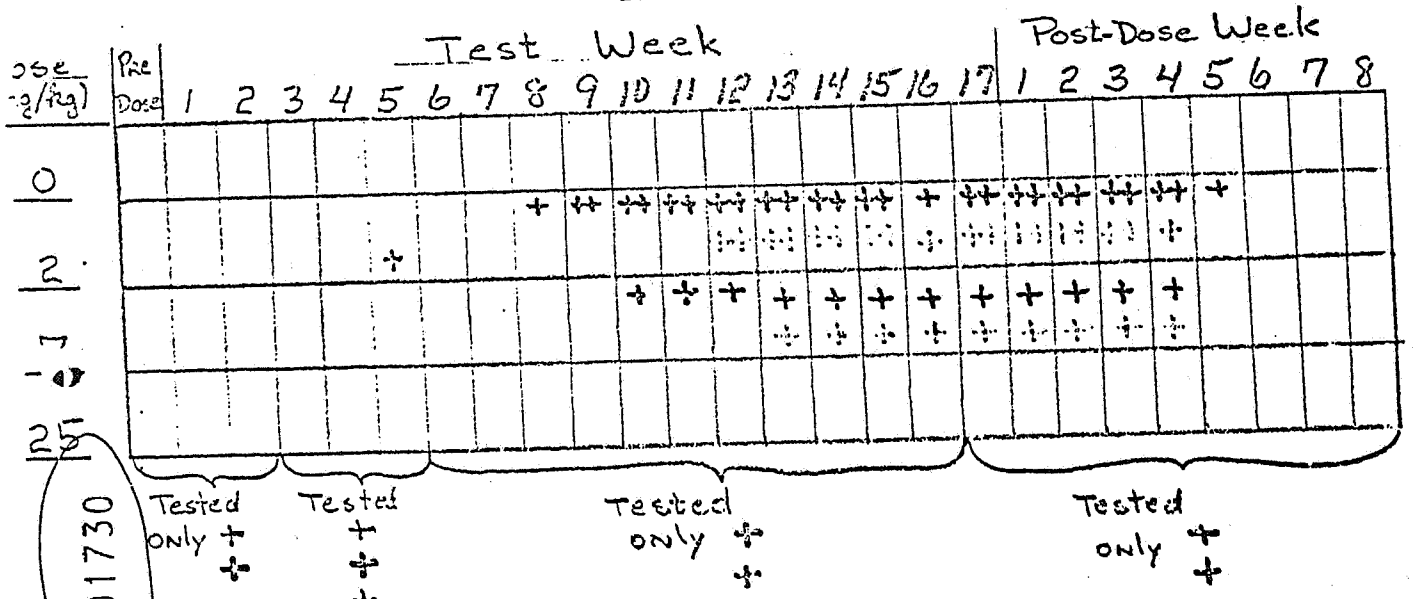
Results: 0 mg/kg - none positive

2 mg/kg - 3 positive (total) (2 + to anti-monkey serum throughout; 3 + on weeks 14, 17; 1 + to all reagents weeks 11, 12 and to anti-monkey globulin week 14).

7 mg/kg - 1 positive (total) (+ to anti-monkey serum week 11, 14, 17 and anti-monkey globulin week 11).

25 mg/kg - 1 positive (total) (+ to anti-monkey serum and globulin throughout; + to all reagents week 12).

DIRECT COOMBS' TEST (Monkey)



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CODE: ✛ = Monkey (Gibco) serum
✙ = Human (Dade) globulin
✛ = Monkey (Gibco) globulin

GRADING:
++++ large agglutinins with clear serum
+++ large agglutinins, a dew free cells
++ small agglutinins, many free cells
+ fine agglutination
± heavy granulation
0 smooth, even suspension (negative)

NOTE: 2 animals reacted in 2 mg/kg group
(1 at week 5; 1 at weeks 8 to post-dose 5)
2 animals reacted in 7 mg/kg group
(1 at week 10-12; 1 at week 13-post-dose 4)
There were 8 monkeys per group.

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Conclusions

Once again, the finding of positive results to the Direct Coombs Test by two different laboratories substantiates the possibility that pirimicarb initiates an anto-immune response in the blocd of monkeys as well as dogs. This effect does not apppear to be dose dependent but more likely due to susceptibility of sensitive individuals.

Rhesus Monkey - Untreated

To detect positive Direct Coombs' Test results in naive (untreated) Rhesus monkeys.

2/6 weak and + 2 to anti-monkey serum

1/6 very weak + to anti-monkey globulin.

Positive results were indicated to be related to an immunological response to possible infection.

Conclusions - None

The overall picture obtained from the Rhesus monkey studies would be that there are apparent effects on the auto-immune response in blood which is supportive of the data presented for dogs. In monkeys, the effects are apparent at 2 mg/kg (40 ppm). However, without the bone marrow cytology evaluations, RBC survivability and certain other biochemical evaluations (i.e. bilirubin) no definite conclusions can be assessed as regards the hemolytic effects. It would appear that the extracorpuscualr effects of pirimicarb are not limited to Beagle Dogs, but possibly the Rhesus Monkey as well.

The Direct and Indicate Coombs Tests are almost always positive at the time of acute hemolysis in man. However, a positive direct antiglobulin test does not always correlate with chemical evidence of hemolytic anemia. Detection of antibody in the serum (indirect test) would depend upon the total amount present and its binding affinity for homologous red cells. The antibody is highly species-specific. (pg. 664, O.W. Schalm)

Now, turning our attention to dogs and addressing the problems noted in item #1 above, additional data has been submitted and reviewed. See below.

16-Week Oral Feeding Study - Foxhound Dog (Hazelton Laboratories Europe)

Animals: Foxhounds

Age: 6 months

Body Weight: 12.55 to 19.6 kg

Chemical: Technical Pirimicarb (98% pure)

Dosing Regimen is very complicated and consequently makes interpretation of results somewhat involved:

		Week		
Group 1	0 mg/kg	0-16		1M/1F
Group 2	2 mg/kg	0-16		1M/1F
Group 3	25 mg/kg	0-7		2M/2F
		0-6		1M
		(11-16	-	1M/3F)
	50 mg/kg	5-6	-	1M/1F
	50 mg/kg	8-11	-	2M/3F

(Note: Group 3 has a total of 3M/3F except for 1M which was killed at week 6; also on many occasions, compound was only administered 1 out of 7 days, 2 out of 7 and 3 out of 7 days per week!) Dogs maintained for 7 weeks after dosing and then necropsied.

Observations:

Body Weight: Weekly

Food Consumption: Weekly

Hematology: Weekly (Hb, RBC, MCH, MCV, MCHC, PCV, platelets, reticulocytes, WBC and differential).

Prothrombin time and activated partial thromboplastin time - week 6 and 16.

Bone Marrow Smear: Week 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 (crest of ileum - 0.2 ml of marrow; 2 smears/dog).

Direct Coombs' Test: Weekly (used serum 3 hours after dosing; taken from jugular vein)

Cholinesterase: 4 times (Plasma, RBC)

Clinical Chemistry: Week 6 and 13 (SGOT, SGPT, SAP, BUN, glucose).

Urinalysis: One dog from Group 3 (693 male) at necropsy, week 6 (Sp. Gravity, pH, protein, glucose, reducing substance, ketones, bile pigments, blood pigments, urobilinogen, micro.)

Necropsy - I.V. pentobarbitone sodium; NOT all animals were necropsied:

1 Control
2 Group 2
4 Group 3

Organ Weighed: adrenals, brain, heart, kidneys, uterus, ovaries, liver, pituitary, prostate, spleen, testes, thymus, thyroids (could not find weights for thyroids).

Microscopy of selected organs was indicated performed, but results not submitted.

Results:

Clinical Symptoms - Group 3 - Vomiting, excessive salivation, loose feces; decreased body weight.

Group 2 - Slight decrease in body weight.

Hematology - Group 3 - Slight to marked signs of anemia (i.e. decrease in Hb, PCV, and erythrocytes count & increase in reticulocytes for weeks 9-17; also increase in MCV and decrease MCHC for weeks 14-17).

Group 2 - 697F slight lower erythrocytes count, PCV and Hb at week 5.

Bone Marrow - The following effects were described; however, a complete table listing the cell types and numbers was not provided for review.

Group 3 - Suppression of bone marrow activity (hypoplasia) when on 50 mg/kg.

- increase in monocytic series
- inclusion bodies (Howell Jolly)
- increase number of normoblasts
- erythrocytes hypochromic and anisocytosis noted

Group 2 - No effects reported.

(Effects noted were reversed, and essentially normal marrow smears were reported for week 17-23).

Direct Coombs Test - Group 3 - Weekly + in 2 dogs at week 10.

Cholinesterase

RBC not effected.

Plasma - Group 3 effected throughout.

Group 2 not effected.

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Clinical Chemistry and Urinalysis - RBC in urine of the only dog evaluated; urobilinogen 0.1 Ehrlich units/dl.

Clinical chemistry values were unremarkable.

Necropsy

No compound related effects in the dogs examined.

Organ Weights - Group 1 - no effects

Group 2 - Increase in pituitary weight and spleen weight (absolute and related).

Reported 694F with increase liver weight - however, this is not a F but a M and is in Group 3, not Group 2.

Group 3 - Increase in liver weight, pituitary weight and spleen weight (absolute and relative in all cases).

Thyroids were not weighed as indicated in the protocol.

No histopathology submitted!

Conclusions:

A NEL has not been demonstrated since organ weight changes were noted in both test groups, as well as certain hematological alterations. Of special concern is the lack of histopathology (specifically for spleen) and bone marrow cytology (including M:E ratio), which would be most beneficial pieces of data. The fact that these were not submitted makes it ever more difficult to assess the occurrence of hemolytic anemia. However, it is clearly evident in group 3 (25 mg/kg) but insufficient information is available to ascertain if this condition exists in group 2 (2 mg/kg) as well. Cursory evidence would indicate that it does.

The Petitioner has submitted numerous data to elucidate the type of anemia observed in dogs and the possible etiology of this occurrence. There has been some discussion and controversy as to whether pirimicarb causes megaloblastic anemia or hemolytic anemia (auto-immune). In order to compare the material submitted the following outlines were obtained from Laboratory Medicine - Hematology by Dr. J.B. Miale (1962); Todd-Sanford Clinical Diagnosis by Laboratory Methods by I. Davidsohn and B.B. Wells [illegible] Veterinary Hematology by J.W. Schalm, N.C. Jain & E.J. Carroll (1975); and Advances in Veterinary Science and Comparative Medicine, Vol. 14 (1970).

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TABLE I

Hematological Findings w/Megaloblastic Anemia (Code: + observed w/pirimicarb; - not observed; ND No DATA)

	Findings	Historical Observation
1.	+	Bone Marrow - Presence of various forms of megaloblasts (erythroid cells are intermediate in morphology between megaloblasts anbd normoblasts).
2.	-	Conditions treatable with Vit B_{12} or folic acid.
3.		Peripheral blood
	+	RBC vary from low to normal
	+	Hb varies from low to normal
	+	MCV increased + MCH increased
	-	MCHC slight increased
	ND	Giant platelets
	-	Reticulocytes seldom higher than 2% (because reticulocyte count in marrow is high).
	+	RBC inclusions - Howell-Jolly bodies, Cabots rings.
4.	ND	Feces - increase urobilinogen content
5.	-	Urine - increase urobilinogen and urobilin
6.	-	Serum iron increased (in relapse)
7.	+	Serum bilirubin increased
8.	+	RBC survival decreased by 1/2 to 1/4 normal

Hemolytic anemia is caused by abnormal and excessive destruction of red blood cells. Specific groups of tests are available, each aimed at the detection of a different mechanism responsible for, involved in, or resulting from the destruction.

I. Intracorpuscular abnormalities. Abnormal RBC structure.

1. Tests for osmotic fragility
2. Tests for mechanical fragility
3. Tests for autohemolysis

II. Abnormal molecular structure of erythrocytes.

4. Tests for sickling
5. Tests for abnormal types of Hb.

III. Extracorpuscular abnormalities: abnormal antibodies.

6. Tests for autoantibodies attached to the RBC (Direct Coombs Test) or circulating in the plasma (Indirect Coombs Test).
7. The gamma globulin neutralization test.

IV. Shortened survival of RBCs.

8. Tests for shortened survival of red cells.

   (a) RBC with defects transfused into normal recipient - survival will be the same as in original host but shorter than recipients RBC.

   (b) Normal RBCs transfused with defective RBC will survive normally.

   (c) RBCs of patient with extracorpuscular defect will survive in a normal recipient as long as his own RBCs.

   (d) Normal RBCs transfused to patient with extracorpuscular defect will survive only about as long as the recipients own RBCs.

V. Excessive destruction of red cells.

9. Chemical tests to demonstrate increased excretion of products of Hb catabolism.

   (a) urine - hemoglobinuria; urobilinogen increase (hemosiderin, methemoglobin, albumin).

   (b) plasma - free Hb; methemalbumin present.

   (c) serum - increase bilirubin; increase iron.

   (d) feces - urobilinogen increased (more dependable than urine because it may show an increase when urine shows none or when serum bilirubin is not raised.)

VI. Excessive regeneration

10. Tests for reticulocytes
11. Examination of bone marrow

TABLE II

Hematological Findings w/Hemolytic Anemia (same codes as above in Table I)

Findings	Historical Observations
1. ND	Peripheral blood demonstrates the presence of normoblasts.
2. -	Peripheral blood demonstrates the presence of Heinz bodies & macrocytosis. -
ND	Giant platelets
+	Platelet count normal or increased
+	RBC decreased
+	Reticulocytes increased
+	MCV normal or increased
+	MCH decreased
+	MCHC normal or decreased
+	WBC normal or moderately elevated with neutrophilia (left shift).
3. +	Osmotic fragility normal
4. +	Direct Coombs Test - positive or negative
+ (monkey)	Antiglobulin serum - cold antibody coated agglutinates best w/low serum dilutions.
-	Gamma globulin neutralization - if agglutination is inhibited by small amounts of gamma globulin, antibody is more like a "warm type".
5. +	Indirect Coombs Test
	Usually positive in severe isoimmune hemolytic anemia.
	Positive or negative in autoimmune hemolytic disease.
6. +	RBC survival decreases
7. +	Serum bilirubin increases
8. +	Plasma Hb increases
9. +	Methemalbumin - present
10. ND	Fecal urobilinogen increases
11. -	Urinary uribilinogen increases
12. +	Urinary Hb increases

TABLE III

Hematological Finds with Pirimicarb (same codes as Tables I and II)

	Findings	Clinical Observations
1.	+	Peripheral blood - change in size and shape of RBC; some nucleated RBCs; inclusion bodies (Howell-Jolly).
2.	+	Hemoglobin decrease
3.	+	Reticulocytosis increased
4.	+	WBC (total and diff.) - normal (dog) left shift (monkey)
5.	+	Platelets - normal
6.	+	Bone marrow - left shift (RBCs) - erythroid hyperplasia
	+	increase in megalobasts and precursors to megaloblasts
7.	+	RBC survival decreased (1/3 normal in dog)
8.	-	Heniz body formation
	-	Osmotic fragility
	-	mechanical fragility
9.	+	Gamma globulin fraction of serum protein increased (possibly involvement of immune mechanisms).
10.	-	Effect of Vit B_{12}, B_6 or folic acid
11.	+	Direct Coombs Test - antigamma globulin serum
12.	+	Free antibody - in serum (positive with own RBCs and other unaffected dogs receiving pirimicarb); possibly IgG - not present before treatment and dose dependent.
13.	-	Neutralization effect of pirimicarb added to serum containing specific antibody.
14.	+	Methemalbumin present

. +	Serum bilirubin (unconjugated) increased
16. +	Serum haptoglobin decreased
17. +	M:E < 1 to << 1
18. +	Splenomegaly (found in Foxhounds)

Much of the above information has been obtained from previous reports submitted by ICI and reviewed by S-L Chan, April 7, 1977. (Special studies, Report No. HO/CTL/P/117B and HO/1H/P/61). However, a correction to this review is in order. That is, 1M (#146) and 1F (#7) were dosed continuously for 110 weeks; but 1M (#992) and 1F (#967) were dosed for a shorter period of time - 48 and 56 weeks respectively. This was due to the severe hemolytic effect noted. Dosing was not reinitiated until 40 weeks (M#992, week 88) and 24 weeks (F#967, week 80) later. The response in both animals was much more immediate and drastic than the original hemolytic episodes and was discontinued after only 8 weeks (F#967, week 88) and 4 weeks (M#992, week 92). Hematological values returned to normal over the remaining weeks at which time the study was stopped after 104 weeks total elapsed time. Various tests were conducted in order to determine the cause of the anemia. However, the actual correlation in time between the 104/110 week evaluation and the subsequent low dose administration (2 mg/kg) involving some of the same dogs is not accurately and clearly presented. Such a presentation would definitely assist in determining the dose-dependence and sensitization to pirimicarb. Dr. S-L Chan's statement that "re-exposure of susceptible dogs to a low dose of pirimicarb (i.e. 2 mg/kg) was sufficient to produce anemia again" is true. However, the elapsed time between the conclusion of the 104/110 week study and the beginning of the low dose administration study is not stated. The submittal of additional data with PP#9F2175 fails to address this point and therefore, remains unanswered.

Going back to the literature, we find that canine autoimmune hemolytic anemia (AHA or AIHA) is marked by severe, recurring hemolytic anemia accompanied by a positive reaction to the direct antiglobulin (Coombs) test, which, in contrast to man, becomes negative during remission in dogs. Clinical signs include pallor, weakness, icterus, hemoglobinuria, anorexia, fever, and malaise. Splenomegaly, peripheral lymphadenopathy, and tachycardia may be detected during physical examination. Clinicopathologically, the anemia is macrocytic and normoblastic, with polychromatophilia, anisocytosis, poikilocytosis, spherocytosis and hyperplasia of the bone marrow. Most importantly, eluates of erythrocytes from affected dogs can possibly sensitize normal canine erythrocytes for the indirect antiglobulin test, thus supporting an autoimmune pathogenesis for the condition. Nevertheless, the etiology and pathogenesis remain largely obscure.

Interim bone marrow cytology data requested by Dr. S-L Chan (2-year dog study) reveals the following information:

1. a) M:E ratio is significantly less than 1 for 4 females and 2 males in the 4.0 mg/kg dose group; signifying an increased erythropoiesis.

   b) M:E ratio is not significantly effected in the other dose groups (1.8, 0.4 and 0.0 mg/kg dose groups).

2. a) Male and female dogs in the 4.0 mg/kg group show significant increase in megaloblastic cell forms throughout the study.

   b) Male and female dogs in the 1.8 mg/kg group show significant increase in megaloblastic cells at 60 and 90 day evaluation, but not at 6 months or longer.

   c) The combined megaloblast and proerythroblast values demonstrate an increase for Group 4 throughout and an increase for Group 3 for 60 and 90 days. There were no tables for evaluating the occurrence of normoblasts in peripheral blood (which are normally confined to marrow in adults). Such information would increase the confidence in the anemia condition as well as increased erythropoiesis.

ICI reevaluated the marrow smears from the 90-day dog studies and concluded that the red cell precursors previosuly identified as megaloblasts were really proerythroblasts; an indicative finding of peripheral red cell destruction or hemolysis. Nonetheless, in the 2-year dog study these cells have not been reevaluated and will be considered megaloblasts as determined by ICI in the interim bone marrow cytology reports.

Noted as a contributory factor to the clinical finding of hemolytic anemia in the subchronic dog studies was an hereditary defect within the colony of dogs used for experimentation. ICI reported the anemia to be caused in the serum and not to the RBC itself. Todd-Sanford, pg 161, indicate that hemolytic anemias are based on the location of the factor responsible for the hemolysis. Those within the RBC are hereditary. The extracorpuscular factors are generally acquired. These latter factors are best demonstrated by RBC survival studies, something which was not adequately evaluated in the 2-year dog study. The sensitivity demonstrated by related dogs (blood lines) has also been demonstrated in man (not for pirimicarb) and therefore, lends greater credence to the importance of such effects as they relate to man himself. That is, the hemolytic effect in sensitive individuals is not a chance finding and one that can be predicted with good reliability. The genetically related dogs had congenital factor VIII deficiency involved in blood coagulation. While such metabolic defects are known to occur in man as well, the hemolytic effects are therefore, even more inportant as they relate to the immunohematological system in man.

The questions of whether or not the megaloblastosis is reversed was partially answered in the Foxhcund study. No adverse effects were noted in the marrow smears when dosing was stopped. However, there were insufficient data provided with respect to a qualitative and quantitative evaluation of the smears examined. The reversibility of this effect is, therefore, not fully answered.

Unless the megaloblasts, identified in the 2-year dog study are later determined to be proerythroblasts (as was done for the special short-term studies), pirimicarb toxicity data presented and reviewed would indicate:

(1) Oral dosing of pirimicarb causes acquired hemolytic anemia of the autoimmune type (having properties similar to those caused by penicillin);

(2) The primary hemolytic effect results in a secondary effect on the marrow demonstrated in the formation of megaloblasts;

(3) The effects noted are apparently reversed when dosing is stopped. However, such a finding has not been fully evaluated (i.e. splenomegaly and liver hypertrophy noted in Foxhounds).

The question which remains is: What is the NOEL for the noted hemolytic effects?

Finding: 0.4 mg/kg (Beagle Dog, 2-Year Study)

The supporting evidence for this conclusion is derived from data submitted on Foxhounds, Beagle Dogs and Rhesus Monkeys:

1. Rhesus Monkey (17-Week Oral) -

   (a) positive Direct Coombs Test at 2 mg/kg (bone marrow cytology, RBC survivability, serum bilirubin data needed)

   (b) WBC - left shift (increase in neutrophilia) at all dose levels

2. Foxhound (16-Week Oral) - Splenomegaly (absolute and relative increase) at 2 mg/kg (No histopathology submitted; No bone marrow cytology submitted).

3. Beagle Dog (2-Year Oral) - Significant increase in megaloblastic cells in marrow at 60 and 90 days for 1.8 mg/kg dose group - (Comment: Even if these are latter identified as proerythroblasts the end result is still an increase in erythropoiesis as a result of hemolysis of the peripheral RBCs.)

4. Beagle Dog (Special short-term studies) - 2 mg/kg administered to previously dosed dogs (at 25 mg/kg) was sufficient stimulus to elicit the reoccurrence of hemolytic anemia. (needed is an accurate and complete explanation of the dosing regimen used and the correlation in time between the 104/110 week study with the low dose administration and subsequent special serological evaluations).

The experimentally produced hemolytic anemia and the involvement of the immunohematological system in more than one species of laboratory animal demonstrates that this is not a chance finding in a presumably sensitive species (i.e. Beagle Dog). Specific key evaluations have not been adequately performed:

(1) RBC survivability evaluations

(2) Bone marrow cytology

(3) Histopathology of specific target organs (namely spleen and liver)

(4) Evaluation of bilirubin (serum) and urobilinogen (feces).

(5) Peripheral blood smear evaluations for normoblasts and abnormal forms of platelets (i.e. giant platelets).

The question of possible development of hemolytic anemia resulting from exposure to two plant metabolites was evaluated in two 14/28 day subchronic feeding studies in rats. These studies are evaluated below:

14/28-Day Feeding (Rat) - Report No. CTL/P/402, Aug. 3, 1978

Metabolite - 5,6-dimethyl-2-methyl formamide-pyrimidin-4-yl dimethylcarbamate

Species: Alderley Park Albino Rat

Weight: 140-190 grams

Sex: M/F

Housing: Group caged - 5/cage (sexes separated)

Dosing: Hematology/Biochem/Histopath - 0 mg (5M/5F) for 14 and 28 days
12.5 mg/kg (10M/10F) for 14 and 28 days
50 mg/kg (10M/10F) for 14 and 28 days

Cholinesterase - 0 mg/kg (5M/5F) for 28 days
3 mg/kg (5M/5F) for 28 days
12.5 mg/kg (5M/5F) for 28 days

Observations:

Clinical: Daily

Body Weight: Daily

Biochemistry: plasma alanine transaminase (ALT)
plasma urea, Na^+, K^+

Urinalysis: pH, glucose, bilirubin, sp. gravity, protein

Hematology: Tail vein (1 ml/rat) - pre-dose, 7 and 14 days

Heart puncture (2 ml/rat) - at death

NOTE: Tail vein: 14 day group - pre-dose, 7 days
28 day group - pre-dose, 7 and 14 days

Heart puncture: 14 day group - 14 days
28 day group - 28 days

Hb, total WBC, RBC, Hct, MCHC, platelet, dif. WBC, reticulocytes, Heniz bodies, prothrombin

Bone marrow smear - 7M/7F

Pathology: All animals

liver, kidney, adrenals, lungs, heart, trachea, thymus, thyroid, spleen, pancreas, salivary gland, esophagus, stomach, duodenum, jejunum, ileum, colon, cecum, mesenteric lymph node, urinary bladder, skeletal muscle, gonads, brain, spinal cord

ChE for plasma, RBC and brain determined - 0.5 - 1 ml/rat by tail vein:

2x in seven days prior to dosing
1 hr. after 14th dose

cardiac puncture - 1 hr. after 28th dose

Results:

No body weight effect.

No clinical abnormalities reported.

No abnormal biochemical effects reported.

Cholinesterase - 50 mg/kg not evaluated (no data submitted)

12.5 mg/kg - 28 day significant decrease in plasma ChE (all other ChE - no effect)

3 mg/kg - no adverse effects

Hematology - No significant dose related symptoms reported.

No bone marrow smear cytology submitted.

Pathology - 12.5 mg/kg - animals not examined

50 mg/kg - 20M/20F a consistent finding of "none" reported, "significant pathological findings".

Conclusion:
Data is considered Supplementary Data because of: 1) insufficient pathology (gross and histo.) submitted; 2) different sites selected for hematology (i.e. tail vein and heart puncture values differ); 3) no bone marrow cytology submitted; 4) biochemical evaluations were insufficient (i.e. no serum bilirubin, no urobilinogen values, etc). The purpose of this study was to determine if anemia can be caused from the administration of one of pirimicarb's plant metabolites - this has not been adequately evaluated in this study. Secondly, the choice of species is questionable. Since hemolytic anemia was demonstrated in the dog following oral dosing of pirimicarb and was not demonstrated in the rat - Why was the rat chosen? It would seem logical to evaluate the hemolytic anemia effect in the same species which demonstrated an effect with parent compound.

14/28-Day Feeding (Rat) - Report No. CTL/P/401, Aug. 10, 1978

Metabolite - 5,6-dimethyl-2-methylamino-pyrimidin-4-yl-dimethylcarbamate

Species: Alderley Park SPF Albino Rat

Weight: 140/260g

Sex: M/F

Housing: Group cages - 5/cage (sexes separated)

Dosing:

0 mg/kg	5M/5F - 14 days	
	5M/5F - 28 days	
25 mg/kg	10M/10F - 14 days	
	10M/10F - 28 days	
100 mg/kg	10M/10F - 14 days	
	10M/10F - 28 days	

Cholinesterase - Doses tested - 0, 1.5, 5, 25 and 100 mg/kg (5M/5F per group)

Observations:

Same as Report CTL/P/402, Aug. 3, 1978.

Results:

Mortality: 1M at 25 mg/kg
5M/6F at 100 mg/kg

Hematology: Group 2&3 increased Hb
Group 3 increased neutrophilia

Bone marrow cytology not submitted.

ChE NEL - 5 mg/kg (plasma)
25 mg/kg - RBC no effect
- Brain no effect
100 mg/kg - RBC no effect
- Brain no effect

Pathology: 25 mg/kg - animal not examined

50 mg/kg - 14F/15M consistent finding of "none" reported.

No adverse effect on body weight.

No abnormal biochemical effects reported.

No adverse effects on prothrombin time.

Conclusion:

Data is considered Supplementary Data because of: 1) insufficient pathology (gross and histo.) submitted; 2) different sites selected for hematology (i.e. tail vein vs heart puncture); 3) No bone marrow cytology submitted; 4) biochemical evaluations were insufficient (i.e. no serum bilirubin, no urobilinogen values - measures of Hb catabolism). Same conclusions as other 14/28 days rat study (plant metabolite).

Therefore, TB considers that the question of hemolytic anemia developing from exposure to plant metabolites of pirimicarb is not answered.

The fourth question, raised previously by TB reviewers with respect to the 2 year rat feeding study, has been adequately addressed by paired feeding and growth studies in the rat. The 8 week dose/8 week recovery (paired feeding study) in rats at 0, 250 and 750 ppm (using 12 female rats) failed to establish a NEL. Body weight was depressed slight at 250 ppm, with marked depression in body weight observed at 750 ppm consistent with finding from previous long-term studies in rats. A follow-up study was performed incorporating two additional dose groups - 100 and 175 ppm (20 female Wistar derived rats per dose). The NEL for growth depression was determined to be 175 ppm, with effects noted at both 250 and 750 ppm. Therefore, the question of growth depression in female rats fed pirimicarb in their daily diet for 2 years has been adequately answered. The NEL for the 2 year rat feeding study is 175 ppm.

Additional data submitted with PP#9F2171 is briefly review below (References 12C, 13C and 14C):

Reference 12C: Residue Transfer and Toxicology Study with Cows Fed Treated Grass Nuts

28-29 day duration - 2/3 killed
- 1/3 recovery for 7 days (then killed)

Dose: 0, 20, 60, 200

3 Friesian Cows per dose

Gross Pathology and Histopath - abomasum, adrenals, aortic arch, colon, duodenum, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs, mesenteric lymph node, ovaries, pancreas, reticulum, rumen, skeletal muscle, spleen, thyroids, uterus.

Tissue samples (for residue) - liver, kidney, muscle (cardiac, abductor, pectoral) and fat (subcu. peritoneal); also milk.

Observations:

Toxic effects - No apparent gross effects (i.e. Body weight, milk yield, feed, etc.)

Pathology - 2 gravid females - conflicts with description of cows being "barren"!!

female #6, 60 ppm - 5 mos. pregnant
female #4, 60 ppm - 2 1/2 mos. pregnant

Animal husbandry was certainly less than adequate to insure that "barren" females were tested.

Histopath - Dosed animals contained hemosiderin laden macrophages in the small intestine, lymph nodes.

0-1/3, 20-0/3, 60-1/3, 200-1/3

No specific dose-related effects observed.

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Reference 13C: The Incorporation of Pirimicarb in the Diet of Laying Hens (Effects on Egg Production, Fertility and Hatchability)

18-weeks old (point-of-lay pullets)
30-week old (cockerels) fed 28 days after 4 week acclim. period.

Group 1	0 ppm	40	F	4F
Group 2	2 ppm	"	"	""
Group 3	6 ppm	"	"	""
Group 4	20 ppm	"	"	"" (males rotated throughtout study)

Suitable eggs laid were incubated and examined for fertility.

Chicks hatched were reared to 10 day of age (Body wt. recorded day 0 and 10).

Eggs examined for residues.

Birds sacrificed on days 21 and 28 of treatment and 35/42 post-treatment.

Post-mortem - liver, kidney, heart, brain, spinal cord, sciatic nerve.

Egg production examined daily.

Hatching - # of fertile eggs
# of early embryonic mortalities
# of later " "
# of dead in shell
chicks hatched alive
of eggs cracked or broken during incubation.

Results:

The following parameters were comparable to control: food consumption, body weight change, total egg production, percent cracked, % broken, % small, % medium, % large, number of eggs incubated, % infertile, embryonic mortalities, % hatched alive, F_1 chick.

Gross and micro - Gross - 10 birds/groups

Histo - 0 and 20 ppm dose groups only

No compound-related effects noted.

NEL 20 ppm.

Core-Minimum

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Reference 14C: Acute Toxicity of Pirimor 50W

Acute Oral LD_{50} (Alderley Park Albino Rat)

Male - 200 mg/kg (154-259)
Female - 173 mg/kg (138-219)

ChE toxicity; muscular fibrillations, urinary incontinence, increased salivation, chromodacryorrhea (blood tears).

Core-Minimum

Acute Dermal LD_{50} (N. Zealand Albino Rabbit)

LD_{50} > 1000 mg/kg (highest dose which could be practically applied).

Draize Method

No systemic effects noted.

Core-Minimum

Dermal Irritation (N. Zealand Albino Rabbit)

Draize Method
P.I. = 1.2/8.0 mild irritant

Slight to mild erythema/edema persistent in 3/6 at 48 hours.

Core-Minimum

Eye Irritation (N. Zealand Albino Rabbit)

Draize Method

Unwashed - 2/6 iritis (mild), clear in 2 days

6/6 conjunctivitis and chemosis (mild), clear in 4 days.

No corneal involvement.

Score 5/110 - 24 hrs.

Washed - No iritis

No corneal involvement.

3/3 conjunctivitis (redness), no chemosis, clear in 2 days.

Score 2/110 - 24 hrs.

Mild Irritant

Core-Guideline

The fifth question raised was with regard to the sensitivity of plasma cholinesterase versus RBC AChE toward pirimicarb. This has been adequately addressed and evaluated in data submitted with PP#9F2715. The results clearly demonstrate that plasma cholinesterase is more sensitive indicator of cholinesterase inhibition caused by pirimicarb than are RBC or brain cholinesterase values (see reference 11C, PP#9F2715).

RCB has requested that TB (10/12/79 and 1/23/80) comment on the following two points:

(1) The content of N-nitrosoamines in cabbage resulting from application of pirimicarb to growing cabbage; and

(2) The toxicological significance of hydroxypyrimidines identified in measurable amounts from animal metabolism studies.

Addressing (1) above, RCB is referenced to a 2/6/80 letter from Chief, TB to Director, HED, subject: Risk Analysis for Carcinogenicity of Nitroso-Prowl. The information in risk analyses of DEN is pertinent to pirimor as well. Using the information provided by RCB (PP#7F1915/FAP#9H5224, 10/12/79) the following is obtained:

0.2 ppb N-nitrosoamines in cabbage (TB will consider this to be worse case DEN because of a lack of adequate qualitative analysis)

0.2 ppb = 0.00001 mg/kg cabbage

1.5 kg (diet) x 0.74 (% cabbage in diet) = 1.11 kg

$$\frac{11.1 \text{ kg} \quad \times 0.00001 \text{ mg/kg}}{60 \text{ kg (man)}} = .000,000,185 \text{ mg/kg/day}$$

This level of exposure from pirimor treated cabbage (as calculated by RCB) would result in:

Log-Probit Model

Upper Limit on Risk	Virtually safe level of DEN (mg/kg/day)
5/100,000,000	0.000,000,160
1/ 10,000,000	0.000,000,215

One-Hit Model

Upper Limit on Risk	Virtually safe level of DEN (mg/kg/day)
1/10,000,000	0.000,000,074,2
5/10,000,000	0.000,000,371

"Depending on the actual upper limit on the risk, a single choice of the extrapolating model can either underestimate or overestimate the 'virtually safe' level of an agent by comparison with another such extrapolating model."

Continuing to comment on the same question raised by RCB, TB is deferring to RCB to resolve the following points:

(a) Since N-nitrosoamine moieties have been identified in the regular sales pack pirimicarb formulation (0.17 ppm) and soluble sales pack (0.51 ppm), why are they not evaluated as residues with regard to PP#9F2235/FAP#5232 (apples and cotton)? All tolerance consideration of pirimicarb will require a 'worst case' calculation of N-nitrosoamine residue in each crop, as was done by RCB for cabbage, in order for TB to assess the toxicological significance of such exposure.

(b) Has RCB considered the time of last application to the time of harvest? What is the degradation or half-life of the n-nitroso compounds applied to each specific RAC for which tolerances are requested?

(c) Which N-nitroso compounds are we concerned with? Owing to a lack of any qualitative information with regard to the N-nitrosoamine moieties involved, TB will consider the worst case - DEN, unless demonstrated to be otherwise.

(d) Is RCB satisfied with the validity of this method for analyzing N-nitrosoamine contaminants in pirimicarb formulation? As questioned in 10/12/79 RCB comments (Deficiency 1.)

The second (2) point raised by RCB (10/12/79) concerns the toxicological significance of measureable terminal residue of hydroxypyrimidine metabolites (V thru VII) in meat and milk. RCB has determined that the metabolism of pirimicarb has been found to be similar in rats, dogs, cows, goats and hens and that the nature of the residue in animals is adeqautely understood. Plant metabolism studies have demonstrated that far less residue ends up as hydroxypyrimidine metabolites (V thru VII). For example, in cabbage:

	% of total residue
met. V	2.7
met. VI	4.1
met. VII	5.4

(plus 6.1% of all three which is conjugated)

This is in contrast to goats:

	% of total residue
met. V	2
met. VI	35
met. VII	29

The animal studies involved feeding of the parent compound and not treated RAC's which can account, in part, for greater percentage of hydroxypyrimidine moieties as a residue. However, TB considers that the feeding of spiked or treated RACs's to animals will result in similar findings (% of total residue) for terminal residues, as animals are apparently more efficient in the conversion of pirimicarb and its carbamate moieties into one of three hydroxypyrimidine metabolites. Also, the fact that such large percentages are found in edible tissue (i.e. meat, milk, liver) causes some concern. The following are rough estimates of the terminal residues (of hydroxypyrimidines) found in animal tissue:

Animal	Dose (ppm)	Residue (ppm	Tissue	Major Metabolite
Cow	33	0.09 - 0.225	milk	VI, VII
		0.036	meat/fat	V, VI, VII
Goat	37	0.06 - 0.15	milk	VI, VII
		0.27	muscle	
		0.108	fat	
		1.08	liver	
		1.38	kidney	
Hen	16	0.025 - 0.03	egg yolk	VI, VII
		0.22	liver	
		0.0126	fat	
		0.0945	muscle	

Certain pyrimidine analogs have been found to cause bone marrow hypoplasia, increased M:E ratio and megaloblastic changes in erythyroid precursors. This finding, coupled with the amount of residue in edible meat and milk, as well as the lack of any toxicity data presented for the hydroxypyrimidine metabolites requires the following information to be submitted:

(a) 90-Day Oral Dosing Study in the Beagle Dog

Study should follow previously submitted ICI 90-Day Dog Study with emphasis on bone marrow cytology, peripheral blood evaluations, M:E ratio, and liver and spleen pathology. Subject study should be performed using hydroxypyrimidine metabolite VI at the same doses used in the 2-year dog feeding study (i.e. 0.4, 1.8, 4.0 mg/kg).

In the event similar toxicicity to the parent compound (i.e. pirimicarb) is displayed by hydroxypyrimidine metab. VI then the tolerances requested will need to be reassessed. The probability of including the hydroxypyrimidine metabolites along with pirimicarb will need to be addressed.

Label review for EPA Reg. No. 10182-7 (7/28/78), Pirimor 50W:

The following additional precautionary statements are needed:

"Avoid breathing spray mist. Avoid contact with eyes, skin and clothing. Remove and wash contaminated clothing. Wash hands, arms and face with soap and water after use and before eating, drinking or smoking."

The following additional first aid statements are needed:

"In case of contact, wash skin with soap and water; for eyes, flush with water. Get medical attention if irritation persists."

Robert B. Jaeger, Physiologist
Toxicology Branch, HED (TS-769)

c. 7/11/80