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TXR-1726

(D)

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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SUBJECT: Request for tolerances for Pirimicarb, 2-(dimethyl amino) 5,6-dimethyl-4-pyrimidyl dimethylcarbamate and metabolites on cole crops (0.5 - 1.0 ppm), lettuce (1.0 ppm) and peppers (0.5 - 2.0 ppm).

DATE: APR 7 1977

FROM: Petition No. 7F1915
Tox, Branch, Sin-Lam Chan Ph.D. *Sin-Lam Chan*

TO: Product Manager, No. 16, Mr. Mautz M.A. *E. Mautz 4/7/77*

Petitioner: Mr. Melford F. Tietze, Manager
Agricultural and General Chemical Affairs
ICI United States Inc.
Concord Pike and New Murphy Rd.
Wilmington, Delaware 19897

Recommendations:

Do not grant the requested tolerances for Pirimicarb on cole crops, lettuce and peppers. The submitted toxicological data are not adequate for the establishment of:

1. The no effect level (NEL) in chronic rat studies.
2. The lack of carcinogenic effect on mice.
3. The NEL and the reversibility on megaloblastosis and antibody elicitation.
4. The lack of immunogenic sensitization.

In addition, the method used for all erythrocyte and brain acetylcholinesterase determination is not suitable.

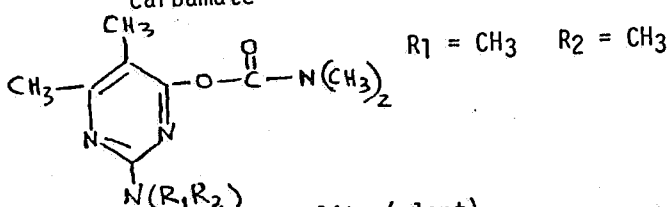
Reviewed and additional toxicology data for these purposes will be required. While the rat and the mouse carcinogenicity studies should be repeated, our main concerns are directed towards studies on bone-marrow cytology, antibody and anemia production and possible immunogenic sensitization. The development of hypochromic anemia from exposure to the two major plant metabolites will require further investigations. The petitioner should be advised to either submit further informations now available or to consult with toxicology branch for the new data required.

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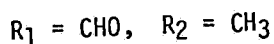
A. Substance Identification:

1. Chemical name and structure

- a. Parent compound
2-(dimethylamino)-5,6-dimethyl-4-pyrimidyl dimethyl-
carbamate



- b. Active metabolite (plant)
2-(formylmethylamino)-5,6-dimethyl-4-pyrimidyl dimethyl-
carbamate



- c. Active metabolite (plant)



2. Synonym: Pirimicarb and metabolites
compound PP062 and metabolites

3. Impurities in technical pirimicarbs

As in previous review

4. Physical and chemical data.

- a. Appearance: A colorless, odorless crystalline solid.
- b. Melting point: 88-90°C
- c. Vapour pressure: Low at 25°C, 1.6×10^{-5} mm Hg
Appreciable at 65°C, 1.8×10^{-3} mm Hg

Note: There is a large increase in vapor pressure as the temperature is raised from 25°C to 65°C. At moderately elevated temperature, the inhalation of vapor Pirimicarb by workers can be substantial.

- d. Solability: Very soluble in organic solvents
In water at 25°C ~ 2700 ppm
- e. Chemical class: A pyrimidine carbamate, a reversible acetylcholinesterase inhibitor

001726

-3-

B. Referenced petitions

Petition No. 5F1608, EPA #10192-7, EPA #10182-7, (Pirimar 50W and Pirimar 50 WP)

C. Formulations

1. Percentage composition

Pirimicarb (>95% active) = 52.6% Active

2. Inert clearance

D. Uses proposed

Pirimar 50 WP is proposed for pre-harvest use as a diluted spray for the control of aphids on cole crops (broccoli, brussel sprouts, cabbage and cauliflower), lettuce, bell and chili peppers. The application rate is recommended as 4-8 oz. diluted to 30-150 gallons per acre, sprayed in a 7-10 days program or as needed.

Toxicological studies

- A. The following studies submitted in Petition No. 5F1608, EPA No. 10182-7 are accepted as presented in a previous toxicology branch review, a copy of which is enclosed here.

1. Studies using the Technical Grade Material
 - a. Oral LD₅₀ for rats
 - b. Oral LD₅₀ for mice
 - c. Oral LD₅₀ for dogs
 - d. I.P. LD₅₀ for rats
 - e. Primary skin irritation study (rabbit)
 - f. Dermal LD₅₀ for rats
 - g. Neurotoicity study with hens
 - h. 10-day rat feeding study
 - i. 14-day rabbit dermal study
 - j. 3 weeks actual exposure inhalation study (rat)
 - k. 3 weeks saturated vapor inhalation study (rat)
 - l. Skin sensitization study with guinea-pigs
 - m. Primary eye irritation study (rabbit)
 - n. Mutagenic study - Mouse dominant lethal test.
 - o. Teratology study (rabbit)
 - p. Dog and rat metaolism study
 - q. Rat absorption and excretion study
 - r. Workers operation experience during product formulation.
2. Studies using the 50% formulation (Pirimor 50 WP)
 - a. Oral LD₅₀ for rats (356 mg/kg, tox. cat. II)
 - b. Primary skin irritation study with rabbits. (moderate, tox. cat. III)
 - c. Primary eye irritation study with rabbits (slight, tox. cat. III)
 - d. Inhalation LC₅₀ for rats (20 mg/l, tox. cat. IV)

B. The following studies are unsatisfactory for support of the current petition.

1. 90-day oral toxicity of Pirimicarb on rats (Report No. 1HR/237)

Protocol: 25M and 25F per group were dosed for levels of 0, 250 and 750 ppm and 25 mg/kg/day. Growth rate, food consumption, plasma and erythrocyte cholinesterase activity were monitored at weekly intervals. Hematology, organ weight, brain AChE and histopathology were conducted at the end of 90 days. 5 of each sex per group were continued for a further 28 days after Pirimicarb withdrawal.

Results: Plasma cholinesterase inhibition among female and male rats fed 25 mg/kg Pirimicarb was observed. The growth rate of female rats was retarded for all test dose levels. No similar effects were seen for male rats. There were no other compound related effects found.

Deficiency: (a) No interim reports on hematology and urinalysis (b) No bone-marrow cytology study conducted. This is especially important because of megakoblastosis development in treated dogs. (c) The no effect level (NEL) on female rat growth rate cannot be satisfactorily established.

2. 2-year rat carcinogenicity study (Report No. H0/1H/P/24) ✓

Protocol: 48M and 48F rats per group were dosed for levels of 0, 250, 500 and 750 ppm of Pirimicarb. The mortality, growth rate, food consumption, hematology for 6M and 6F were examined at 0, 26, 52, 78, 91 and 104 weeks. RBC and plasma cholinesterase activity was assayed for 0 and 750 ppm levels at 6 month intervals. Brain AChE, histology, organ weight and gross examinations were conducted upon animal death and terminally.

Results: No carcinogenic effects of Pirimicarb was found. The female growth rate was retarded for all dose levels used. This effect was not seen in male rats. Relatively high incidences of respiratory disease and mammary tumors were seen but these were not treatment related. The mean survival rate for 2 years was 64%.

Deficiency: (a) A NEL cannot be established for female rats. (b) No interim reports on hemachemistry, urinalysis, and bone-marrow cytology.

3. 80-week mouse carcinogenicity study (Report No. HO/CTL/P121/B)

Protocol: 50M and 50F mice per group were tested for dose levels of 0, 300 and 1500 ppm of Pirimicarb. Food intake and body weight gain were monitored weekly for the first 12 weeks and thereafter, monthly. Inspection was made daily for general behavior and clinical abnormalities. Gross examinations and histopathology were conducted upon death and termination of test.

Results: Body weight gain reduction was seen at 1500 ppm level. This study was marred by a very high incidence of respiratory disease, with only about 30% of the animals surviving the 80 weeks period. The scanty results obtained are suggestive of pulmonary tumors.

Deficiency: (a) Only 2 dose levels were used. (b) Less than 40% of the animals surviving at termination of test; mortality in part due to disease. (c) There was a suggestion of pulmonary tumors.

4. 90-day dog feeding study (Report No. 1HR/241)

Protocol: 4M and 4F dogs per group were dosed at levels of 0, 4, 10 and 25 mg/kg/day of Pirimicarb. Half the number of animals were continued for an additional 28 days undosed. Examinations included were body weight, food intake, mortality, hematology, hemachemistry, urinalysis, cholinesterase activity, macroscopic and microscopic examinations, organ weight and bone-marrow cytology.

Results: Inhibition of plasma cholinesterase activity was shown at 10 mg/kg and above. Megaloblastosis was produced in certain animals in all test doses. 3 dogs at 10 mg/kg and 25 mg/kg manifested anemia. Full recovery from anemia but only partial reversal from megaloblastosis resulted 28 days after cessation of treatment.

Deficiency: (a) No NEL can be established, (b) No full recovery of megaloblastosis achieved.

5. 90 and 180-day dog feeding study (Report No. 1HR/248)

Protocol: 4M and 4F dogs per group were treated for dose levels of 0, 0.4, 1.8 (90 days) and 4.0 mg/kg/day (180 days). Examinations conducted were body weight gain, food intake, hematology, cholinesterase activity, gross and microscopic examinations, organ weight and bone-marrow cytology.

Results: No anemia was produced in dogs at any dose level. There was some increase in megaloblastosis in dogs fed 1.8 mg/kg ($P < 0.01$, > 0.05) but became marked at 4 mg/kg.

NEL established at 0.4 mg/kg/day for 90 days study.

Deficiency: No attempt is made to determine (a) if megaloblastosis is fully reversible and (b) if this effect has any sensitization potential.

6. 2-year dog feeding study (Report No. HO/1H/R/337)

Protocol: 4M and 4F dogs per group were dosed at levels of 0, 0.4, 1.8 and 4.0 mg/kg/day. Body weight was measured weekly for the first 12 weeks and thereafter monthly. Urinalysis, hematology, hemachemistry, bone marrow cytology and cholinesterase activity were monitored at intervals of 3-6 months. Histopathology, gross examinations and organ weight were conducted upon death and termination of test.

Results: No adverse effects were found on gross and histological examinations. No anemia was produced in any dog. There was an increase in megaloblastosis and in the erythroid/myeloid ratio for dogs fed 4 mg/kg dose. Other parameters examined were found normal.

Deficiency: (a) Interim bone-marrow cytology data were not presented and (b) No study was conducted on the complete reversal of megaloblastosis.

7. Pirimicarb induced anemia in dogs (Special studies, Report No. HO/CTL/P/117B and HO/1H/P/61)

Protocol: 1M and 1F dogs per group were dosed at levels of 25 and 50 mg/kg/day for 110 weeks. Examinations included were, general behavior, hematology, hemachemistry, urinalysis and bone-marrow cytology. The presence of anti-body was studied for complex formation with RBC treated with Pirimicarb either in Vitro or in Vivo. Similarly RBC agglutination in the presence of serum from Pirimicarb treated animals was studied.

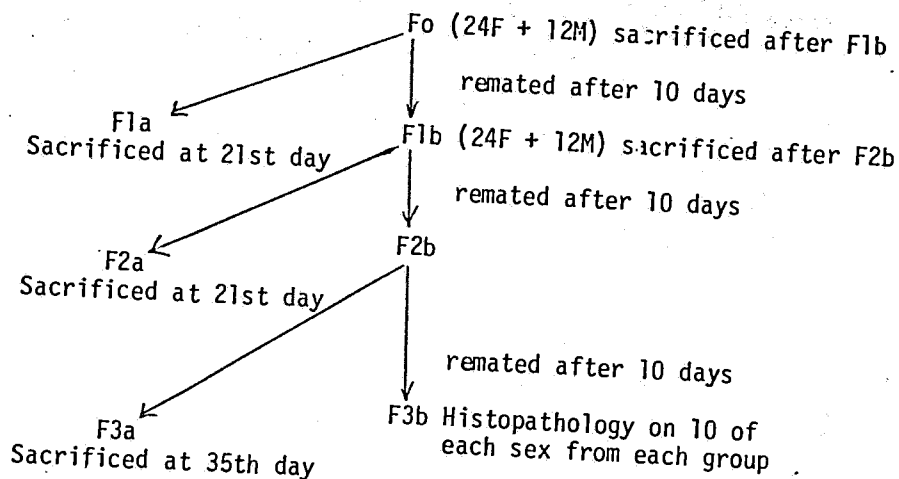
Results: Pirimicarb treated dogs developed markedly disturbed erythropoiesis, reticulocytosis and falling hemoglobin. Hemolysis was prominent. Hemachemistry was however normal. The presence of an antibody, reactive towards RBC from the same or other Pirimicarb treated dogs was shown in anemic dogs. This agglutination cannot be overcome by the addition of free Pirimicarb. This observation led to the conclusion that RBC from treated

dogs have become antigenic to elicit the production of its own antibody. The immunological system is implicated in the development of hemolysis and consequential anemia. The anemia developed is reversible upon cessation of treatment. However, re-exposure of susceptible dogs to a low dose of Pirimicarb, i.e. 2 mg/kg for 3 months was sufficient to produce anemia again.

Deficiency: The involvement of the immunological system in Pirimicarb exposure may lead to sensitization, the absence of which is not examined.

8. 3 generation rat reproduction study (Report No. H0/1H/R/339)

Protocol: 12M and 24F per group were tested for dose levels of 0, 250 and 750 ppm. The outline of study is presented below:



Examinations and observations included are: mortality, body weight gain, food intake, No. of pregnancies, litter size, live births, still births, sex, pup weight fertility index and pup abnormality.

Results: Female (Fo, F1b and F2b) body weight gains were retarded for all test doses. This effect was less for male rats and only evident at the 750 ppm level. Food utilization efficiency was however, not altered. Reproductive performance and litter data were not unusual.

Deficiency: The deficiency here is minor, but serves to confirm the effect on female growth rate of the rat at similar dose levels.

9. Three 2-year rat feeding studies (Report No. CTL/P/183)

Protocol: A different strain of rats was used in each study. Tests and examinations included body weight gain, food intake, clinical abnormality, macroscopic and microscopic examinations upon animal death and at the termination of studies.

Study (a): 48M Sprague-Dawley rats were dosed at 0, 750 and 2500 ppm.

Study (b): 48M Alderly Park rats were dosed at 0, 750 and 2500 ppm.

Study (c): 24M and 24F Wistar rats were dosed at 0 and 750 ppm from the day of inception and for 2 years after weaning.

Results: All 3 studies suffered from high mortalities due to an outbreak of respiratory disease.

Study (a): There was a reduction in growth rate at both test dose levels. The food utilization efficiency was decreased only at the 2500 ppm level. An increase in the number of tumors in surviving test animals was seen. The number of tumors/live rats for control and test animals were respectively: 2/7, 12/15, 14/14.

Study (b): A growth rate retardation accompanied by a food utilization efficiency decrease was seen at the 2500 ppm level. There was no evidence of an increase of tumors in test animals.

Study (c): A growth rate reduction was seen for both male and female test animals. No difference in food utilization efficiency was noted. No significant difference in tumor incidence was found.

Deficiency: These studies are of minimal value due to a high mortality rate as a result of an outbreak of respiratory disease. The average percentages of rats surviving the test period were 25, 29 and 43% respectively. From these scanty results there was a suggestion of carcinogenic effect in Sprague-Dawley rats.

Study (c) suffers from a small number of test animals employed. A NEL from these studies cannot be established.

Toxicity of Metabolites

The following studies are accepted as in the previous tox. branch review.

Acute rat oral LD50 for:

1. R34885, 2-(formylmethylamino)-5,6-dimethyl-4-pyrimidyl dimethylcarbamate, (plant)
2. R34836, 2-(methylamino)-5,6-dimethyl-4-pyrimidyl dimethylcarbamate, (plant)
3. R31805, 2-(dimethylamino)-5,6, dimethyl-4-hydroxy pyrimidine (plant and animal)
4. R34865, 2-(methylamino)-5,6-dimethyl-4-hydroxy pyrimidine (plant and animal)
5. R31680, 2-amino-5,6-dimethyl-4- ^{hydroxy pyrimidine} pyrimidyl dimethylcarbamate
6. R35140, 2-amino-5,6-dimethyl-4- pyrimidyl dimethylcarbamate
7. Guanidine
8. Methyl guanidine sulfate
9. Dimethyl guanidine hydrochloride

1. Subacute rat intubation study with Desmethyl pirimicarb - a major plant metabolite (Rep. No. H0/1H/T/842)

Protocol: 10M and 10F were dosed with 100 mg/kg/day for 2 weeks. Studies included mortality, body weight gain, cholinesterase activity. Hematology, macro and microscopic examinations were conducted terminally.

Results: Plasma ChE was inhibited by 25%. Female rats exhibited hypochromic anemia and male rats, showed reticulocytosis some hypochromic anemia. Histopathology revealed an increased hemopoietic activity in the spleen and thymus of some animals. Other parameters were unaffected.

Deficiency: (a) Only one single dose used for a relatively short exposure period. No NEL possible (b) No attempt was made to determine the reversibility of the hypochromic anemia produced.

2. Subacute rat intubation study with Desmethyl formamidopirimicarb - a major plant metabolite (Report No. H0/1H/T/843)

Protocol: 10M and 10F were dosed with 25 mg/kg/day for 2 weeks. Studies included body weight gain, food intake, hematology, hemachemistry and pathology.

-11-

Results: Some female rats showed hypochromic anemia while male rats exhibited only hypochromia. An increased hemopoietic activity in spleen and an increased mitotic activity in livers of some animals were observed. Other parameters were not unusual.

Deficiency: The same as those given in the study with Desmethylpirimicarb.

D. Literature Review:

1. Not in ARC monographs or Revocation list.
2. Only a brief mention in Toxic Substances lists.
3. Reviewed in World Health Organization Meeting, Rome, Nov./22-30/1976.
 - a. "Pirimicarb" by Dr. R.L. Baron
 - b. "Pirimicarb" by Dr. F.K. Ohnesorge
 - c. "Pirimicarb on Cholinesterase by A.R. Main.

E. Evaluation of ADI.

Not possible.

F. Conclusion:

The acute studies on the technical and formulation are acceptable. Certain special and longer term studies including teratology, mutagenicity, metabolism, neurotoxicity, subacute dermal, inhalation and skin sensitization tests are satisfactory.

However, there are several major short comings that make safety evaluation of the tolerances requested on cole crops, lettuce and peppers impossible. These inadequacies are presented below:

1. Pirimicarb is a carbamate, a reversible inhibitor of true acetylcholinesterase (AChE) and a partially reversible inhibitor of plasma cholinesterase, (ChE), a pseudocholinesterase. For the correct determination of the enzyme inhibition by a reversible inhibitor, no dilution or as little as possible of the inhibitor concentration should be made. No consideration toward such an end has been registered. Δ PH esterase assay method used involves 10-100 folds dilution of the inhibitor concentration from brain to erythrocyte and plasma cholinesterase determinations. Since Pirimicarb is only partially reversible towards

plasma ChE; the values obtained for the plasma enzyme are to a large extent correct. All the values presented for brain and erythrocyte AChE are however not valid.

2. No-effect levels (NELs) cannot be satisfactorily established for rats from long term feeding studies. All dose levels employed in the 2-year feeding and the 3-generation reproduction study, 250, 500 and 750 ppm exerted a retardation on the growth rate of female rats. Another three 2-year feeding studies using 3 different strains of rats are of no value due to very high mortalities as a result of an outbreak of respiratory disease. In addition the dose levels, 750 and 2500 ppm, used were too high to include a NEL.
3. The carcinogenicity study with mice is not acceptable for 2 main reasons. These are (1) very high mortalities from respiratory disease with only an average of 30% of the animals surviving the test period and (2) from the data available there may be a development of pulmonary tumors in test mice.
4. From the subacute, special and the 2-year dog feeding studies, the development of megaloblastosis, anemia and anti-body elicitation are documented.

The NEL for the 90-day study may be established at 0.4 mg/kg/day. However an NEL for the 2-year study cannot be satisfactorily established because of the lack of any interim data on bone-marrow cytology. An NEL for the production of anti-body in longer term study is not approached. While recovery from anemia upon cessation of dosing has been shown, only partial reversal from megaloblastosis has been presented. The involvement of the immunological system may lead to sensitization development towards Pirimicarb re-exposure, the lack of which is not investigated.

5. A review by Chemistry Branch, EPA on the residues in cole-crops, lettuce and peppers is requested. Toxicology data are only presented for metabolites in potato tubers. The subacute studies on the 2 major potato metabolites presented are not adequate in design and the lack of a NEL. The development of hypochromic anemia will require further investigations.